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Phenolic composition of some Tunisian medicinal plants associated with anti-proliferative effect on human breast cancer MCF-7 cells

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Abstract

Plants have been seen to possess the potential to be excellent biological matrices to serve as a basis for investigating the presence of promising therapeutic agents for cancer treatment. Several successful anti-cancer medicines - or their analogues - nowadays in use are plant derived and many more are under clinical trials. Under current circumstances, the purpose of this work was to test aqueous and ethanolic extracts of five aromatic and medicinal plants from arid zones on some tumor cell lines. These plants, *Cymbopogon schoenanthus* (L.) Spreng, *Crithmum maritimum* (L.) Spreng, *Hammada scoparia* (Pomel) Iljin, *Retama raetam* (Forssk.) and *Zizyphus lotus* (L.) Desf., widely used in Tunisian ethnomedicine, were assessed for their phenolic compounds, antioxidants and anticancer activities in aqueous and ethanol extracts. Total polyphenols, flavonoid and tannin contents were determined colorimetrically and some of these molecules were identified using RP-HPLC. A significant difference on phenolic contents and composition were found among the investigated plants. *Cymbopogon schoenanthus* was the richest in phenolic compounds (approx. 72%) with quercetine-3-o-rhamnoside (approx. 33%) as main contributor. For all the tested plants, the highest antioxidant capacity was detected in the ethanolic extracts rather than in the aqueous ones. The highest antiproliferative potential was observed for the ethanolic extracts. *Hammada scoparia, Retama raetam* and *Zizyphus lotus* exhibited important antiproliferative effect that reached 67% at a 1% extract concentration. Taken together, the present study supports the potential development of chemotherapeutic agents from, at least, four of the five studied Tunisian ethnomedicinal plants.

Keywords: Antioxidant capacity, antiproliferative potential, breast cancer, phenolic compounds, traditional medicinal plants

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Introduction

The incidence of cancer in Mediterranean countries is generally lower than in the rest of European countries and in the United States (1); the major reason of this, in addition to genetic factors, could be attributed to their eating habits. The traditional Mediterranean diet is characterized by a high consumption of plant-based foods, rich in compounds with nutraceutical properties (2). With this respect, it is well known that plants (fruits, vegetables, medicinal herbs, etc.) usually contain a important variety of antiradical compounds, such as phenolic (e.g. phenolic acids, flavonoids, coumarins, lignans, stilbenes, tannins), nitrogen (alkaloids, amines, betalains) and terpenoids (including carotenoids) molecules which are recognized for their efficient antioxidant activities (3). Moreover, it has been proposed that there is an inverse relationship between dietary intake of antioxidants-rich food and the incidence of many human diseases, such cancer (4).

Accordingly, the National Cancer Institute (USA) began to screen plant extracts with antitumor potency (5) and many researchers pay attention to ethnomedicinal plants as rich sources of novel anticancer drugs. With this respect, the ethnobotanical survey and documentation of traditional knowledge, mainly on the medicinal uses of plants, has provided important clues for the discovery of new drugs (6).

