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Potential Antioxidant Capacities and Neuroprotective Properties of Six Tunisian Medicinal Species

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Abstract: *Potential antioxidant activities of methanolic extracts from six Tunisian halophytes were assessed. Significant differences were observed in phenolic contents and composition and antioxidant capacities between species. Frankenia pulverulenta extract exhibited the highest phenolic content and the strongest radical scavenging activities and oxygen radical absorbance capacity (ORAC). High correlations were found between total phenolics with scavenging capacities and ORAC, indicating an important role of these compounds as chain-breaking antioxidants. Nevertheless, no correlation was found between ferrous ion-chelating activity and polyphenols, suggesting that other components were more effective chelators. Protective effect of extracts on the Aβ -induced toxicity in PC12 cells was investigated. Both Frankenia species exhibited an extremely efficient protection. Other species except Solanumsodomaeum show a moderate neuroprotective capacity. Thus, these results suggest the value of these species and especially F. pulverulenta as a new source of bioactive compounds and in particular for its preventive effect against neurodegenerative diseases.*

Keywords: *Neuroprotective activity, HPLC, antioxidant capacity, polyphenol, medicinal plants.*

Abbreviations:*$ABTS^+$:2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH:2,2-Diphenyl-1-picryl hydrazyl; MCA: Metal chelating activity; MTT : 3-(4,5-diméthylethiazol-2-yl)-2,5-diphényltetrazoluim bromide; ORAC: Oxygen Radical Absorbance Capacity; TPC: Total Phenolic Content.*

1. INTRODUCTION

Among the various categories of natural antioxidants, phenolics exhibit a wide range of physiological properties, such as anti-allergenic, anti-inflammatory, anti-viral, antibacterial, cardioprotective and neuroprotective effects. Antioxidant molecules are known to prevent abnormal oxidative modifications in the human tissues by protecting the cellular components from the oxidation effect of free radicals (Jeya et al., 2015). These substances can have an important role in the absorption and neutralize reactive oxygen species (ROS), and the extinction of the shirt and triple oxygen or peroxide decomposing (Bencherchar et al. 2017). Several reports have shown different biological effects of phenolic compounds such as the effects of anti-inflammatory, anti-cancer and anti-aging as a result of the anti-oxidant activity. For example, Al-Awadi et al. (2004) showed that polyphenols are able to prevent cardiac hypertrophy and production of free radicals in the insulin resistant fructose-fed rat. In the same context, Auger et al. (2004) reported, antioxidant flavonoids, especially catechin and procyanidins, have a significant protective effect against coronary heart disease (CHD) red wine and some fruits and vegetables, owing to their flavonoids. Alzheimer's disease (AD), which is the main cause of dementia, is a slowly progressive neurodegenerative disorder that leads to decline in memory and other cognitive abilities (Kumar et al. 2015). Several reports have shown that phenolics have potent neuroprotective activities *in vitro* (Pamplona et al. 2015) and *in vivo* (Liu et al. 2015). Indeed, one of the major properties of polyphenols is the important interaction with peptides and proteins particularly in the neurodegenerative disease (Richard et al. 2011). For that, there is an increasing interest to identify among Tunisian halophytic species those with high antioxidant capacities to use them as a new source of medicinal preventive compounds. For instance, *Frankenia thymifolia* and *F. Pulverulenta* belonging to Frankeniaceae family are two endemic xero-halophyte species, which have

not been the subject of many chemical investigations. The only phytochemical studies consist to the identification of sulphated phenolic compounds (Harkat et al. 2007; Hussein, 2004; Harborne 1975). *F. pulverulenta* is used in local medicine for its analgesic and carminative properties (Youssef 2013).The specie *Glaucium flavum* (Papaveraceae family) was used for its laxative and antitussive properties (Leporatti and Ivancheva 2003). Bournine et al. (2013) reported anti-tumoral activity of *G. flavum* root extract against human cancer cells *in vitro* and *in vivo*. These authors demonstrate that the specific anticancer effect has attributed to bocconoline alkaloid compound. *Drimia maritima* L. (Liliaceae) has been used for centuries for its positive effects on the cardiovascular system (Knittel et al. 2015) and for its rodenticidal effects. *Lycium europaeum* (Solanaceae) was known as a medicinal plant and used in several traditional remedies to reduce the incidence of cancer and also to inhibit or to stop the growth of tumoral cells (Ghali et al. 2015). Hydro-alcoholic fruit extract of *L. europeum* seemed to protect lipids, proteins and DNA against oxidative stress damages induced by oxygen peroxyde via scavenging reactive oxygen species (Ghali et al. 2015). Moreover, *Solanum sodomaeum* (Solanaceae family) is locally used to treat rheumatism, eczema and haemorrhoids (Zouiten et al. 2006).

