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Mesembryanthemum edule (*Aizoaceae*) is an edible halophyte widely used as a traditional remedy against fungal and bacterial infections. This study investigates phenolic contents and biological activities of aque-ous methanolic fractions (methanol/acidified water, v/v: 20/80, 40/60 and 60/40) of *M. edule* leaves, stems and roots. The most phenol-rich fractions were leaf 20%, stem 60%, and root 40% (from 671 to 989 mg GAE g^{-1} DR). The highest ferric reducing power was found in leaf 40% and stem 40% (86 and 94 $\mu g\ ml^{-1}$, respectively) whereas the highest total antioxidant activity was noted in root 40% (395 mg GAE g^{-1} DR). Concerning the antimicrobial activity, organ extracts were assayed for their activity against food borne bacteria and fungi. The inhibitory percentages ranged from 0 to 94%. Stems showed the strongest antibac-terial activity (inhibition of the growth in 6 of the 7 strains tested) especially against *Micrococcus luteus* (82%) followed by leaves and roots. Regarding fraction polarity, 60% and 20% aqueous methanolic frac-tions were the most and the less active fractions, respectively. All the organ fractions showed a high antifungal activity, notably against *Kloeckera apiculata* (85% for leaf 40%) and *Candida albicans* (77% for stem 40%). Even more, leaf, stem and root fractions were very potent in inhibiting growth of filamen-tous fungi, with inhibition percentages varying between 23% and 99%. Overall, the fractionation process enabled a better assessment of *M. edule* antioxidant and antimicrobial activities, which were attributed to various metabolites with different polarities. Our findings also indicate that *M. edule* organs could be used as a potent source of natural antioxidants and antibiotics.

1. Introduction

Medicinal plants are a dependable source of therapeutics for the treatment of various ailments (Hoareau and Da Silve, 1999). The increase in antibiotic resistance and the growing interest in natural products have placed medicinal plants in the front lines as a reliable source for the discovery of active antimicrobial agents and possibly even novel classes of antibiotics (Saleem et al., 2010). It has long been established that naturally occurring substances in plants have antibacterial and antifungal activities. The presence of a wide range of phytochemicals such as phenolic compounds has been suggested to exert antibacterial, antiviral, and antiseptic effects (Romani et al., 2006) as well as protecting the human body against oxidative damage (Ksouri et al., 2008). In addition, some phenolics (tannins) have the capacity to inhibit certain viruses and bacteria, such as human immunodeficiency virus (*HIV*) and *Helicobacter pylori* that cause ulcers (Funatogawa et al., 2004). In this context, herbal medicines are further requested by several industry mainly pharmaceutical ones. Moreover, numerous useful drugs from higher plants have been discovered by following up ethnomedical uses (Fabricant and Farnsworth, 2001). The diversity of plants offers a huge possibility of finding novel structures with antioxidant, antibacterial and antifungal properties. Thus, recent works showed that some medicinal halophytes display high polyphenol levels, powerful scavenging capacity against free radicals and strong antimicrobial activity (Falleh et al., 2009; Ksouri et al., 2009; Meot-Duros et al., 2010).

Nowadays different chromatographic processing methods can be employed to enhance the bioaccessibility of phenolic compounds by modifying the crude extract compositions (Amarowicz et al., 2010; Falleh et al., 2011; Martinez-Correaa et al., 2011; Meot-Duros et al., 2010). Currently, column chromatographies of Sephadex LH 20 or reversed C18 phase are often used for fractionation of phytochemicals into several fractions according to the phenolic properties (weight, solubility and polarity). The

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fractionation is a suitable approach to facilitate the study of these secondary metabolites activities (Martinez-Correaa et al., 2011; Myjavcovà et al., 2010). In fact, it should be noted that in the case of polyphenol mixtures in a solution, molecules may be rearranged and their structure and cross-orientation changed, leading to a decrease of their biological activity in the mixture (Sokol-Letowska et al., 2007). Amarowicz et al. (2010) showed that antioxidant activity of *Lens culinaris* fractions was significantly higher compared to the crude extract. Therefore, the separation of polyphenolic extracts into several fractions seems to be beneficial in respect of their biological activity (Martinez-Correaa et al., 2011).

Mesembryanthemum edule L. is a multipurpose and edible halophyte that is used traditionally for the treatment of various diseases, e.g. sinusitis, diarrhoea, infantile eczema and tuberculosis (Wisura and Glen, 1993). Moreover, it is used by traditional healers as remedy against fungal and bacterial infections. The widespread use of *M. edule* as an anti-infective medicine led us to further investigate its biological activities especially the antimicrobial one. We have previously shown that raw methanolic extract of *M. edule* shoots had a strong antioxidant activity and was rich in phenolic compounds (Falleh et al., 2009) and that leaf and stem fractions contain procyanidins and propelargondins with strong antioxidant activity (Falleh et al., 2011). In the present work, we report the first attempt of antimicrobial activity analyses in extract fractions of *M. edule* organs by focusing on antibacterial, anti-yeast, and anti filamentous fungal activities.