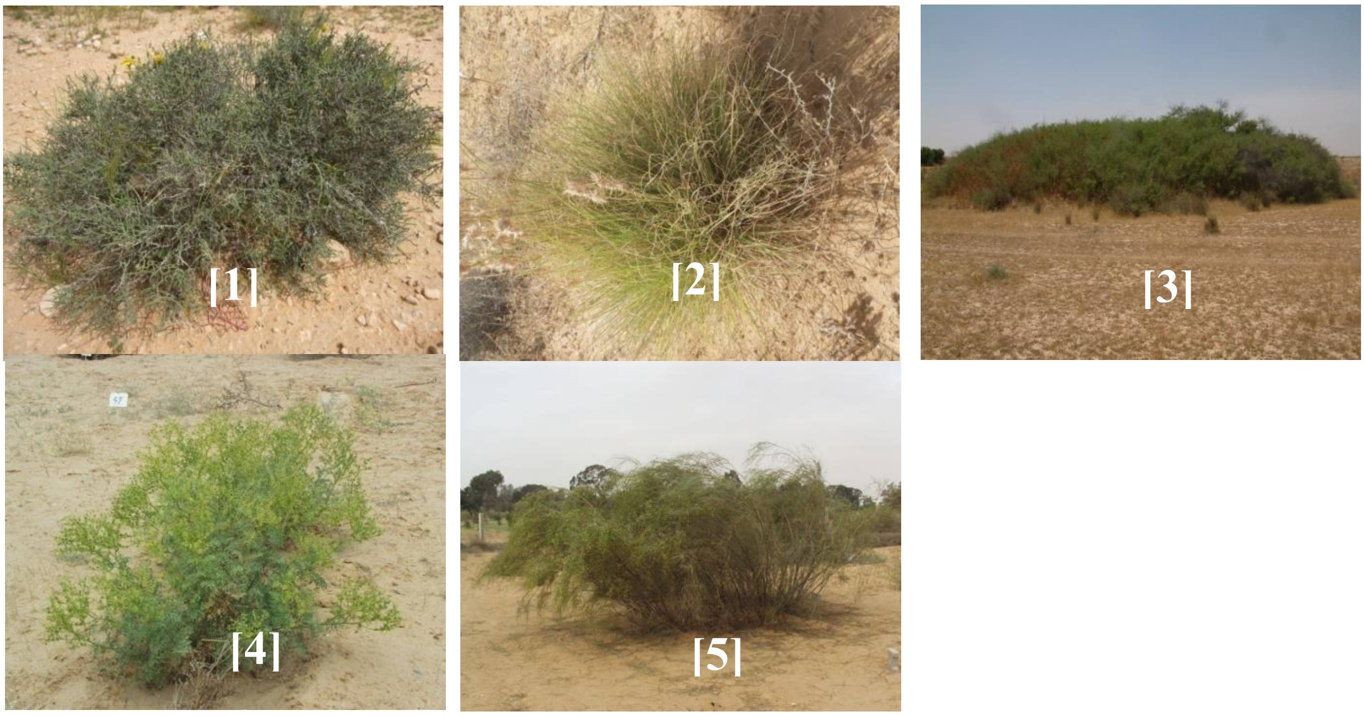

Figure 1. Aromatic and Medicinal species: **[1]** *Hamada scoparia = Arthrophytum scoparium,* **[2]** *Cymbopogon schoenanthus,* **[3]** *Ziziphus lotus,* **[4]** *Crithmum maritimum,* **[5]** *Retama raetam.*

In Tunisia, thanks to the diversity of its climate, the traditional pharmacopoeia comprises a large arsenal of medicinal plants (7). Although several of these species are nearly facing extinction (8), only a small proportion of them have been scientifically studied. It is also important to highlight that Tunisian medicinal and aromatic plant; especially those growing in arid and desertic lands are equipped with powerful antioxidant systems and were labeled as valuables to treat several human diseases (7,6,9). For that reason, five traditional Tunisian medicinal plants (TMP): *Cymbopogon schoenanthus* (L.) Spreng, *Crithmum maritimum* (L.) Spreng, *Hammada scoparia* (Pomel) Iljin, *Retama raetam* (Forssk.) and *Zizyphus lotus* (L.) Desf., were selected to be tested in this study (**Fig. 1**). The main aims of this paper were: 1- to determine the bioactive compounds (especially phenolic ones) present in aqueous and ethanolic extracts of the five selected TMP; 2- to assay their antioxidants capacities (AC); 3- to explore their antiproliferative on a breast cancer cell lines (MCF-7) using the MTT tetrazolium salt colorimetric assay.

Material and Methods

Plant material

The all plant material used in the present study were obtained from wild plants which were collected from the Southeast of Tunisia. This area is arid to semi-arid with a typical Mediterranean climate, characterized by irregular rainfall events and a harsh dry summer period. Annual precipitation is around 186 mm and annual mean temperature is 19.4°C with a minimum temperature 3.9°C in January and 35.9°C maximum in August correspond to climatic data where original population of the plants occurred. These plants species, belonging to five different plant families from South East of Tunisia (arid land), were collected (200–250 g per species) during the vegetative phase. The harvested plants were authenticated by botanist Dr. Mohammed Neffati according to the "Flora of Tunisia" catalogue (10). Voucher specimens were deposited at the herbarium of the IRA. The scientific names and the parts used in folk medicine are detailed (**Table 1**). The samples were harvested, processed (dried in the shade at ambient temperature for two week, then powdered at around 100 msh), finally stored in dark condition until use.

Preparation of the extracts

For the aqueous extraction, an amount of 5g of the powdered sample was extracted with 100 mL Milli Q water at 105°C for 15 minutes. After cooling, each extract was centrifuged at 800 x g for 10 minutes and filtered using a 0.45μm nylon membrane (Millipore). The filtrated extracts were finally stored in shady condition until use.