The objective of this study was to carry out preliminary phytochemical screening and to determine total phenolic contents and compositions by HPLC and antioxidant capacities (DPPH, ABTS, MCA and ORAC) of six medicinal halophytic species. Extracts were also evaluated for their neuroprotective activities against (Aβ)-induced neurotoxicity in cultured PC12 cells.

2. MATERIALS AND METHODS

2.1. Plants Material and Extraction Procedure

Aerial parts from *F. pulverulenta*, *F. thymifolia*, *S. sodomaeum*, *L. europium*, *D. maritima* and *G. flavum* were collected during the vegetative stage in March 2015. Amount of 10 g of each plant material was extracted with 100 ml of methanol 80% and then filtered. Each extract was evaporated under reduced pressure. Dry residue was weighed and removed in methanol 50% and then stored at 4°C until analysis (1mg/mL).

2.2. Phenolic Compounds by HPLC

The identification of phenolic compounds was done using HPLC system equipped with a reversed phase C_{18} analytical column of 4.6 x 100 mm and 3.5µm particle size (Zorbax Eclipse XDB C_{18}). The DAD detector was set to a scanning range of 200-400 nm. Temperature of column was maintained at 25°C. The volume of injected extract was 2 µl and 0.4 ml/min was the mobile phase flow-rate. Mobile phase B was milli-Q water constituted of 0.1% formic acid and mobile phase A was methanol. The optimized chromatographic condition was as follows: 0-5 min: 10% A- 90% B; 5-10 min: 20% A- 80% B; 10-30 min: 30% A-70% B; 30-40 min: 50% A- 50% B; 40-45 min: 60% A- 40 % B; 45-50 min 70% A- 30% B; 50-55min: 90% A- 10% B; 55-60 min: 50% A- 50% B and at 60min 10% A- 90% B. Phenolic compounds identification were obtained by comparing their retention time and the UV spectra with those of pure standards (Table 1).

Table1. *Retention time of the used thirty standards phenolic acids and flavonoids at 280nm*

N°	***Standards***	***R.T*(min)**	***N°***	***Standards***	***R.T* (min)**
1	Gallic acid	4.080	**16**	Rutin trihydrat	15.700
2	Gallocatechin	5.465	**17**	Sinapic acid	16.493
3	Protocatechic acid	6.680	**18**	*Trans*-4-hydroxy-3-methoxycinnamic acid	16.567
4	3,4-dihydroxyphenol acetic acid	6.835	**19**	3,4 dimethoxybenzoïc acid	16.737
5	Epigallocatechin	7.596	**20**	*Trans*-2-hydroxycinamic acid	19.384
6	Catechin	7.785	**21**	*O*-coumaric acid	20.040
7	Chlorogenic acid	8.873	**22**	Rosmarinic acid	20.231
8	4-hydroxybenzoïc acid	9.764	**23**	Salicylic acid	21.231
9	2,5-dihydroxybenzoïc acid	10.121	**24**	Naphtoresorcinol	24.039
10	Vanillic acid	10.897	**25**	*trans*-Cinnamic acid	25.999
11	Caffeic acid	11.309	**26**	Quercitin dihydrate	26.760
12	3,5 dimethoxy-4-hydoxybenzoïc acid	11.332	**27**	Apigenine	27.273
13	Epigallocatechin-3-o-gallate	11.512	**28**	4, methoxycinnamic acid	27.693
14	*p*-coumanic acid	14.771	**29**	4',5,7 trihydroxyflavone	30.326
15	Rutin hydrate	15.679	**30**	Kaempferol	31.840

2.3. Analysis of Total Phenolic Content

TPC of each aqueous methanolic extract were assessed by the Folin–Ciocalteu colorimetric method (Singleton and Rossi 1965) adapted to 96-wells plate. To 20 µl of extract (1mg/ml) or methanol (blank), 100 µl of Folin-Ciocalteu's reagent were added. A volume of 80 µl of Na_2CO_3 (75 g/l) were added after 2-3 min of incubation. The mixture was then kept in obscurity for 1h. The absorbance was measured using a plate reader at 765 nm (Fluostar Optima; BMG Labtech). The TPC was expressed as mg gallic acid equivalent per g of extract (mg GAE/g E).