2. Materials and methods

2.1. Plant sampling

M. edule (*Carpobrotus edulis* L.) plants were collected from Jerba (600 km south of Tunis, arid with temperate winter bioclimatic stage, mean annual rainfall 50–200 mm). The samples were rinsed with distilled water and separated into groups of leaves, stems and roots. Each organ was then freeze-dried and ground to a fine powder in a Mettler AE 200 (Dangoumau type) grinder.

2.2. Extraction, clean up and fractionation

The powder (5 g) of each organ was extracted three times by 50 ml hexane to remove lipids and pigments. The resulting residue was re-suspended in 50 ml methanol (three times). Each extraction was carried out by maceration in the solvent for 5 min under magnetic straining, and the mixture was filtered through a Whatman No. 4 filter paper.

Methanol filtrates were combined, concentrated under vacuum fractionated on reverse phase silica gel (Waters, Milford, MA, USA). Methanolic extracts were loaded onto the column containing 30 g C18 resin per g of dry extract, followed by 80 ml of 2.5% acetic acid to remove sugars and other polar compounds. Phenolics adsorbed onto the resin were then eluted with 20 ml of different increasing MeOH/diluted acetic acid mixtures (successively 20, 40, 60, 80 and 100% methanol in 2.5% acetic acid, v/v). The obtained fractions for each organ were separately evaporated to dryness, weighed to obtain the "dry residue" and re-suspended in 5 ml pure methanol.

2.3. Polyphenol content and antioxidant activity

2.3.1. Quantification of total polyphenol

The level of total phenolics in *M. edule* organs was determined with the Folin–Ciocalteu reagent using the method described by Dewanto et al. (2002). To 125 μl of each extract or fraction, 500 μl of reagent and 125 μl of distilled water were added. The mixture was shaken, added with 1250 μl of Na_2CO_3 (7%, w/v) and adjusted with distilled water to a final volume of 3 ml. After incubation for 90 min at 23 °C in the dark, the absorbance *vs.* prepared blank was read at 760 nm in UV–visible spectrophotometer (Anthelie Advanced 2, Secoman). Total phenolic content was expressed as mg gallic acid equivalents per gram of dry residue (mg GAE g^{-1} DR) using a calibration curve with gallic acid (0–400 μg ml^{-1}). All samples were analyzed in triplicate.

2.3.2. Determination of total antioxidant activity

Total antioxidant capacity of methanolic extracts was evaluated through the assay of a green phosphate/Mo^{5+} complex according to the method described by Prieto et al. (1999). An aliquot (0.1 ml) of diluted extracts/fractions was combined with 1 ml of reagent solution (0.3 N sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Methanol was used instead of sample for the blank. The tubes were incubated in a boiling water bath for 90 min. Then, the samples were cooled to room temperature and the absorbance was measured at 695 nm against blank. Antioxidant capacity was expressed as mg gallic acid equivalent per gram of extract dry residue (mg GAE g^{-1} DR). All samples were analyzed in triplicate.

2.3.3. Evaluation of iron reducing power

The reducing power of *M. edule* organs was determined through the transformation of Fe^{3+} to Fe^{2+} induced by plant extracts according to the method of Oyaizu (1986). Sample solutions at different concentrations were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%, w/v). The mixture was incubated at 50 °C for 20 min. Afterwards, 2.5 ml of TCA (10%) were added and the mixture was centrifuged for 10 min at 1000 × *g*. Supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1%, w/v), and the absorbance was read at 700 nm against ascorbic acid as authentic standard. Higher absorbance of the reaction mixture indicates greater reducing power. EC_{50} value (μg ml^{-1}) is the effective concentration of the extract at which the absorbance was 0.5 and it was obtained from linear regression analysis.

2.4. Evaluation of the antibacterial and antifungal activities

2.4.1. Microorganisms

Antimicrobial activity was tested against a panel of microorganisms, mainly food-borne pathogens and clinical isolates: four Gram-positive bacteria (cocci and bacilli), four Gram-negative bacteria, yeasts and filamentous fungi (Table 1). For each type of bacteria, at least one strain was obtained from American Type Culture Collection (ATCC), all the others were from Brittany Culture Collection (BCC).

2.4.2. Antibacterial bioassay

Strains were grown in liquid nutrient broth (Difco, Surrey, England) at 37 °C for 24 h and 48 h, for bacteria and yeast respectively, before being used for antimicrobial test. The microplate bioassay (microdilution) was used to study the antimicrobial activities of *M. edule* organs. Aliquot corresponding to 100 μg of dry plant extracts were dropped in sterile 96-well plates (NUNC microplate, Fisher Bioblock). After complete evaporation of the solvent, 100 μl of microorganism suspensions (10^2 cells per ml) obtained by dilution from the culture tube (10^8 cells/ml), were added in each well. Microbial suspension was used alone as positive control or in the presence of antibiotic mixture (5 mg ml^{-1} streptomycin and 10 mg ml^{-1} penicillin G) as negative control. Finally, the microplate was aseptically sealed, agitated and incubated at 30 °C for 24 h. Then, microorganism growth was estimated by reading the absorbance in microplate wells at 405 nm with a microplate spectrophotometer (Multiskan MCC/340, Titertek).

