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Antioxidant Properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* Extracts

I. BOUFTIRA*†, C. ABDELLY† and S. SFAR
Laboratory of Galenic Pharmacy, Faculty of Pharmacy of Monastir
Rue Ibn Sina 5000 Monastir, Tunisia
Fax: (216)73461830; Tel: (216)73461000; E-mail: ibtissem.bouftira@laposte.net

Extraction efficiency using different solvents on antioxidant capacities of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* was measured. The dried extracts were screened for their radical scavenging activity using α,α-diphenyl-β-picrylhydrazyl (DPPH) method. The order of antioxidant capacity of *M. crystallinum* in different solvents was found to be petroleum ether > ethyl acetate > chloroform > methanol > flavonoids and water. The order of antioxidant capacity of *C. edulis* was found to be flavonoids > methanol > chloroform > water and petroleum ether. Petroleum ether and flavonoids extracts from *M. crystallinum* and *C. edulis* showed highest antioxidant activity at 500 µg/mL. Results of the present study may be due to the extent of antioxidant capacity of each extract is in accordance with the amount of chrolophylls, carotenoids, phenolics and others different antioxidant compounds that can be presents in the extracts.

Key Words: Antioxidant activity, DPPH, Aizoacea, Extraction methods, Flavonoids.

INTRODUCTION

Reactive oxygen species (ROS) are involved in the organism's vital activities including phagocytosis, regulation of cell proliferation, intracellular signalling and synthesis of biologically active compounds and ATP[1]. With an insufficiency of the antioxidant protective system or under an intense influence of radical-initiating factors (ionizing radiation, hard ultraviolet radiation, xenobiotics, mineral dust), ROS are overproduced and oxidative stress develops. Oxidative stress is a specific feature in the pathogenesis of various diseases, including cancer, cardiovascular diseases, diabetes, tumours, rheumatoid arthritis and epilepsy[2,3].

†Laboratory of Plant Adaptation to Abiotic Stresses, Center of Biotechnology Technoparc of Borj Cédria, Tunisia.

In recent years, considerable attention has been paid to antioxidant properties of plants that may be used for human consumption. Plant-derived natural products are highly abundant; many exhibit numerous biological activities and some can be employed as food additives. Synthetic antioxidants have been used in the food industry since the 1940s, but trends in natural sources. Therefore, investigation of natural antioxidants has been a major research interest for the past two decades as many research groups and institutions have been screening plant materials for possible antioxidant properties. Extraction procedures to obtain active principles are mainly focussed on the use of methanol or ethanol as solvents. Since active compounds in plants exhibiting biological activity are in low concentrations, selective extraction methods should be used. Activity may be varied when different solvents are used for conventional extraction. The antioxidant activity of plant origin is dependent on the type and polarity of the extracting solvent as well as on the test system and the substrate to be protected by the antioxidant[4-6]. Solvent extraction is frequently used for isolation of the antioxidants and both extraction yield and antioxidant activity of the extracts are strongly dependent on the solvent, due to the different antioxidant potentials of compounds with different polarity. For these reasons, comparative studies for selecting the optimal solvent providing maximum antioxidant activity are required for each substrate. Although the use of different polarity substances can provide more exhaustive information on the properties of the extracts, the literature contains few reports of the polarity-based solvent extraction of medicinal plants.

The species *Mesembryanthemum crystallinum* and *Mesembryanthemum edule* (*Carpobrotus edulis*) are two halophyte plants belonging to Aisoaceae family. They are surviving in coastal area. The main stressful factor in such harsh environment is the salinity resulting from salt spray from the ocean. Many studies were conducted to evaluate the response of *M. crystallinum* to abiotic stress, mainly salinity, drought and high irradiance. *M. crystallinum* is very tolerant towards photosynthetic active radiation and UV irradiance[7]. The plant is extremely tolerant to high salinity, within the last decade it has become synonymous with halophytic stress responses modeled at the molecular level[8]. *M. crystallinum* can tolerate > 500 mM NaCl at the flowering and seedpod stage of growth[8]. However, few studies investigated *M. edule* regarding its response to abiotic stress. The two species were traditionally used for their medicinal properties. In ancient periods, Physicians used leaf juice of *M. crystallinum* to soothe inflammation of the mucous membranes of the respiratory or urinary system. In Europe, its fresh juice has been used to treat water retention and to painful urination and to soothe lung inflammation[9]. *M. edule* leaf juice is effective in soothing itching caused by spider and tick bites[10]. The leaves also contain an astringent antiseptic juice which can be taken orally for treating sore throat and mouth infections[11].

