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AUSTRALIAN INDIGENOUS EDIBLE HALOPHYTES — NUTRITIOUS AND FUNCTIONAL FOR A SUSTAINABLE FUTURE: ANTIOXIDANT CAPACITY AND ANTIMICROBIAL PROPERTIES

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ABSTRACT: In recent years, edible halophytes have received more attention due to their ability to tolerate a wide range of salinities. Furthermore, halophytes have long been used for food, feed and medicinal purposes. However, available information on their nutritional profile (including antioxidant compounds) and bioactivity is still very limited. Therefore, the present study investigated the antioxidant capacity and antibacterial activity of three important Australian indigenous edible halophytes, *Sesuvium* sp. (Seapurslane), *Suaeda* sp. (Seablite) and *Atriplex* sp. (Saltbush), to assess their bioactive properties and potential to be used as functional food ingredients. The antioxidant capacity was determined by total phenolic content (TPC), total flavonoid content (TFC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and the antimicrobial activity was evaluated using the agar well diffusion method. The methanolic extract of Seapurslane showed the highest TPC (12.5 mg GAE/g DW), TFC (4.3 mg QE/g DW) and DPPH (102.6 µM TE/g DW), followed by Seablite and Saltbush. The ethanolic extract of Seapurslane had antimicrobial activity against Gram positive *Staphylococcus aureus* bacterium, a predominant food pathogen causing gastroenteritis and other health issues. These initial results are very promising and indicate that Australian-grown halophytes may have the potential to be utilised as novel sources of antioxidant and antimicrobial compounds for different food applications.

Keywords: halophytes, saltbush, seapurslane, seablite, antioxidant capacity, antimicrobial activity

INTRODUCTION

Halophytes, biologically specialised plants, are capable of growing in a saline environment of more than 200 mM NaCl (Flowers & Colmer 2008). Adaptive responses of halophytes against salinity and drought consist of both biochemical mechanisms and specific compounds such as phenolics, alkaloids, polysaccharides and lipids (Ksouri et al. 2012). Although representing less than 2% of the plants worldwide, halophytes are expected to have a significant contribution to the production of sustainable plant food in the future due to their demonstrated salt and drought tolerance (Sharma et al. 2016; Srivarathan et al. 2023). Halophytes can thrive in saline environments where 99% of other (salt-sensitive) plants perish due to ion toxicity induced by high salinity (Flowers & Colmer 2008). Furthermore, it has been reported that 45 million ha (20% of total farming land) are salt-affected worldwide. Therefore, there is a high potential for halophytes in utilising saline lands for sustainable food production in the future (Srivarathan et al. 2021). According to the World Health Organization, about 65–80% of the world's population, particularly in developing countries, depend on medicinal plants including halophytes for primary health care (Ksouri et al. 2012; Safari & Ahmady-Asbchin 2019). However, reports about the utilisation of Australian indigenous edible halophytes (AIEH) for medicinal purposes as well as functional food applications are still very limited (Faustino et al. 2019; Ksouri et al. 2012; Srivarathan et al. 2022). Two recent studies by Srivarathan et al. (2021, 2023) on important AIEH could identify Samphire, Saltbush, Seablite and Seapurslane as valuable dietary sources of minerals and trace elements, particularly K, Zn, Mg, Ca and Fe, fibre, protein, vitamin C and some phytochemicals.

Among the bacteria responsible for approximately 167 known food-borne diseases, *S. aureus* is a predominant pathogen, which can cause gastroenteritis and other serious health issues as a result of the consumption of contaminated food (Le Loir et al. 2003). However, screening indigenous and underutilised edible plants, such as halophytes, for their antioxidant capacity and

antimicrobial activity, could be an alternative pathway for the discovery of natural antimicrobials to inactivate *S. aureus* and other food-borne pathogens. Therefore, three important AIEH, Saltbush, Seapurslane and Seablite, were investigated in the present study for their antioxidant capacity and antimicrobial properties, as a measure of their bioactivity and functionality.

MATERIALS AND METHODS

A total of 1 kg of wild harvested Seablite (*Suaeda arbusculoides*) and Seapurslane (*Sesuvium portulacastrum*) was collected from Twin Lakes Cultural Park (Kimberley, WA, Australia). The samples were packed in airtight polythene bags, frozen and transported to the laboratory at the Health and Food Sciences Precinct, Coopers Plains, Queensland, in temperature-controlled containers. The dried leaves of Saltbush (*Atriplex nummularia*) were sourced from NATIF Australian Superfoods, Fruits, Herbs, Spices and Mixes (Glen Eira, VIC, Australia). Freeze-dried ground samples were stored at -35 °C prior to extraction and analysis.