Table 1
Bacterial and fungal (yeasts and mould) strains investigated. (BCC: Brittany Culture Collection http://www.univ-brest.fr/souchotheque.)

Strain	Species	Culture collection, identification number	Pathology
Cocci Gram positive	*Enterococcus faecium*	ATCC 6569	Human pathogen causing meningitis, endocarditis and urinary tract infections (Darby, 2005)
	Micrococcus luteus	ATCC 10240	Common environmental contaminant (Harisha, 2007)
	Staphylococcus aureus	ATCC 33862	Human pathogen causing skin infections, food poisoning, toxic shock syndrome and septic shock (Darby, 2005).
Bacilli Gram positive	*Bacillus cereus*	ATCC 6464	Human pathogen causing foodborne illnesses (Friedman et al., 2007)
Gram negative	*Escherichia coli*	ATCC 1053	Pathogen causing foodborne illnesses (Friedman et al., 2007)
	Erwinia carotovora subs, atroseptica	BCC	Phytopathogen (Darby, 2005)
	Pseudomonas aeruginosa	BCC	Pathogen to human, animals and plants (Darby, 2005).
	Salmonella typhimurium	ATCC 49416	Food contaminant and pathogen (Friedman et al., 2007)
Fungi			
Yeast	*Candida albicans*	BCC	Human pathogens, hard to eradicate in the hospital environment (Baltch et al., 2000).
	Candida krusei	BCC	Human pathogens, hard to eradicate in the hospital environment (Baltch et al., 2000).
	Kloeckera apiculata	BCC	Economic importance (agent of fermentation) (Pscheidt and Glieder, 2008)
	Pichia anomala	BCC	Economic importance (agent of fermentation) (Pscheidt and Glieder, 2008)
	Schizosaccharomyces pombe	BCC	Economic importance (agent of fermentation) (Pscheidt and Glieder, 2008)
Filamentous fungi	*Aspergillus niger*	BCC	Economic importance (agent of fermentation) (Pscheidt and Glieder, 2008)
	Penicillium glabrum	BCC	Food contaminant, opportunistic in the respiratory tract (Harisha, 2007)

2.5. Antifungal bioassay

2.5.1. Anti-yeast activity

This activity against yeast strains was assessed using the same protocol than bacteria ones except that plates were incubated at 25 °C and absorbance was measured at $\lambda = 405$ nm.

2.5.2. Anti-filamentous fungi activity

Concerning the filamentous fungi, *Aspergillus niger* and *Penicillium glabrum* were grown on potato dextrose agar (PDA, Difco) for 1 week at 25 °C in the dark. Then, inocula were prepared by transferring sporulating mycelium (scraped from the agar surface by a sterile loop) to sterile distilled water containing 0.05% of Tween 80. The suspensions were mixed to ensure homogeneity and subsequently diluted in 2.5% potato dextrose broth (PDB, Difco) to get a final concentration of about 10^3 spores ml^{-1} (concentration was checked on a Malassez cell). The antifungal activity of each fraction was determined with the modified (National Committee for Clinical Laboratory Standards) broth microdilution method of Liu et al. (2007) recently adapted to filamentous fungi (Bonenfant–Magné et al., unpublished results). The microplate bioassay (microdilution) previously described for the antibacterial activity was performed excepted that 5 μl of resazurin (1 mg ml^{-1} in water) was added as a redox indicator of fungal growth. Resazurin did not affect the efficacy of antibiotics and this widely used indicator has no inhibitory activity against fungi (Liu et al., 2007). Negative control was carried out using 10 μl of an antibiotic mixture containing pimaricin (1 mg ml^{-1}), amphotericin B (2.5 mg ml^{-1}) and aminotriazol (1 mg ml^{-1}). Plates were incubated at 25 °C and absorbance of the bright pink resorufin issued from resazurin oxidation was measured at $\lambda = 492$ nm after four days. The background for each sample, measured at $t = 0$, was subtracted from each final reading.

2.5.3. Quantitative evaluation of antimicrobial activity

The antimicrobial activity of *M. edule* extracts may be expressed in different ways based on the methodology used. In this work, antimicrobial efficiency was expressed as described by Fabri et al. (2008) in percentage of growth inhibition, percentage of activity (proportion of microorganisms inhibited by one extract), as well as microbial susceptibility index MSI (proportion of extracts inhibiting one microorganism). The absorbance data allowed calculating the percentage of growth inhibition using the formula (1):

$$\text{Growth inhibition}\,(\%) = 100 - \left[100 \times \frac{A_{\text{sample}} - A_{\text{SC}}}{A_{\text{GC}} - A_{\text{SC}}}\right] \quad (1)$$

where A_{SC}, absorbance of the sterility control (negative control); A_{GC}, absorbance of the growth control (positive control).