The present study was undertaken to perform the screening of antioxidant properties of two halophyte plants *Mesembryanthemum crystallinum* and *Carpobrotus edulis* using ethyl acetate, methanol, chloroform, petroleum ether and water as extract solvents which permit comparison of the antioxidant properties among the polarity-based solvent extracts of medicinal plants.

EXPERIMENTAL

Fresh leaves from the two halophyte plants Mesembryanthemum crystallinum and Carpobrotus edulis were isolated at the period of December 2006 at Monastir.

A Soxhlet extractor was used for extraction processes. Rotary evaporator was used in the evaporation steps. Sample (100 g dry leaf) was introduced into a glass extracted in a Soxhlet equipment with 300 mL of each one of the solvents; petroleum ether, ethyl acetate, chloroform and methanol. The extract was concentrated to 80 mL using a rotary evaporator.

Total flavonoids oligomer extraction: The extraction of the total flavonoids oligomer was performed by the methods of Ghdira[12]. 100 g dry leaf was macerated for 24 h under agitation in the presence of water/acetone (1:2). After filtration (0.2 mm), acetone was evaporated under vaccum. The filtrate was saturated with sodium chloride and kept for over night at 4 °C. A precipitate rich in tannins was formed and immediately eliminated by filtration. The filtrate was mixed with ethyl acetate inducing the formation of two phases. The organic phase was concentrated under vaccum and finaly mixed with chloroform. Flavonoids were separated after centrifugation at 4000 rpm for 25 min.

Water extraction: 500 g of fresh leaves were chopped into small parts in a blender with 250 mL of distilled water, boiled for 15 min followed by filtration. The filtrate was freeze-dried.

DPPH free radical scavenging activity: Free radical scavenging activity of plant extract was determined by using a stable free radical, (1,1-diphenyl-2-picrylhydrazyl) DPPH[13]. DPPH solution was prepared at the concentration of (0.024 mg/mL DPPH in ethanol). During assay 1 mL of the crude extract was mixed with 1 mL DPPH solution. The mixture was incubated in the room temperature for 0.5 h. The absorbance was recorded at 517 nm (Cam spec M230/330 UV visible spectrophotometer, United Kingdom). Butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA) and vitamin C were used as a standard for the investigation of the antiradical activity.

The percentage of remaining $DPPH^{\bullet}$ (% $DPPH_{REM}$) at the steady state was determined as follows:

$$\% \ DPPH_{REM} = 100 \ C_{DPPH}/C_{DPPH\ (t=0)}$$

where $C_{DPPH\ (t=0)}$ is the initial DPPH concentration and C_{DPPH} is the DPPH concentration at the steady state.

RESULTS AND DISCUSSION

Yield of each extract: Results (Fig. 1) showed, in the two plant extract, the highest yield was obtained in the methanol extract (10.08 % in *M. crystallinum* and 22.92 % in *C. edulis*). The extract of total oligomer flavonoids showed a very low yield (0.03 %) in *M. crystallinum* in comparison with *C. edulis* (1.91 %). For the other extract (in the two plant), yield is between 0.45 and 1.91 %.