For the alcoholic extraction, the same amount (5 g) of powedered sample was extracted using 100 mL of 70% ethanol at room temperature for 24 hours in a shaking condition. After centrifugation and filtration, as well as for the aqueous extract, the plant extracts were stored at 4°C before assays.

Quantification of total phenolics, total flavonoids, total condensed tannins and identification of individual phenolic components

Total phenolic (TPC), flavonoid (TFC) and Total Condensed Tannins Content (TCTC) contents in the extracts were determined as gallic acid and catechin equivalents, respectively, by spectrophotometry, while Reversed-Phase High Performance

Table 1. Medicinal properties of the plant species investigated

Family and scientific name	Medicinal used parts	Use(s)	Major active compounds	Reference(s)
Poaceae *Cymbopogon schoenanthus* L. Spreng	Arial parts	Indicated for the treatment of rheumatism and fever. Diuretic, insecticide	Essential oil: limonene (10.5–27.3%), β-phellandrene (8.2–16.3%), α-terpinene (4.3–21.2%) and α –terpineol	(11)
Apiaceae *Crithmum maritimum* L.	Leaves, Seeds	Appetizers and condiments. Antiscorbutic, tonic, carminative, diuretic, depurative and vermifuge	vitamin C, carotenoids, flavonoids, essential oil (γ-terpinene, β-phellandrene, and sabinene)	(12) (13)
Chenopodiaceae *Hammada scoparia* Pomel. Iljin (*Arthrophytum scoparium*)	Shoots	Indicated for the treatment of eye disorders, a poultice for mould, diarrhea, bites of snakes and against rheumatisms Snuff powder	Alkaloids, flavonol triglycoside, isorhamnetin (3-O-beta-D-xylopyranosyl-(1-3)-alpha-L rhamnopyranosyl-(1′′′-6 »)-beta-D-galactopyranoside, Isorhamnetin 3-O-beta-D-apiofuranosyl-(1-2) (alpha-L-rhamnopyranosyl-(1-6))-beta-D-galactopyranoside, Isorhamnetin3-O-alpha-L-rhamnopyranosyl-(1′-2 »))	(14) (15)
Fabaceae *Retama raetam* Forssk.	Leaves	Used for the treatment of some renal deseases wounds, bites of snakes, sheep scabies, hypertension and for making eye wash for eye troubles. Diuretic, febrifuge, purgative and vermifuge.	Alkaloids and flavonoids (daidzein, vicenin-2, naringenin, apigenin, kaempferol, quercetin and kaempferol-7-O-glucoside, daidzein 7, 4′-dimethyl ether, chrysoeriol 7-O-glucoside and orientin)	(16) (17)
Rhamnaceae *Zizyphus lotus* L.	Fruits, leaves, seeds, roots bark	Treatment of digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhea, fatigue, distress, eyes inflammation, vitamin B deficiencies, emollient, sedative, spleen tonic, aphrodisiac, cleans blood, appetizing, facilitate digestion.	Cyclopeptide alkaloids, (lotusines B, C, E and F dammarane saponins) Flavonoids and phenolic acids (catechin, caffeic acid, p-coumaric acid, ferulicacid, rutin, apigenin-7-glucoside, eriodictyol, quercetin, p-hydroxybenzoic acid, chlorogenic acid andsyringic acid)	(18) (19)

Liquid Chromatography (RP-HPLC) was used for their qualitative analyses. The analysis was performed by Agilent Technologies 121100 series HPLC system. The separation was carried out using a reverse phase ODSC18 (250mm × 4.6mm ID, 5 μm particle size Hypersil) column in gradient elution as mobile phase using acetonitrile (Solvent A) and (0.2%) water sulphuric acid (Solvent B). Experimental conditions are given in the supplementary material in detail.

Determination of antioxidant activity

Total Antioxidant Capacity (TAC) of plant extracts was determined monitoring the capacity of the extract to reduce Mo (VI) to Mo (V) with the subsequent formation of a green phosphate / Mo (V) complex at acidic pH (22). The ability of plant extracts to scavenging the DPPH radical was measured according to Hanato and colleagues (23). Iron reducing power, defined as the capacity of plant extracts to reduce Fe^{3+}, was assessed by the method of Oyaizu (24). The ferrous ion chelating activity of the extracts was assessed as described by Falleh and colleagues (22). Experimental conditions are given in the supplementary material in detail.