Then, the antimicrobial activity of *M. edule* extracts was expressed as percent activity values, using the formula (2), and as the microbial susceptibility index (MSI) using the formula (3):

$$\text{Activity}\ (\%) = 100 \times \frac{\text{No. of susceptible strains}}{\text{Total no. of strains}} \quad (2)$$

The percent activity value highlights the total antimicrobial potency of particular extracts. It shows the number of microbial strains susceptible to one particular extract.

$$\text{MSI} = 100 \times \frac{\text{No. of extracts effective against each microbial strains}}{\text{No. of total samples}} \quad (3)$$

MSI values ranges from '0' (resistant to all samples) to '100' (susceptible to all samples).

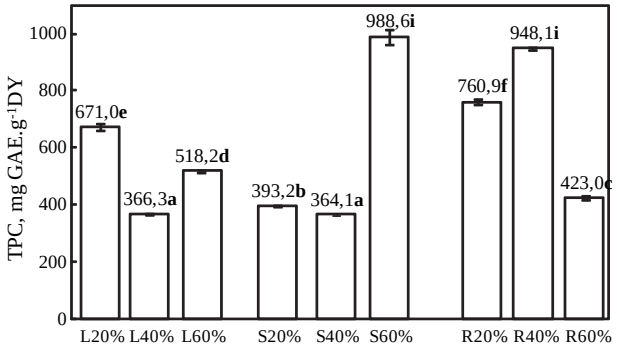

Fig. 1. Total polyphenol content, expressed as mg GAE g^{-1}DR, in *Mesembryanthemum edule* leaf, stem and root fractions. Means of three replicates followed by at least one same letter are not significantly different at $p < 0.05$.

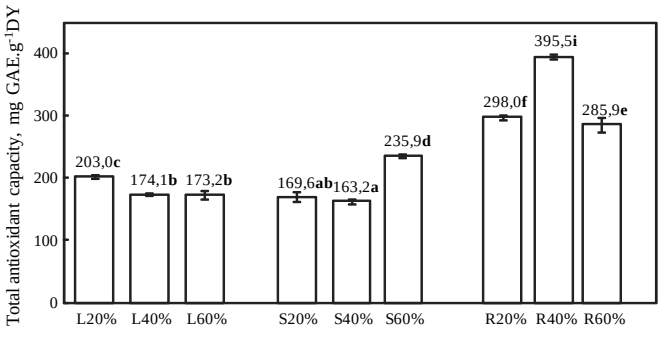

Fig. 2. Total antioxidant capacity of different fractions of *Mesembryanthemum edule* leaf, stem and root extracts expressed in mg GAE g^{-1}DR. Means of three replicates followed by at least one same letter are not significantly different at $p < 0.05$.

2.6. Statistical analysis

Results were expressed as mean ± standard deviation of 3 or 6 replicates (antioxidant and antimicrobial tests, respectively). Multiple sample comparison was performed using the software Statgraphics Plus program version 5.1 for Windows. Analysis of variance (ANOVA) followed by Duncan's multiple comparison test were used. Whenever the one way ANOVA could not be used, Kruskal–Wallis test was applied after checking for normal distribution of the groups and homogeneity of variances. The level of significance was $p < 0.05$.

3. Results

M. edule organ extracts were fractionated through a C18 column into 5 fractions (20, 40, 60, 80, 100% aqueous methanol). ^{1}H NMR analysis of each methanolic fraction showed that no aromatic compounds were present in the 80% and 100% methanol fractions (data not shown). Therefore, phenolic content and biological activities were determined only for 20%, 40%, and 60% methanol fractions.

3.1. Antioxidant content and activity of M. edule organs

3.1.1. Total polyphenol contents

For all organs, the three fractions (20%, 40%, and 60% methanol) showed high total polyphenol content (TPC), despite a significant variability occurred depending on solvent polarity and organ nature (Fig. 1). The highest phenolic contents were registered in stem 60% and root 40% fractions (over 940 mg GAE g^{-1} DR). A second group, composed by root 20%, leaf 20% and leaf 60%, showed intermediary TPC values (from 518 to 760 mg GAE g^{-1} DR), and a third one included root 60%, stem 20%, stem 40% and leaf 40% fractions, characterized by TPC values between 364 and 423 mg GAE g^{-1} DR.

3.1.2. Total antioxidant activity

Total antioxidant capacity of *M. edule* fractions differed significantly as function of organ and polarity (Fig. 2). The highest values were found in root extracts (over 280 mg GAE g^{-1} DR in all fractions), followed by stem and leaf extracts (below to 236 and 203 mg GAE g^{-1} DR respectively).