2.4. DPPH Radical-Scavenging Activity

Radical scavenging ability of extracts against DPPH radical was measured according to Blois (1958). A volume of 50 µl of each samples (1mg/ml) was mixed with 150 µL of 200 µM methanolic solution of DPPH in a 96-well plate. The plate was allowed to stand at ambient temperature in dark for 20 min. The absorbance was measured at 520 nm against methanol as a blank. A standard curve of Trolox was performed and results were expressed as mg of Trolox equivalent per g of extract (mgTE/g E).

2.5. ABTS Radical-Scavenging Activity

The scavenging activity of the six extracts on $ABTS^+$ radical was determined according to Re et al. (1999). $ABTS^+$ was generated by reacting ABTS stock solution with 2.45 mMof $K_2S_2O_8$ (in equal quantities) in obscurity at room temperature for 12–16 h before use. The $ABTS^+$ solution was adjusted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm. 250 µl of the diluted $ABTS^+$ solution were added to 10 µl of extracts at the concentration of 1mg/ml. Six min after initial mixing, the absorbance was measured at 734 nm at 30°C. Results were expressed as mg of Trolox equivalent per g of extract (mg TE/g E) using a standard curve for Trolox(100; 150; 200; 250; 300 and 400µM).

2.6. Metal Chelating Activity

The chelating activity of the six methanolic extracts for ferrous ions was determined by the procedure of Dinis et al. (1994). A volume of 80 µl of deionized water and 40 µl of $FeSO_4$ (0.2 mM) were added to extract (40µl, 1mg/ml) and mixed in 96-well microplate. Addition of 40 µl of ferrozine (2 mM) initiates the reaction with the divalent iron to form stable magenta complex species. After 10 min at ambient temperature, Fe^{2+}-ferrozine complex was read at 562 nm. Methanol, instead of sample, was used as positive control and distilled water as blank, instead of ferrozine. EDTA was used as standard and results were expressed as mg EDTA per gram of extract (mg EDTA/g E).

2.7. $ORAC_{FL}$ Assay

ORAC assay was carried out using Ou et al. (2001) method with some modification in 96-well microplates (Costar). All the samples (extracts, fluorescein and AAPH) were diluted in phosphate buffer (75 mM, pH 7.4). Thirty microliters of extracts (1mg/ml) or phosphate buffer (blank) were mixed with 180 µL of fluorescein solution (117 nM final concentrations) in a 96-well black plate and incubated for 5 min at 37°C. A volume of 90 µl of AAPH solution (40 mM) were added and fluorescence was immediately monitored with an automated plate reader (Fluostar Optima; BMG Labtech), using 485 nm excitation and 520 nm emission wavelengths at 1 min intervals for 70 min. The antioxidant capacities of the extracts were expressed as mg of Trolox equivalent per g of extract (mgTE/g E) using standard curve for Trolox (1.25, 2.5, 5, 10, 15 and 20µM). All samples were analyzed in quadruplicate and at least in three different experiments.

2.8. Cell Culture and MTT Assay

PC12 cells were obtained from the American Type Culture Collection (ATCC, Manassas, USA) were growing in DMEM-Glutamax (Dulbecco's Modified Eagle Medium Glutamax) added with 15% heat-inactivated horse serum, 2.5% fetal bovine serum and 1% penicillin/streptomycin antibiotics at 37°C and 10% CO_2. All cells were cultured in poly-D-lysine-coated culture dishes.

Cells were harvested from flasks and plated at a density of 10^3 cells per well in 96-well plates and incubated at 37 °C for 24 h. $A\beta_{(25-35)}$preincubated with 0, 100, 200 and 400µM of extract at 37 °C for 48 h, was diluted with fresh DMEM-Glutamax and added to individual wells. The final concentration of Aβ was 5 µM. After 24 h of incubation, cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction test.

Cells were treated with MTT solution (final concentration, 0.5 mg/ml (DMEM-Glutamax)) for 3 h at 37 °C. The dark blue formazan crystals formed in viable cells were solubilized with DMSO for 30 min. The absorbance was measured with a microplate reader (Dynex, USA) at 595 nm. Results were expressed as the percentage of MTT reduction in relation to the absorbance of control cells at 100%. All data represent the average of three tests.