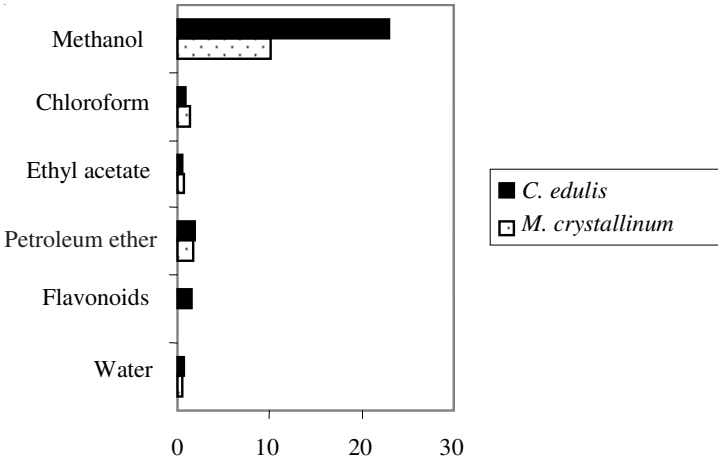

Fig. 1. Yield of each extract from *M. crystallinum* and *C. edulis*

Antioxidant activity

Water extract: The results of the antioxidant activity of the water extract in the two plant *Mesembryanthemum crystallinum* and *Carpobrotus edulis* are shown in Fig. 2. The antioxidant activity was higher in *C. edulis* in comparison with *M. crystallinum* extract. From the concentration of 5 mg/mL, the antioxidant activity of *M. crystallinum* extract became higher than 80 % of DPPH inhibition. For the *C. edulis* extract, a concentration of 1 mg/mL induced an antioxidant activity which was higher than the synthetic antioxidant BHA but still lower than BHT and vitamin C.

Total flavonoids extract: From Fig. 3, an important antioxidant activity in the extract of the total flavonoids in *C. edulis* has been observed. This was higher than 90 % of DPPH inhibition (at a concentration of 5 mg/mL). The antioxidant activity in *C. edulis* extract was higher than BHA, BHT and vitamin C. In *M. crystallinum*, a very low antioxidant activity was noted which exceed 70 % of DPPH inhibition at the concentration up to 5 mg/mL.

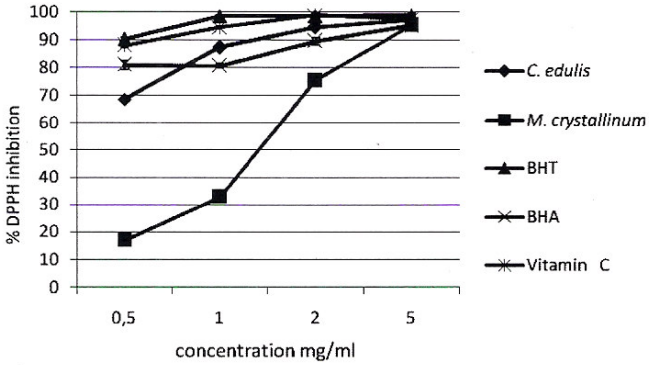

Fig. 2. Comparison of antioxidant activity in water extract from *M. crystallinum* and *C. edulis*

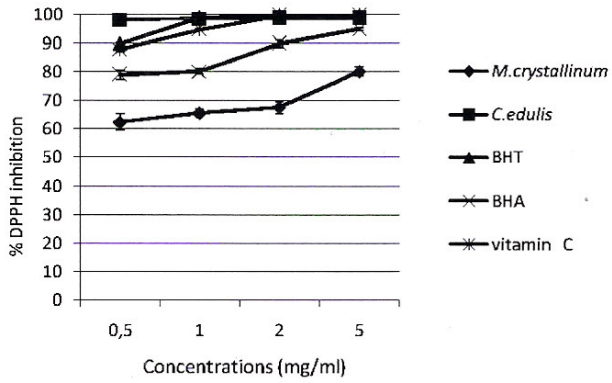

Fig. 3. Comparison of antioxidant activity in total flavonoids extract from *M. crystallinum* and *C. edulis*

Ethyl acetate extract: The most important antioxidant activity was observed in ethyl acetate extract of *M. crystallinum* which was higher than the synthetic antioxidant BHT (0.5 mg/mL induces 95.5 ± 0.5 % of DPPH inhibition). The antioxidant activity in case of *C. edulis* extract in ethyl acetate was lower than BHT and vitamin C (Fig. 4).

Chloroform extract: The results (Fig. 5) showed that the chloroform extract demonstrate a high antioxidant activity in *M. crystallinum* (91.5 ± 0.5 % of DPPH inhibition for a concentration of extract 0.5 mg/mL) than in *C. edulis* (81 ± 1.17 % of DPPH inhibition). The antioxidant activity of *M. crystallinum* in chloroform extract was higher than BHT, BHA and vitamin C.