3.1.3. Iron reducing power

Analysing EC_{50} values revealed that all *M. edule* fractions were able to reduce iron (III). EC_{50} values ranged from 86 to 238 μg ml^{-1} (leaf 40% and root 60%, respectively) and the reducing activity was significantly higher in shoots as compared to roots (Fig. 3). Whether for 40% aqueous methanol or for 20% aqueous methanol fractions, almost no statistical differences were recorded when comparing leaf and stem samples. Interestingly, the elution solvent had more influence on the reducing activity than on TPC or total antioxidant capacity. For each organ, the highest and the lowest reducing power was recorded for 40% MeOH fraction and 60% MeOH fraction, respectively.

3.2. Antimicrobial activity of M. edule organs

3.2.1. Antibacterial activity

The microplate bioassay used to determine the antimicrobial activity against selected food-borne pathogens as well as clinical isolates indicated that the inhibition percentage of bacterial growth was differently affected by *M. edule* fractions depending on organ nature, solvent polarity, and strain sensitivity (Table 2). Concerning the organ activity, regardless of solvent polarity, stems had the most potent antimicrobial activity (up to 85%). It is worth mentioning that 40% MeOH fraction was able to significantly inhibit the growth of 7 among the 8 tested bacteria. On the contrary, antibacterial activity of leaf extracts was low (activity between 28.6% and 57.4%).

Regarding to the solvent polarity, the most potent fraction differed depending on the organ: 60% MeOH fraction in roots and leaves, 40% MeOH in the stems (Table 2). Interestingly, in about 70% of the 21 tested combinations (bacteria *vs.* fractions *vs.* organs), methanol 60% was the most efficient fraction whereas 20% was the less active one. This trend was also observed at the single bacteria level, except *E. faecium* for leaf extracts and *M. luteus* for stem extracts. Considering bacterial susceptibility, MSI values were useful in evaluating the sensitivity of the different strains towards the

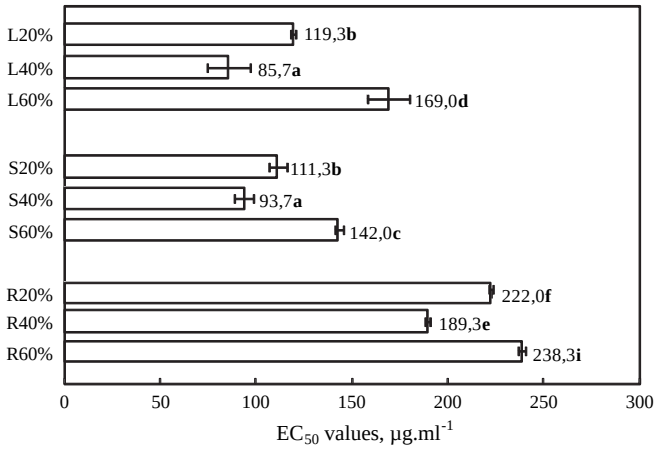

Fig. 3. Iron reducing power, expressed as EC_{50} (μg ml^{-1}) by *Mesembryanthemum edule* leaf, stem and root fractions. Means of three replicates followed by at least one same letter are not significantly different at $p < 0.05$.

Table 2
In vitro evaluation of antibacterial activity of *Mesembryanthemum edule* leaf, stem and root fractions against pathogenic bacteria. Percentage of Growth Inhibition, Percent Activity Value and Microbial Susceptibility Index represent comparative numerical scales for evaluation of antimicrobial efficiency of extracts and susceptibility of microbe isolates (refer to text for details). Means (six replicates) followed by at least one same letter within a row are not significantly different at $p < 0.05$.

	Pseudomonas aeruginosa	*Escherichia coli*	*Bacillus cereus*	*Erwinia carotovora*	*Staphylococcus aureus*	*Micrococcus luteus*	*Salmonella typhimurium*	*Enterococcus faecium*	Activity values (%)
L20%	0.0 ± 0.0a	0.0 ± 0.0a	14.9 ± 4.5bc	0.0 ± 0.0a	74.2 ± 3.7f	0.0 ± 0.0a	0.0 ± 0.0a	25.9 ± 4.2b	28.6%
L40%	67.2 ± 6.1e	0.0 ± 0.0a	10.7 ± 3.6b	1.7 ± 1.2ab	37.3 ± 7.0bc	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	28.6%
L60%	86.8 ± 0.9f	26.0 ± 1.1c	15.4 ± 1.4c	15.0 ± 2.3c	76.1 ± 3.7f	79.7 ± 6.2c	0.0 ± 0.0a	0.0 ± 0.0a	57.4%
S20%	59.7 ± 6.3d	2.0± 2.0a	3.1 ± 1.3a	0.0 ± 0.0a	32.3 ± 8.5ab	30.9 ± 5.7b	0.0 ± 0.0a	0.0 ± 0.0a	42.7%
S40%	70.4 ± 2.2e	36.2 ± 2.6c	26.9 ± 6.7e	4.1 ± 3.0b	26.5 ± 5.5a	28.6 ± 6.7b	14.2 ± 4.2b	41.0 ± 4.3c	85.7%
S60%	26.1 ± 9.2b	11.1 ± 2.1b	7.3 ± 3.8ab	0.0 ± 0.0a	43.1 ± 2.6 cd	82.4 ± 8.6c	0.0 ± 0.0a	0.0 ± 0.0a	42.9%
R20%	50.9 ± 4.2c	0.0 ± 0.0a	11.0 ± 5.1b	0.0 ±0.0a	71.5 ± 6.6ef	2.0 ± 3.8a	0.0 ± 0.0a	0.0 ± 0.0a	28.6%
R40%	71.5 ± 0.2e	0.0 ± 0.0a	22.2 ± 5.3de	0.0 ± 0.0a	63.1 ± 4.1e	22.0 ± 3.5b	0.0 ± 0.0a	0.0 ± 0.0a	57.4%
R60%	69.7 ± 0.4e	9.8 ± 1.7b	94.3 ± 0.6 f	0.0 ± 0.0a	71.4 ± 5.1ef	79.9 ± 8.4c	0.0 ± 0.0a	38.6 ± 3.4c	71.4%
MSI	88. 9%	22.2%	33.3%	0.0%	100.0%	66.7%	0.0	33.3%	