2.9. Statistical Analysis

Means were statistically compared using the Statgraphics Plus program (version 5.1). Analysis of variance (ANOVA) followed by Duncan's multiple range tests were carried. Kruskal-Wallis test was assessed (when ANOVA could not be used) after checking for normal distribution of the groups and homogeneity of variances. The Pearson correlation test was used to compare the different values of antioxidant activities obtained in our extracts after all types of antioxidant measurement. The level of significance was $P<0.05$. GraphPad Prism 5.03 for Windows (GraphPad Software, San Diego, CA, USA) was used for these analyses.

3. RESULTS

3.1. Determination of Phenolic Content and Composition in Halophytic Plants

Figure 1 presented typical HPLC chromatograms of each plant methanolic extract. Among the identified compounds, peak chromatograms revealed a phenolic fingerprint principally composed by flavonoids such as catechin, epicatechine 3-*O*gallate, epigallocatechin, luteolin- 7-*O*-glucoside, kaempferol-3-*O*-rutinoside and hyperoside followed by phenolic acids (gallic acid, resorcinol, trans-hydroxycinnamic acid, ellagic acid) and other compounds like coumarin and catechol.

Among flavonoid family, those belong flavan-3-ol group (epigallocatechin, catechin, epicatechin 3-*O*-gallate) were highly represented in all studied species expect *S. sodomaeum*. Epigallocatechin (peak 4) was the most representative compound with amount ranged between 1.13 mg/g DW in *F. pulverulenta* and 25.97 mg/g DW in *D. maritima*. Catechin (peak 5) was present at appreciable amount in *F.pulverulenta* (0.77 mg/g DW), *G.flavum* (0.994 mg/g DW) and *D.maritima* (1.311 mg/g DW). Among other flavonoids, isorhamnetin-3-*O*-glucoside (peak 17) amount was well represented at 5.808 mg/g DW in *S. sodomaeum* extract.

Phenolic acids profiles showed differences in quantitative and qualitative composition between species. *F. thymifolia* was shown to contain a high amount of phenolic acids (hydroxycinnamic acid, caffeic acid, chlorogenic acid and sinapic acid). The most representative compound in *F. thymifolia* was chlorogenic acid with 4.12 mg/g DW.

Among the other identified compounds, resorcinol and catechol, two dihydroxybenzene, were found at appreciable amount in both *F. pulverulenta* and *D. maritima* species.

TPC of the six methanolic extracts obtained by Folin-Ciocalteu assay were given in Table 2. Phenolic contents varied significantly between extracts and ranged from 3.64to 34.28 mg GAE/g E. *F. pulverulenta* exhibited the highest value, which was about 9.4 times higher than those determined in *F. thymifolia*. In an increased order, plant phenolic richness were the following: *F. thymifolia*, *L. europeum*, *D. maritima*, *G. flavum*, *S. sodomaeum* and *F. pulverulenta*.

Table2. *Phenolic compounds identified and quantified by HPLC from six plant methanolic extracts.*

Compounds	Methanolic aerial parts extracts: amount of compound (mg/g DW)					
	L.europeum	*F.thymifolia*	*S.sodomaeum*	*F.pulverulenta*	*G.flavum*	*D.maritima*
Phenolic acid						
Gallic acid	0.063	0.125	-	0.078	0.125	0.255
Chlorogenic acid	-	4.120	-	-	-	-
Caffeic acid	-	0.146	-	-	-	-
Sinapic acid	-	0.182	-	-	-	0.255
Ellagic acid	-	-	0.007	-	0.028	-
trans-Hydroxycinnamic acid	-	-	0.021	-	-	-
Flavonoids						
Catechin	-	-	-	0.768	0.993	1.310

Epigallocatechin	25.887	1.134	-	2.278	7.259	25.976
Epicatechin3-*O*-gallate	0.570	-	-	-	0.705	0.307
Luteolin-7-O glucoside	-	1.392	0.385	-	-	0.846
Kaempferol 3-O rutinoside	-	-	1.282	0.189	-	-
Isorhamnetin-3-O-glucoside	-	-	5.808	-	-	-
Quercetingalactoside	-	-	-	-	0.114	-
Others						
Coumarin	-	-	-	0.124	-	-
Catechol	0.495	-	-	1.410	0.209	0.043
Resorcinol	-	2.326	-	5.557	-	13.614
Polydatin	-	-	0.280	-	-	0.166