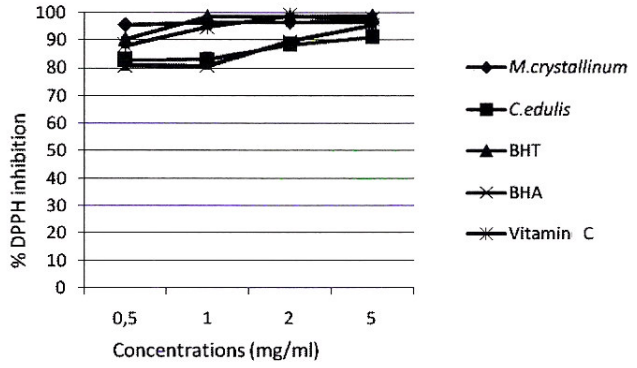

Fig. 4. Comparison of the antioxidant activity in the ethyl acetate extract of *M. Crystallinum* and *C. edulis*

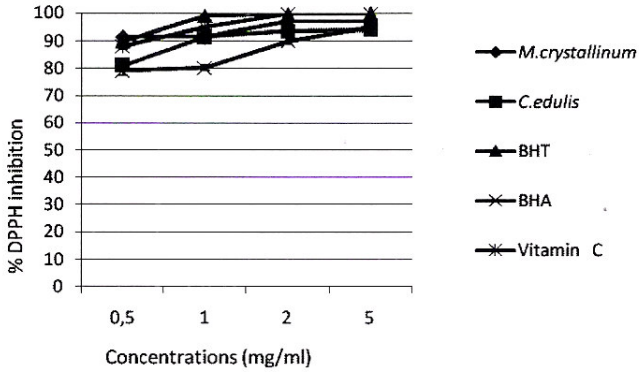

Fig. 5. Comparison of the antioxidant activity in the chloroform extract of *M. Crystallinum* and *C. edulis*

Petroleum ether extract: The antioxidant activity was higher in *M. crystallinum* in comparison with *C. edulis*, BHT, BHA and vitamin C, when petroleum ether was used as an extraction solvent (Fig. 6). In *M. crystallinum*, antioxidant activity was (98.3 ± 0.6 % of DPPH inhibition for a concentration of 0.5 mg/mL). The antioxidant activity was very low in *C. edulis* (33.2 ± 2.4 % of DPPH inhibition for a concentration of 0.5 mg/mL) extract in comparison with synthetic antioxidant (BHT and BHA) and vitamin C.

Methanol extract: The results of the antioxidant activity in *M. crystallinum* and *C. edulis* showed a little difference between the two plants extracts in methanol depending on the extract concentrations (Fig. 7). The antioxidant activity increased by the augmentation of extract concentrations in *C. edulis* and *M. crystallinum*. The two plants showed a higher antioxidant activity than BHA.

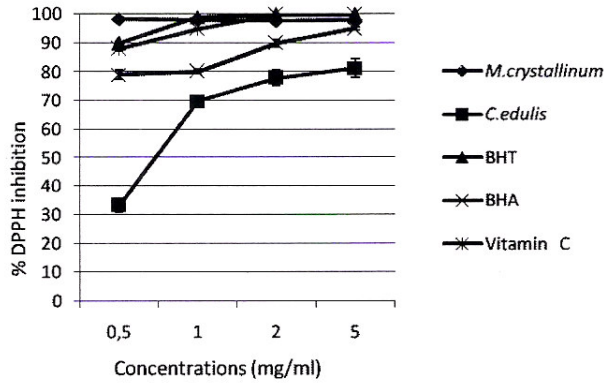

Fig. 6. Comparison of the antioxidant activity in the pethrolium ether extract of *M. crystallinum* and *C. edulis*