organ fraction investigated. According to Table 2, a classification of bacterial strains on the basis of their sensitivity may be proposed. The first class comprises the most sensitive bacteria, with MSI ranging from 67% to 100%: *M. luteus*, *Pseudomonas aeruginosa* and *S. aureus*. The second group of bacteria showed a moderate sensitivity, with MSI around 22% to 33%, and was composed of *E. faecium*, *Escherichia coli* and *B. cereus*. Finally, the third class comprised the most resistant pathogens (*Erwinia carotovora* and *Salmonella typhimurium*), which exhibited a strong resistance toward *M. edule* fractions.

3.3. Antifungal activity

3.3.1. Antimicrobial activity against yeasts

Yeast species are quite resistant toward *M. edule* organ fractions as shown by the absence of effects observed in most of the cases (Table 3). Among plant organs, stems displayed the most powerful activity with five growth inhibition over to 50% notably against *K. apiculata* and *C. albicans*. Moreover, and contrasting with antibacterial result, root extracts were more efficient than leaf ones against yeasts growth. In fact, according to the activity percentage, 0% activity was recorded 6-times for roots whereas in leaves it reached 9. Regarding the solvent polarity influences, data showed that 40% methanol fraction exhibited the highest antifungal activity as compared to 20% and 60% methanol fractions. Indeed, most fungi were very sensitive to the compounds eluted by 40% methanol, with growth inhibition reaching 84.8% (*K. apiculata*). The highest antifungal activity of 20% and 60% fractions was recorded against *K. apiculata* (75%) and *C. albicans* (68%), respectively. Regarding strains sensitivity, about 70% of the tests displayed 0% activity and 1 (*Schizosaccharomyces pombe*) of the 5 strains studied was resistant to all organ fractions (MSI = 0%). Conversely, *C. albicans*, and to a lesser extent *K. apiculata*, appeared susceptible to almost every organ fractions.

3.3.2. Antimicrobial activity against filamentous fungi

Leaf, stem and root fractions of *M. edule* showed a significant and variable efficiency against the two fungal strains *A. niger* and *P. glabrum* (Table 4). The growth inhibition percentage against *A. niger* varied from 24% to 94% whereas it was ranged between 4% and 98% against *P. glabrum*. Among organs, roots appeared as the most active ones against *A. niger* (IP from 87% to 94%) whereas stems displayed the highest activity toward *P. glabrum* (IP from 47% to 99%). It is noteworthy that leaves exhibited moderate to high activity against these two fungal strains (24–58% and 37–68% growth inhibition percentage against *A. niger* and *P. glabrum*, respectively). Activity variability was more pronounced in stem as compared to root and leaf fractions, suggesting the presence of several antimicrobial compounds in the last two organs. Considering the fraction polarity, 60% MeOH was the most potent fungal inhibitor particularly against *A. niger* (58%, 89% and 94% inhibition percentage for leaves, stems and roots, respectively). *A. niger* and *P. glabrum* growth was also significantly affected by *M. edule* organ fractions. In particular, *P. glabrum* was strongly sensitive to the three stem fractions (growth inhibition reached 99%) and *A. niger* growth was considerably inhibited by root fractions (inhibition percentage over 87%).

4. Discussion

In the present study, total polyphenol contents and biological activities of different *M. edule* organ fractions were investigated. Antioxidant activity assessed via total antioxidant capacity and reducing power and antimicrobial capacity was assayed against pathogenic bacteria, yeast, and filamentous fungi strains. Our findings revealed significant variability in phenolic contents and biological activities between *M. edule* organ fractions. Stems and roots were characterized by the highest polyphenol amounts, particularly the 60% and 40% fractions. The organ nature, the solvent polarity, and the extraction process largely influence the yield of

Table 3
In vitro evaluation of antifungal activity of *Mesembryanthemum edule* leaf, stem and root fractions against six mould strains. Percentage of Growth Inhibition, Percent Activity Value and Microbial Susceptibility Index represent comparative numerical scales for evaluation of antimicrobial efficiency of extracts and susceptibility of moulds isolates (refer to text for details). Means (six replicates) followed by at least one same letter within a row are not significantly different at $p < 0.05$.