Antioxidant capacity

Extraction

The extraction of the samples was performed as described by Hong et al. (2020). Briefly, 0.5 g of dried powder of AIEH was vortexed with 3 mL of 80% aqueous methanol containing 0.1 M HCl. Then the mixture was shaken using a reciprocating shaker (RP1812, Paton Scientific, Victor Harbor, SA) for 10 minutes at 200 rpm and centrifuged (Eppendorf Centrifuge 5804, Hamburg-Eppendorf, Germany) at 3900 rpm for 10 minutes at 4 °C. The supernatant was collected, and the residue was re-extracted with the extracting solvent, followed by ultra-sonication at 4 °C, shaking and centrifugation as described above until the supernatant was colourless. Finally, the supernatants were combined and filtered through a 0.2 µm PP membrane filter prior to the determination of the total phenolic content, total flavonoid content and DPPH radical scavenging capacity. All extractions were performed in triplicate.

Total phenolic content (TPC)

The TPC (Folin-Ciocalteu assay) was determined as described previously by Phan et al. (2019), using a micro-plate absorbance reader (Sunrise, Tecan, Maennedorf, Switzerland) at 700 nm. TPC was expressed as milligrams of gallic acid equivalents per gram dry weight extract (mg GAE/g DW), based on an external gallic acid standard curve.

DPPH radical scavenging capacity

The methanolic sample extract was evaporated at 40 °C, using a miVac sample Duo concentrator (Genevac Inc., Gardiner, NY, USA). The dried extract was re-dissolved in absolute methanol and further diluted to different concentrations for the DPPH assay. The DPPH radical scavenging capacity was determined as previously described by Moore and Yu (2008) with slight modifications using a microplate absorbance reader (Sunrise, Tecan) at 517 nm. The radical scavenging capacity was expressed as µM Trolox equivalents (TE)/g DW, based on an external Trolox standard curve.

Total Flavonoid content (TFC)

The TFC was determined as previously described by Wang et al. (2019) with slight modifications using a microplate absorbance reader (Sunrise, Tecan) at 415 nm. Briefly, 100 µL of sample extracts were mixed with the same volume of 2% aluminium trichloride ($AlCl_3$) in methanol. Absorption readings were taken after 10 minutes against a blank sample consisting of a 100 µL extract solution with 100 µL methanol without $AlCl_3$. TFC results were expressed as mg quercetin equivalents (QE)/g DW, based on an external quercetin standard curve.

Antimicrobial activity

Sample preparation

Freeze dried AIEH samples (0.5 g) were extracted with water using three different extraction methods (cold extraction, decoction and infusion) and acidified aqueous ethanol (80%; cold extraction) as previously explained by Guimarães et al. (2011) with modifications:

1. Cold extraction: Powder sample was macerated with 5 mL of water or acidified aqueous ethanol in a container and placed on a shaker (150 rpm) for 24 h in an incubator at RT.
2. Decoction: Water (5 mL) was poured into a container and boiled in a water bath, then powdered sample was placed into the container while boiling, stirred and kept for 10 minutes.
3. Infusion: Powdered sample was placed into a container. The hot extraction solvent (5 mL water at 70°C) was poured into the container and kept for 10 minutes.

Then the mixture was centrifuged (5000 rpm, 10 minutes; Eppendorf Centrifuge 5804) and the supernatant was collected and filtered (Whatman No. 1 filter paper; Sigma-Aldrich, Castle Hill, NSW, Australia). The filtered solution was evaporated at 60 °C using a miVac sample Duo concentrator (Genevac Inc.). Dried extracts were reconstituted with water and 20% aqueous ethanol, and the extraction yield was calculated as follows:

$$Yield\ (\%) = \frac{Weight\ of\ extracts\ after\ solvent\ evaporation}{Weight\ of\ freeze\ dried\ plant\ powder} \times 100$$

Microbial strains and preparation of inoculums

The antimicrobial activity of AIEH powdered samples was tested against two bacterial strains including Gram-positive bacteria *Staphylococcus aureus* NCTCC 6571 and Gram-negative bacteria *Escherichia coli* NCTCC 9001, as well as the fungi *Candida albicans* ATCC 10231, causing food-borne diseases. The microbial strains (bacterial and fungi) were obtained from the American Type Culture Collection (ATCC), USA or the National Collection of Type Cultures (NCTC), UK. Each microbial strain was precultured overnight in Mueller Hinton Agar (MHA) at 37 °C and 30 °C for bacterial and fungal strains, respectively. The inoculum was prepared in saline solution (0.9% NaCl) and the absorbance was adjusted to 0.08–0.1 (according to 0.5 McFarland turbidity standard) at 600 nm using a Thermo Fisher Scientific GeneSys 20 spectrophotometer (Thermo Fisher Scientific, Melbourne, VIC, Australia).