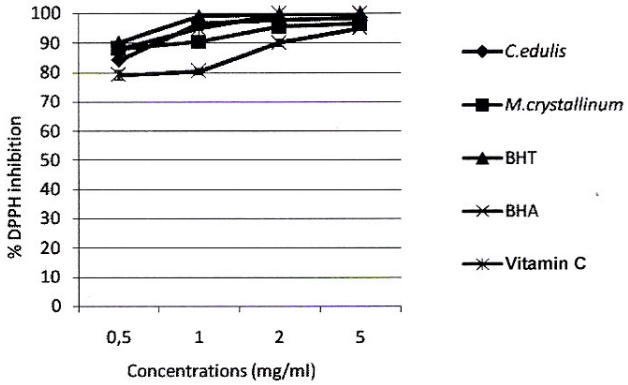

Fig. 7. Comparison of the antioxidant activity in the methanol extract of *M. crystallinum* and *C. edulis*

Comparison of the antioxidant activity in the different extract from *M. crystallinum*: The comparison of the antioxidant properties of different extracts in *M. crystallinum* was illustrated in Fig. 8. The most important antioxidant activity was found in petroleum ether extract. The lower activity was found in water extract.

Comparison of the antioxidant activity in the different extracts of *C. edulis*: The present results of the comparison of the antioxidant potential in different solvent extracts from *C. edulis* are shown in Fig. 9. The most important antioxidant activity was found in flavonoids extract. The lower antioxidant activity was found in petroleum ether extract.

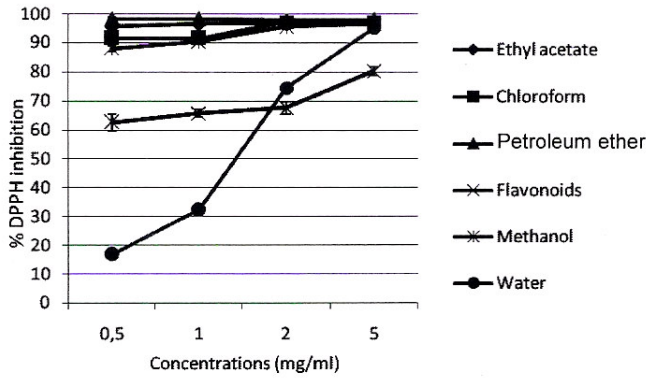

Fig. 8. Comparison of the antioxidant activity in the different solvents extracts of *M. crystallinum*

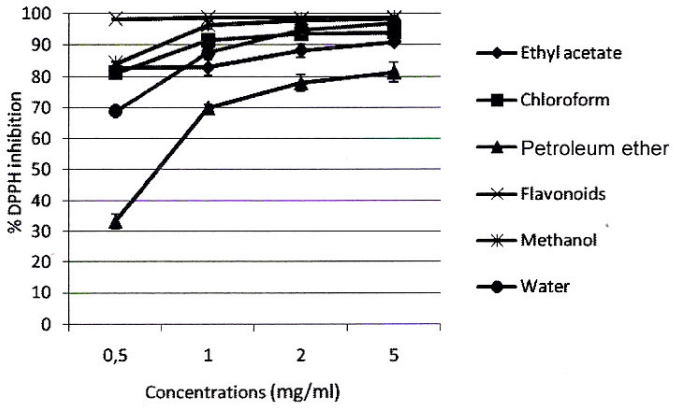

Fig. 9. Comparison of the antioxidant activity in the different solvents extracts of *M. edulis*

Different solvent systems have been used for the extraction of polyphenols from plant material[14]. Extraction yield is dependent on the solvent and the method of extraction[15]. The extraction method must allow complete extraction of the compounds of interest and it must avoid their chemical modification[16]. Water and aqueous mixtures of ethanol, methanol and acetone, are commonly used solvents in plant extraction[17]. Wang and Helliwell[18] reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, in another work[19], water was found to be a better solvent, for extracting tea catechins, than were 80 % methanol or 70 % ethanol. Moreover, in the extraction of polyphenol, a single extraction compared to multiple extraction procedure is not sufficient[15]. Despite the medicinal potential of plants in Tunisia being considerable, knowledge

of this area and studies on these plants remain scarce. The choice of present investigated plants is based on two criteria: first, that these plants have ethnopharmacological activity indicating their utilization in folk medicine; second, that in this domain, there is no study in Tunisia which deals with the biological activities of various extracts of such halophytes plants.