	Candida albicans	*Candida krusei*	*Schizosaccharomyces pombe*	*Pichia anomala*	*Kloeckera apiculata*	Activity values (%)
L20%	28.7 ± 5.7c	0.0 ± 0.0a	14.9 ± 3.0b	0.0 ± 0.0a	0.0 ± 0.0a	33.3
L40%	37.8 ± 1.1d	0.0 ± 0.0a	0.0 ± 0.0a	29.6 ± 3.7c	84.8 ± 3.4d	50.0
L60%	22.6 ± 3.0b	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	16.7
S20%	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	75.2 ± 2.2d	16.7
S40%	77.0 ± 4.0f	36.5 ± 5.3b	0.0 ± 0.0a	0.0 ± 0.0a	76.2 ± 5.9d	66.7
S60%	67.6 ± 1.1e	53.8 ± 6.2c	0.0 ± 0.0a	32.0 ± 4.2c	0.0 ± 0.0a	50.0
R20%	22.0 ± 1.6b	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	56.2 ± 4.9c	33.3
R40%	27.3 ± 1.2c	51.8 ± 3.6c	14.1 ± 0.6b	12.8 ± 2.7b	50.0 ± 3.8c	83.3
R60%	34.0 ± 1.9c	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	22.0 ± 2.9b	33.3
MSI	88.9	33.3	0.0	22.2	66.7	

Table 4
In vitro evaluation of antifungal activity of *Mesembryanthemum edule* leaf, stem and root fractions against two filamentous fungi. Activity expressed as percent of growth inhibition. Means (six replicates) followed by at least one same letter within a row are not significantly different at $p < 0.05$.

	Aspergillus niger	*Penicillium glabrum*
L20%	23.9 ± 2.8a	35.6 ± 2.7a
L40%	48.6 ± 4.1b	47.3 ± 1.5a
L60%	58.2 ± 2.3c	68.0 ± 3.4b
S20%	29.27 ± 2.5a	98.6 ± 0.6c
S40%	44.7 ± 3.6b	46.7 ± 1.2a
S60%	89.5 ± 5.9d	63.0 ± 2.0b
R20%	91.3 ± 6.3d	47.6 ± 3.5a
R40%	87.5 ± 8.5d	61.6 ± 5.1b
R60%	94.4 ± 5.0d	50.1 ± 2.2a

phenolic compound extracted from plants (Trabelsi et al., 2010; Zhao et al., 2006). This is also true for the halophyte *Salsola kali*, in which the significant difference between leaves, stems and roots was found, suggesting that organ effect on phenolics was more potent than other endogenous factors notably developmental stage (Boulâaba, 2007). The ability of solvents to extract polyphenols is greatly influenced by their polarity. According to Chua et al. (2011), the amount of total phenolic compounds may increase with increasing solvent polarity. With this respect, the most polar solvent (notably aqueous methanol) was the most effective in extracting phenolic compounds from *Limoniastrum monopetalum* leaves (Trabelsi et al., 2010). When extracted with 60% methanol/water, *Labisia pumila* leaves phenolic content was 4 times higher than that obtained with 100% acetonitrile (Chua et al., 2011).

Antioxidant activity. The heterogeneous distribution of total antioxidant capacity and reducing power between *M. edule* leaves, stems, and roots as well as between the different fractions confirms the significant effect of these two factors (plant organs and solvent polarity). Considering plant organs, roots exhibited better total antioxidant activity whereas leaves and stems were more potent in reducing Fe^{3+} iron. This organ dependency is in agreement with previous studies showing that antioxidant metabolite distribution differed between plant organs due to their specificity (Lisiewska et al., 2006; Maisuthisakul et al., 2007). This organ-related variability in antioxidant activity was reported in the halophyte *Tamarix gallica* in which flowers had higher antioxidant activity than leaves (Ksouri et al., 2009). Similar differences were found for the antioxidant activity of *Salvia* sp. leaves and roots (Matkowski et al., 2008). Considering the fraction polarity, the most potent antioxidant fractions were for 40% aqueous methanol notably for the reducing power. These results confirm those of Chua et al. (2011) who found that *L. pumila* leaf antioxidant activity was significantly higher in 40% MeOH fraction (SC_{50} = 1060 ppm) than for 60%, 80% and 100% MeOH fractions (SC_{50} > 1386 ppm). Interestingly, *M. edule* most phenolic rich fractions did not correspond to the most effective ones, which points out the interest of fractionation. Indeed, the most active compounds responsible for the antioxidant activity in each fractions are not necessarily the most represented ones (Mariod et al., 2010), and fractionation process allows separation from complex mixtures of phenolic compounds that may present antagonistic interaction (Falleh et al., 2008; Martinez-Correaa et al., 2011).