Antimicrobial screening

Antibacterial and antifungal activities of different solvent extracts were determined using the agar well diffusion method as previously explained by Seididamyeh et al. (2023) with slight modifications. Briefly, MHA plates were inoculated with the prepared microbial suspensions, and 8-mm wells were aseptically made in the inoculated agar plates. Then, 100 µL of plant extracts were added to each well. Water and 20% ethanol were included as negative control to investigate the effect of solvent on microbial growth. The plates were incubated at 37 °C (for bacteria) and 30 °C (for fungi) for 18 hours. Antimicrobial activity was determined by measuring the zone of inhibition around the respective wells after the incubation period. Results were reported as mm of inhibition zone (excluded the 8 mm of the well). The inhibition zone was classified as low (1–6 mm), moderate (7–10 mm), high (11–15 mm), and very high antimicrobial activity (16–20 mm) (Mosbah et al. 2018). The experiment was carried out in triplicate.

Statistical analysis

The results were expressed as mean ± standard deviations (SD) and analysed using a multivariate general linear model (IBM SPSS statistics 26; IBM, Sydney, NSW). Pearson's correlation coefficient (R) and the coefficient of determination (R^2) was calculated for testing the correlation between the DPPH radical scavenging capacity, TPC and TFC. The means were compared using ANOVA and Duncan's multiple range tests, and probability was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

TPC, TFC and DPPH free radical scavenging capacity

The TPC ranged from 6.4 to 12.5 mg GAE/g DW, with Saltbush having the lowest value and Seapurslane the highest (Figure 1). Overall, these values were lower than that of Samphire, another AIEH wild harvested in the Kimberly Region, Western Australia (12.6 to 54.2 mg GAE/g DW; Srivarathan et al. (2021)). Saltbush and Seablite had similar TPC values (6.4–7.8 mg GAE/g DW). However, the TPC of Seablite in the present study (7.8 mg GAE/g DW) was lower than that reported for *Suaeda fruticosa*, *Suaeda pruinosa* and *Suaeda mollis* (24.5–32.7 mg GAE/g DW), but higher than that in *Suaeda maritima* (3.5 mg GAE/g DW) (Oueslati et al. (2012). Saltbush, on the other hand, had considerably higher TPC values than that reported in the literature for the same species (6.4 vs. 3.0 mg GAE/g DW; Ben Salem et al. (2002)).

Phenolic compounds act as antioxidants by scavenging radical species, chelating trace metals including Cu^{+} or Fe^{2+} which are responsible for the production of free radicals (Chintalapani et al. 2018) and can exert a stronger antioxidant capacity than Vitamin C, E and carotenoids. Flavonoids are the most common polyphenols in our diets (Dai & Mumper 2010). Seapurslane had the highest TFC and Saltbush the lowest (4.3 vs. 2.2 mg QE/g DW),

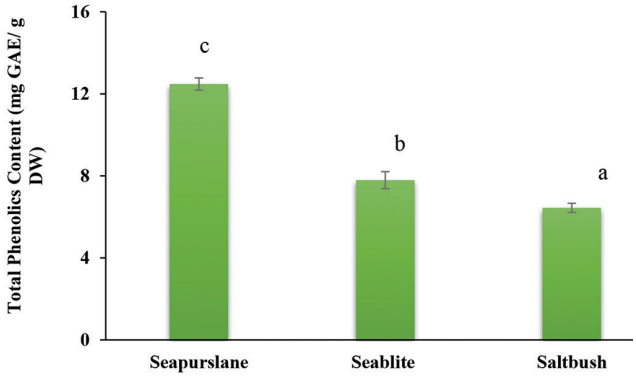

Figure 1: TPC in the studied AIEH species. Data are means ± SD (n=3); Different letters indicate significant ($p < 0.05$) differences.