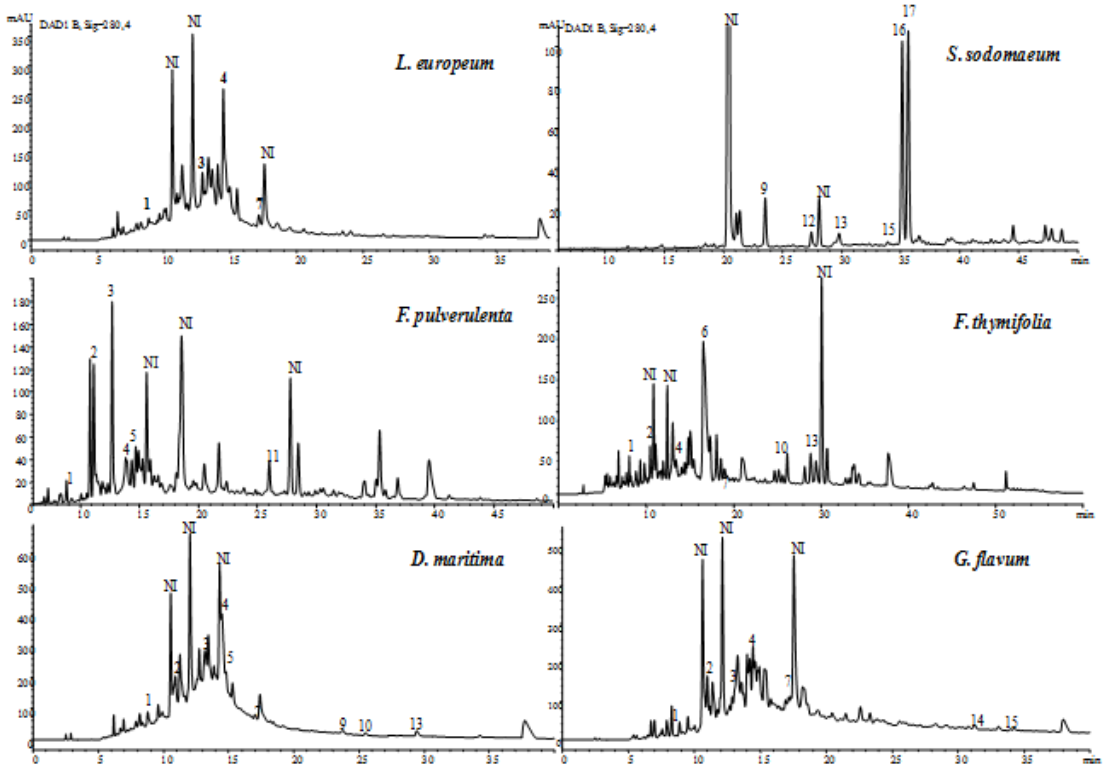

Figure1. *RP-HPLC chromatogram of methanolic extract of six Tunisian species. Signal was collected at 280nm. Peaks bearing NI letters correspond to not determined compounds. Peak numbers corresponded to:* ***1:*** *gallic acid;* ***2****: resorcinol****; 3:*** *catechol****; 4:****epigallocatechin;* ***5****: catechin****;6:****chlorogenic acid****; 7:*** *epicatechine 3-O-gallate;* ***8:*** *caffeic acid* ***9:*** *polydatin****; 10:*** *sinapic acid****; 11****: coumarin****; 12:*** *trans-hydroxycinnamic acid;* ***13:*** *luteolin-7-O-glucoside****; 14:*** *quercetingalactoside;* ***15 :*** *ellagic acid;* ***16:****kaempferol 3-O-rutinoside;* ***17****: isorhamnetin-3-O-glucoside.*

3.2. Antioxidant Capacities of Tunisian Halophytic Plants

All studied plant extracts were able to scavenge DPPH radical (Table 3). *F. pulverulenta* displayed the highest activity (1090.4 mg TE/g E) followed by *L. europeum* (621.7 mg TE/E). The lowest values were found for *S. sodomaeum* and *F. thymifolia* (80.8 and 128.44 mg TE/g E, respectively), whereas *D. maritima*and *G. flavum* exhibited similar and moderate DPPH activity (377.85 and 331.4 mg TE/g E, respectively).