Antimicrobial activity against bacterial and yeast strains. The microplate bioassay results showed a significant variability in microbial growth inhibition depending on both the organ nature and the fraction polarity. Stems were the most potent antimicrobial organs whereas methanol 60% and 40% were the most active fractions against bacteria and yeasts, respectively. These results agree with the antimicrobial effects observed upon the traditional uses of *M. edule* since the healers use shoots (leaves and stems) in order to cure infectious diseases (Martins et al., 2005; Van der Watt and Pretorius, 2001). However, as far as our literature survey could ascertain no information was available on *M. edule* stem or root antimicrobial activity. Nevertheless, similar data were reported previously on antimicrobial activity of stems and leaves. *Serjania lethalis* and *Bolusanthus speciosus* stems exhibited a significantly better antibacterial activity than leaves against *S. aureus* and toward *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia* and *S. aureus*, respectively (Lima et al., 2006; Mulaudzi et al., 2011)

Antimicrobial activity of *M. edule* was significantly variable as function of fraction polarity. Our findings suggest that the most polar solvents are the most active, confirming previous results (Ho et al., 2010; Kim et al., 2008; Rangasamy et al., 2007). For instance, methanolic extracts of several medicinal plants of the Mauritians flora were more efficient in inhibiting *Salmonella enteritidis*, *Enterobacter cloacae* and *B. subtilis* growth than hexane and dichloromethane extracts (Rangasamy et al., 2007). Recently, Ho et al. (2010) found that antimicrobial activity of *Orthosiphon stamineus* shoot fractions was greatly influenced by methanol/water proportion. 100% water fraction was more effective in inhibiting the growth of *S. aureus*, *Listeria monocytogenes* and *K. pneumoniae* as compared to 75% MeOH and 100% methanol fractions. This finding corroborates the ethnobotanical claims on *M. edule*, as in the local folklore, the beneficial medicinal properties of this halophyte are derived in most cases from decoctions and infusions in aqueous medium (Wisura and Glen, 1993). Moreover, *M. edule* fractions exhibiting the highest antibacterial activity were different from those presenting the best antioxidant capacity suggesting the presence of different compounds with variable biological activity in each fraction.

Antimicrobial activity against filamentous fungi. M. edule organ fractions showed a variable efficiency against fungal strains. Roots appeared as the most active organs, and as for the antibacterial activity, 60% MeOH fraction was the most potent fungal growth inhibitor. Contrasting with the antibacterial activity findings, root extracts showed the highest antifungal activity. In *Breynia cernua*, root methanolic extracts were also more efficient against *A. niger* and *Penicillium notatum* as compared to leaves and stems (Khan and Omoloso, 2008). In addition, the strong activity displayed by *M. edule* extracts against both fungi indicates the presence of a large number of antifungal molecules in the fraction eluted with 60% MeOH. Comparing these results to the literature data may be difficult because of different extracting solvents and systems, which produces extracts with diverse compositions, both qualitatively and quantitatively. Yet, the presence of antifungal molecules in the fractions with high polarity is consistent with previous reports on *Astragalus verrucosus* shoots (Pistelli et al., 2002) and *B. cernua* antifungal activity (Khan and Omoloso, 2008).

Overall, *M. edule* is already used against a wide range of bacterial infection (Thring and Weitz, 2006). The fact that *M. edule* extracts showed activity against pathogenic bacteria, yeasts and filamentous fungi supports the ethnopharmacological use of this species and makes it worthy for further evaluation. Interestingly, among the microbial strains tested, the most sensitive were *S. aureus*, *P. aeruginosa* and the two filamentous fungi, which are common food contaminants and human pathogens (Darby, 2005; Harisha, 2007). This suggests the possibility of using *M. edule* fractions as starting points for the isolation of antibacterial and antifungal agents that selectively inhibit common bacteria and fungi in food industry. Moreover, using the fractionation allowed to better highlight the variability in phenolic compounds activity, which may facilitate their isolation. These fractions are currently submitted to further bioassay-guided fractionations in order to isolate the compounds responsible for the antioxidant, antibacterial and antifungal activities.

5. Conclusion

We reported the antioxidant and antimicrobial activities evaluation of the edible halophyte *M. edule* fractions. The leaf, stem and root extracts using aqueous methanol yield fractions with high polyphenol content, significant though variable antioxidant activity, and a variable level of broad spectrum of antimicrobial potency. The rapid and functional fractionation method allows the separation of raw organ extracts into distinct fractions with different molecules exhibiting various biological potentialities. Generally, the best biological activities were found in 40 and 60% MeOH/H_2O stem and root fractions which exhibited the highest amount of polyphenols together with the highest level of antioxidant, antibacterial and antifungal activities particularly against filamentous fungi. The overall results provide baseline information for the potential use of this fractionation process as quick and efficient method in order to separate active compounds that would be promising as natural antioxidant and antibacterial agents in food and/or pharmaceutical industries.

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