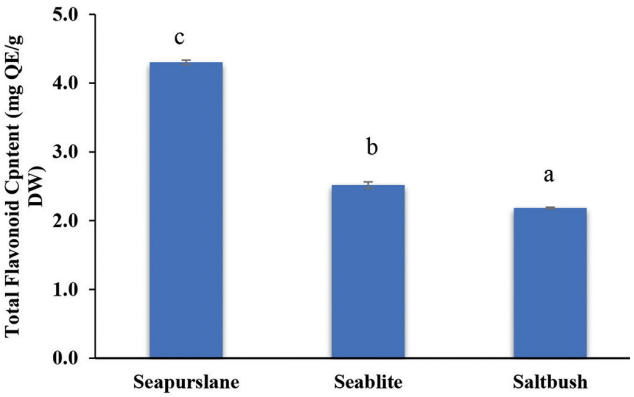

Figure 2: TFC in the studied AIEH species. Data are means ± SD (n=3); Different letters indicate significant ($p < 0.05$) differences.

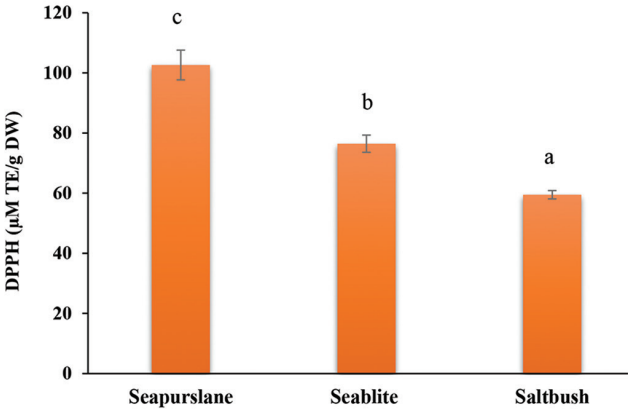

Figure 3: DPPH free radical scavenging capacity in the studied AIEH species. Data are means ± SD (n=3); Different letters indicate significant ($p < 0.05$) differences.

reflecting the TPC results (Figure 2). However, the TFC in both AIEH was considerably lower than that reported by others for the same species: 4.3 vs. 22.0-56.8 mg QE/g DW for Seapurslane (Chintalapani et al. 2018) and 2.2 vs. 56.7-98.1 mg rutin equivalents/g DW for *A. halimus* (*Atriplex* species) (Souad et al. 2019). It should be noted that the TFC reported by Souad et al. (2019) was expressed as rutin equivalents and not quercetin equivalents, as in the present study. As already observed for TPC and TFC, Seapurslane had also the highest DPPH radical scavenging capacity and Saltbush the lowest (102.6 vs. 59.5 µM TE/g DW; Figure 3). Furthermore, TPC and TFC were found to have a strong positive correlation to DPPH antioxidant activity with the Pearson correlation coefficients (R) of 0.96 and 0.92, respectively. This finding is a clear indication that phenolic compounds are most likely the main antioxidants in the studied AIEH. However, further experiments are warranted to identify and quantify individual phenolic compounds and their potential contribution to the observed antioxidant capacity. Finally, it should be mentioned that the observed differences in TPC, TFC and DPPH between the three AIEH species are most likely caused by species dependent characteristics: *Atriplex* sp., *Sesuvium* sp. and *Suaeda* sp. have all specific matrix related features and traits, affecting their metabolic and biochemical pathways and processes, and subsequently their nutritional composition. However, differences between the AIEH investigated in the present study and data reported in the literature for the same species are most likely caused by different environmental factors (e.g. growing location, soil quality, rainfall), stage of maturity at harvest, pre- and post-harvest treatment, including storage and transport as well as differences in the applied analytical methods.