The ABTS test is widely used for the evaluation of antioxidant activity of various substances. The ABTS of methanolic plant extracts was consistent with the data obtained from the DPPH test. Comparison of obtained ABTS values of methanolic plant extracts depicted significant variability (Table 3). The best antioxidant activity was obtained in *F. pulverulenta* extract with a value equal to 3621.43 mg TE/gE followed by *D. maritima* (783.81 mg TE/gE). The less effective activity was found in *F. thymifolia* (335.93 mg TE/gE) and *L. europeum* (372.86 mg TE/gE).

The metal chelating activity (MCA) was used to investigate the ferrous ion chelating ability of different methanolic plant extracts. Among tested samples, *F. thymifolia* extract exhibited the strongest iron chelating capacity (1566.35 mg EDTA/gE) as compared to the other species that displayed significantly varying values comprised between 103 and 350 mg EDTA/gE (Table 3). Measuring the ability of oxygen radical absorbance (ORAC) was widely encountered in the field of antioxidants and oxidative stress.Methanolic extract of *F. pulverulenta*exhibit the better antioxidant capacity, with ORAC value of 58.08 mg TE/gE, followed by *D. maritima* and *G. flavum* extracts (17.87 and 17.45 mg TE/gE, respectively), while *F.thymifolia* extract showed very low oxygen radical absorbance capacity, which was around 9 times weaker than *F.pulverulenta*(Table 3).

Table3. *Antioxidant assays from six plant methanolic extracts*

	TPC (mg GAE/g E)	ORAC (mg TE/g E)	DPPH (mg TE/g E)	ABTS (mg TE/g E)	MCA (mg EDTA/g E)
F. pulverulenta	34.28±0.61a	58.08±1.7a	1090.4±18.14a	3621.43±101.02a	71.98±2.38f
F. thymifolia	3.64±0.11f	6.83±0.61e	128.44±4.95e	335.93±32.84d	1566.35±21.8a
S. sodomaeum	8.66±0.58b	14.79±1.43c	80.80±4.87c	406.19±25.32c	103.27±4.68e
G. flavum	7.49±0.23c	17.45±0.79b	331.4±13.89c	409.76±58.5c	264.57±10.02c
D. maritima	6.75±0.45d	17.87±0.36b	377.85±36.69b	783.81±116.55b	207.24±17.64d
L. europeum	5.38±0.27e	11.39±0.82d	621.7±28.64d	372.86±45.12c	350.05±13.77b

3.3. Neuroprotective Activity of Methanolic Extracts

The cytotoxic potential of each extract on PC12 cells was measured by MTT assay. All plant extracts, except *S.sodomaeum*, did not significantly affect cells viabilityat concentration up to 400 µM (Figure 2a).Indeed, incubation of PC12 cells with 5 µM $A\beta_{(25-35)}$, for 24 h decreased the cell viability compared to control to 44%(Figure 2b). In order to evaluate the protective effect of extracts, Aβ was added to PC12 cells simultaneously to the extracts (100, 200 and 400 µg/ml). Among all tested extracts, both *Frankenia* species exhibited an extremely efficient neuroprotective activity. At the lowest tested concentration (100 µM), *F. thymifolia* and *F. pulverulenta* extracts restored the cells viability (80%) and the percentage increased with the dose of extract applied. The extracts of *D. maritima, L. europeum* and *G. flavum* show a moderate neuroprotective capacity.