The purple-coloured DPPH is a stable free radical, which is reduced to α,α-diphenyl-β-picrylhydrazine (yellow coloured) by reacting with an antioxidant. Antioxidants interrupt free radical chain oxidation by using hydrogen from hydroxyl groups to form a stable end product, which does not initiate or propagate further oxidation of lipids[20]. The results of the free radical scavenging activity of the extracts using solvents at different polarities showed that all extracts demonstrated a significant inhibitory activity against the DPPH radical at a final concentration of 0.5 mg/mL.

The data obtained showed a big difference in the antioxidant activity of the two plant extracts, the flavonoids extracts showed the highest antioxidant activity in *C. edulis* extracts but it was very low in *M. crystallinum* extract. The petroleum ether extract showed the highest antioxidant activity in *M. crystallinum* but showed a very low antioxidant activity in *C. edulis* extract. In the two plant extract water extract showed a low antioxidant activity in comparison with organic solvents extracts. It was reported that the leaves of *M. edule* from South Africa contain flavonoids (rutin, neohesperidin, hyperoside), catechin, ferulic acid and catechol tannins[21]. In this study, the flavonoids are the most important components responsible for the antioxidant activity in *C. edulis* extracts. Flavonoids are a group of polyphenolic compounds[22] common in leaves of plants and protect them against the damaging effect caused by UV-radiation and antimicrobial infections[23]. Common family members of flavonoids include flavones, flavanes, flavonols, catechins and anthocyanins[24]. Different flavonoids and phenolic compounds react with free radical to reduce the degradation of membranes by preventing the reaction between free radicals and phospholipids[23]. They can also be used as antioxidants and *in vitro* as enzyme inhibitors[25]. Flavonoids antioxidants function as scavengers of free radicals by rapid donation of a hydrogen atom to radicals[26]. Many phenolics, such as flavonoids, have antioxidants capacities that are more[27] than those of vitamin C and E.

However, not much reports are available on the medicinal properties of the halophyte plant *M. crystallinum*, most of the research focused on its physiological responses to abiotic stresses.

It was reported that *M. crystallinum* tissues contains negligible amounts of phenolics compounds[8]. Vogt *et al.*[28] indicate that UV light induced the accumulation of β-cyanin and flavonoids in leaf bladder cells of *M. crystallinum*. In present study, it is found that the flavonoids extract has not the highest antioxidant activity in comparison with petroleum ether

extract. This can be supported by the low amounts of flavonoids in *M. crystallinum* tissues and by the presence of other antioxidant compounds that can be extracted by petroleum ether solvent. *M. crystallinum* can accumulate in response to abiotic stresses like salinity, a large number of antioxidant compounds. It was reported *M. crystallinum* accumulates free polyamines[29]. It was found that matured leaves and roots under normal conditions or salinity (400 mM NaCl) contained all types of free polyamines (putrescine, spermidine, spermine and cadaverine). The polyamines (spermine, spermidine and putrescine) are ornitine metabolic products[24]. The primary and secondary amine moieties of polyamines always carry a charge at physiological pH, resulting in low molecular weight 'organic cations', the most important characteristic required for 'aldehyde scavengers'. These amines are involved with numerous cellular functions, including free radical scavenger, antioxidant and antiinflammatory properties[30]. Msakni *et al.*[31] isolated water-soluble polysaccharides from the leaves of *M. crystallinum*. Plant polysaccharides have exhibited strong antioxidant properties and can be explored as novel potential antioxidants[32-34].

In conclusion to this work, we found that the potential of the antioxidant activity in the two plants still dependent by the extraction method and by the solvent used for the extraction.

In *Mesembryanthemum crystallinum*, petroleum ether extract showed the highest antioxidant activity, but in *Carpobrotus edulis* extract total flavonoids extract showed the highest antioxidant potential. This may be related to the presence of flavonoids with an important concentration in *C. edulis* and in low concentration in *M. crystallinum*. In the two plants, the water extract showed a low antioxidant activity in comparison with solvents extracts which can be related to polarity of the water and the cell walls.

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