Antimicrobial activity

The extraction yields of AIEH obtained with different extraction methods and solvents are summarised in Table 1. Seablite delivered the highest extraction yields with 40.5% and 25%, respectively, using the water maceration and ethanol maceration method. However, only the ethanolic (maceration) extract of Seapurslane showed an inhibition zone against *S. aureus* of 5.1 mm, which was lower than that reported by Magwa and co-workers for essential oil from Seapurslane leaves (9.5 mm; 100% v/v). The observed higher antimicrobial activity against *S. aureus* was most likely caused by the presence of monoterpenes in the essential oil extracted from the leaves (Magwa et al. 2006). The observed inhibition zone (5.1 mm) indicates a 'low' antimicrobial activity of the ethanolic Seapurslane extract against the Gram-positive bacteria *S. aureus* (Figure 4). However, the ethanolic Seapurslane extract was not effective against *E. coli* (Gram-negative bacteria) and *C. albicans* (yeast). The observed difference in antimicrobial activity of the ethanolic Seapurslane extract can most likely be attributed to the structural differences of the microorganisms, such as the presence of hydrophobic phospholipid membranes in Gram-negative bacteria which prevents the passage of bioactive compounds (Delcour 2009). Interestingly, neither of the water or ethanolic extracts of Saltbush and Seablite showed any antimicrobial activity against the three tested microorganisms. However, the methanolic extract of *A. halimus*, another Saltbush species, showed antimicrobial activity against both Gram-positive bacteria including *Bacillus cereus*, *Listeria ivanovii* and *S. aureus* and Gram-negative bacteria including *Klebsiella oxytoca* and *Klebsiella pneumonia* as reported by Rahman and co-workers (Rahman et al. 2011). It should be noted that the authors used a different extraction procedure. *S. australis* and *S. maritima*, both belonging to the *Suaeda* species, also demonstrated antimicrobial activity against Gram-positive (*S. aureus*, *Listeria monocytogenes*, *Streptococcus pyogenes*, *Enterococcus faecalis* etc.) and Gram-negative (*Pseudomonas aeruginosa*, *Proteus mirabilis*, *K. pneumonia* etc.) bacteria as previously demonstrated by Kim and co-workers (Kim et al. 2016). The differences observed in antimicrobial activity of Saltbush and Seablite in the present study and the literature can most likely be attributed to different extraction protocols and solvents (Cowan 1999; Masoko et al. 2007). Therefore, further studies are warranted, using different extraction methods and solvents as well as a broader spectrum of microorganisms, to elucidate the antimicrobial potential of the studied AIEH. However, in the case of Seapurslane, the identification and quantification of individual bioactive compounds in the ethanolic extract are required to explain its antimicrobial activity.

Table 1: Antimicrobial activity of AIEH extracts against three microorganisms using different extraction methods.

Extraction Method	Yield (%)				Inhibition Zone (mm)		
	Maceration	Water Decoction	Infusion	Ethanol Maceration	*S. aureus*	*E. coli*	*C. albicans*
Seapurslane	7.9 ± 1.3	5.7 ± 2.1	15.9 ± 3.0	15.5 ± 1.9	5.1 ± 0.3*	-	-
Seablite	40.5 ± 2.0	31.3 ± 2.7	38.1± 2.2	25.0 ± 2.8	-	-	-
Saltbush	18.4 ± 1.9	14.4 ± 2.6	13.4 ± 1.5	12.5 ± 1.6	-	-	-

*Ethanolic extract; -: no observed inhibition zone; Yield %: relative amount of extracted sample material in dry mass; values are means ± standard deviations (n=3)

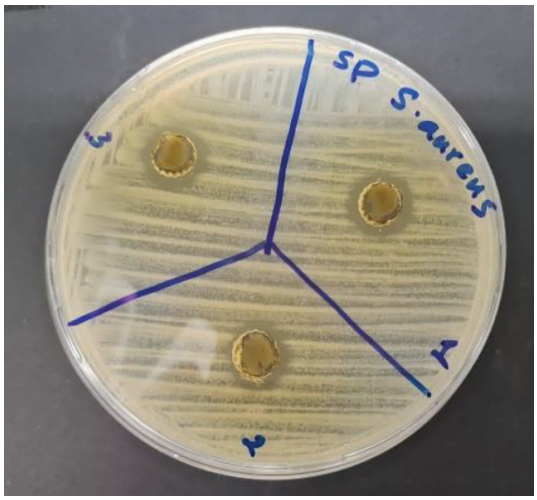

Figure 4: Inhibition zones (mm) of ethanolic extracts of Seapurslane against *S. aureus*.

CONCLUSION

The present study provided crucial information on the antioxidant capacity and antimicrobial activity of three important Australian indigenous edible halophytes (AIEH). The generated information is also useful for the food and functional food industry regarding the development of new products and ingredients. Furthermore, follow-up studies with a larger sample size in terms of total sample number, replicates, seasons and locations/sub-locations are needed to better understand the impact of species and environment on the bioactivity of these underutilised Australian indigenous edible plants.

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Conflict of interest

The authors declare no conflicts of interest.

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