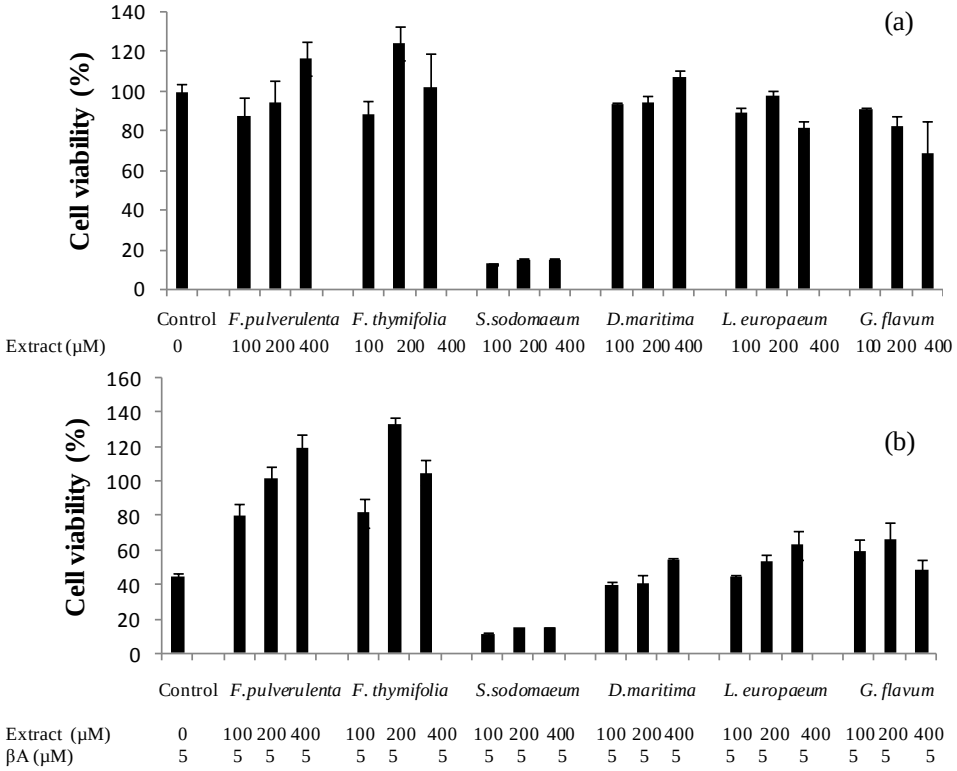

Figure2. *Cytotoxic activity* ***(a)*** *and neuroprotective activity on Aβ-induced toxicity in PC12 cell line* ***(b)*** *of six methanolic extracts. The experiment was repeated three times.*

4. DISCUSSION

In our study, results indicate that major phenolic compounds are flavonoids especially those belong to flavan-3-ol group. Among these compounds, catechin is proven potent antioxidant, due to the presence of the *O*-dihydroxy and *O*-hydroxyketo groups (Vinson et al. 1995). Epicatechin 3-*O*- gallate is also an efficient bioactive compound which is able to act as a powerful ferric reducing agent (Trabelsiet al. 2013). Flavonoids such as proanthocyanidins found in different berries and cocoa that are derived from flavon-3-ols such as catechin or epicatechin, found in parsley or chamomile and other phenolic compounds, but also natural products from different chemical classes such a terpenoids, have been shown to exert anti-inflammatory effects in different pathological conditions (Tili and Michaille, 2016). Indeed, Zeng et al. (2011) showed that quercetin-galactoside (hyperoside) which is present in the methanolic extract of *G. flavum* significantly inhibited $A\beta_{25–35}$-induced cytotoxicity and apoptosis by reversing Aβ-induced mitochondrial dysfunction, including mitochondrial membrane potential decrease, reactive oxygen species production, and mitochondrial release of cytochrome c. Recently, numerous studies have suggested that a wide range of polyphenols may have neuroprotective effects both *in vitro* and *in vivo* by exerting protective effects through their ability to scavenge ROS or by directly inhibiting the formation of Aβ fibril deposits in the brain (Richard et al. 2011). *F. thymifolia* was shown to contain high amount of phenolic acids (caffeic, chlorogenic, sinapic and gallic acids). These compounds and specially caffeic and chlorogenic acids were found to possess the strongest antiviral activity (Karar et al. 2016). It is interesting to note that the analysis of different plant extracts resulted in the different amounts of phenolics. Significant difference in contents ($P< 0.05$) was observed between two *Frankenia* species. In this study, slight variation between the TPC in the *F. Thymifolia* plant, with those reported by previous authors, was observed. For instance, higher phenolic amounts (14.18 mg GAE g^{-1} DW) were reported by Megdiche et al. (2011) in *F. thymifolia* fraction. Amount of total phenolics of *F. pulverulenta*was higher than some medicinal glycophytes such as *Nigella sativa* (Karray-Bouraoui et al. 2010) or *Diplotaxis harra* (Falleh et al. 2013). Indeed, chromatogram profile of two species belongs to the Frankinaceae family revealed the presence of number of no identified compounds. As reported by authors, these compounds seemed to be flavonoid and phenolic sodium sulfates (Harkat et al. 2007; Hussein 2004; Harborne, 1975). Different reports are in accordance with the hypothesis that flavonoids sulfates play a role in the cancer prevention or inhibition (Hertog, 1996).

The highest amount of total phenolics in *F. pulverulenta* extract corroborated with the higher DPPH and ABTS scavenging abilities. In the other way, we can hypothesize that the antioxidant capacity may be related to the amount of phenolics in this species. Indeed, studies have shown that polyphenols possess a powerful inhibitory capacity against lipid oxidation through radical-scavenging (López-Cobo et al. 2015). Subsequently, extracts were also tested for their metal chelating activity which was lower in *F. pulverulenta* (71.98 mg EDTA/g), thus reflecting humble antioxidant effects when compared with other medicinal halophytes (Oueslatiet al. 2012). Indeed, Jallali et al. (2014) suggest that the chelating activity against the ferrous ion in the halophytic species *I. crithmoïdes* can be related to specific phenolic compounds rather than the whole phenolic content. Authors have noted that phenolic compounds exert their metal chelating potency based on their phenolic structure and the number and position of the OH^- groups (Santoso et al. 2004). Thus, *F. thymifolia* which displaying the lowest phenolic content but the strongest iron chelating activity could be correlated to the nature of its compounds able to chelate metal ions. As for the other *in vitro* antioxidant tests, the highest capacity of *F. pulverulenta* to scavenge peroxyl radicals may be due to the presence of potent antioxidant phenolic compounds such as quercetin and catechin derivated compounds. The higher content of phenolics in *F. pulverulenta* is corroborated with the higher antioxidant activity determined by antiradical scavenging and ORAC tests. From our data, it appears that the ORAC values in the present study were comparable to or even higher than those reported in the literature (Remila et al. 2015).

The correlation coefficients between the antioxidant capacities (DPPH, ABTS, MCA and ORAC) and the total phenolic were determined (Table 4). Significant positive relationships were obtained between the TPC and the antioxidant capacities assessed by DPPH, ABTS and ORAC methods. These results are in accordance with those of Biglari et al. (2008) who showed that phenolic compounds contribute significantly to the antioxidant capacities. However, no correlation was established between chelating power assay and total phenolics. In this context, some studies have demonstrated that metal chelating potency of some phenolic molecules was far lower as compared to polysaccharides, EDTA and some peptides as well as proteins (Santoso et al. 2004).

Aβ aggregation is a risk factor common to a several neurodegenerative diseases leads to the formation and the deposition of senile plaques and neurofibrillary tangles which promote pro-inflammatory responses and induce neurodegenerative disorders. This situation is responsible of the dysfunction and death of brain cells (Trabelsi et al. 2013). There are extensive investigations which have been made to establish the neuroprotectiverole of polyphenols *in vitro* and *in vivo* tests. As previously reported by Richard et al. (2011), a submicromolar concentration of Aβ peptide was sufficient to elicit metabolic interruptions in PC12 cells. Both *Frankenia* species have efficient protective effect against cell death induced by Aβ peptide. The great antioxidant activities of methanolic extracts and their phenolic composition can explain their highest capacities to inhibit Aβ aggregation. Thus it might be reasonable to consider that phenolic compounds might prevent neurodegenerative diseases, not only by scavenging reactive oxygen species but also by directly inhibiting the aggregation of Aβ fibrils in the brain by complex formation (Richard et al. 2011). Furthermore, the toxicity of *S. sodomaeum* against PC12 cells viability was may be due to the presence of alkaloids.

Table4. *Pearson's correlation coefficients of antioxidant activities and total phenolic contents*[a]

	DPPH	ABTS	ORAC	MCA
TPC	0,8192*	0,986***	0,9904***	-0,4175 ns
DPPH		0,8585*	0,8466*	-0,4272 ns
ABTS			0,9855***	-0,3477 ns
ORAC				-0,4756 ns

[a]*Data represents Pearson Correlation Coefficient R. ns: indicates non significant, ** and *** indicates significant at p < 0.01 and p < 0.001, respectively.*

5. Conclusion

Species investigated in this work showed an important and a wide range of polyphenol contents and antioxidant capacities. Thus, these results suggest the importance of these halophytic species and in particular *F. pulverulenta* as a new source of phenolic compounds with potent neuroprotective capacity.

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