Cytotoxity towards the MCF-7 breast cancer cell line MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

In order to investigate the antiproliferative effect of the examined plant extracts towards the MCF-7. The cell viability and proliferation were assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide or MTTassay. Data are presented as percentage of cell proliferation against a control (100% of cell proliferation) using different concentration of the plant extracts. Experimental conditions are given in the supplementary material in detail.

Statistical analyzes

For all the studies, samples were analyzed in triplicates. Data are shown as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) with Duncan's test was carried out to test any significant differences between species at $P < 0.05$. Statistical analyses were carried out using SPSS v.20 software.

Results

Phenolic contents in plant extracts

In this study, both aqueous and ethanolic extracts of the five TMPs were compared. Total Polyphenolic Content (TPC), Total Flavonoids Content (TFC) and Total Condensed Tannins Content (TCTC) indicated large differences among all the plant extracts (**Table 2**).

TPC ranged from 62.5 to 139.1 mg GAE DW^{-1} for the ethanolic extracts and from 35.2 to 128.6 mg GAE DW^{-1} for the aqueous ones. TFC ranged from 6.1 to 70.7 mg CE DW^{-1} for the ethanolic extracts and it resulted lower than 30 mg CE DW^{-1} for the aqueous ones in all the plants. Finally, TCTC reached 129.2 mg CE DW^{-1} in alcoholic extracts, while it was limited to 60 mg CE DW^{-1} in the aqueous ones. Interestingly, among the ethanolic extracts, the one obtained from *Z. lotus* exhibited the highest amount of TPC, TFC and TCTC. On the other hand, the *C. schoenanthus* aqueous extracts presented the highest TPC and TCTC content. According to these results, relevant interspecific variations regarding the accumulation of total phenolics, flavonoids and condensed tannins were observed. Furthermore, a significant difference in the phenolic content between aqueous and ethanolic extracts was registered. *C. schoenanthus* and *C. maritimum* showed the highest TPC in the aqueous extracts, while the ethanolic extracts of *H. scoparia* and *Z. lotus* presented the highest levels of TPC.

Antioxidant activities

Antioxidant activities of fruits and vegetables are measured by using different assays. Some of these methods were applied to quench and scavenge Reactive Oxygen Species, (ROS) which include both free ($O2^{•-}$, $OH^{•}$, $HO_2^{•}$, and $RO^{•}$) and non-radical forms (35). Several analyses such as total antioxidant activity, DPPH and ABTS assays, ROS quenching assay, metal chelating, reductive potential, β-carotene linoleate system and linoleic acid method are commonly used for the determination of antioxidant activities of plant extracts (36,7). In this study, according to the results of the total polyphenols, results showed that antioxidant activity (TAA, antiradical activity, chelating effect and iron reducing power) measured on each plant extract, was dependent from the extraction method (**Table 3**). In fact, TAA of the aqueous extracts ranged from 73.5 to 139 mg EGA g DW^{-1} for *Z. lotus* and and *R. raetam* respectively; moreover they were found to be lower than those measured from the ethanolic extracts that reached the concentration of 248.9 mg EGA g DW^{-1} in *R. raetam*, which displayed the highest total antioxidant activity for both the extracts (139 and 248.89 mg GAE g DW^{-1} for the aqueous and ethanolic extracts, respectively). Interestingly, TAA of *R. reatam* ethanolic extract was around 17-fold higher than that observed in the traditional and very used medicinal plant *Tamarix gallica* (L.) with 14.66 mg GAE g DW^{-1} (7).

Regarding the values of IC_{50}, referred to the antiradical activity (**Table 3**), most of the tested medicinal plants reported a concentration below 0.6 mg mL^{-1} for aqueous extracts and below 0.8 mg mL^{-1} for ethanolic ones. Precisely, *Z. lotus* and *C. schoenanthus* ethanolic and water extracts exhibited the highest antiradical potency. Considering the iron reducing power, EC_{50} values of aqueous extracts ranged from 0.11 *(C. schoenanthus)* to 0.4 mg mL^{-1} (*Z. lotus* and *C. maritimum*) and for the etha-

Table 2. Total polyphenolics, flavonoid and condensed tannin content in the five investigated plant extracts. Data are expressed in mg g^{-1} of dry matter

	Extracts	*Cymbopogon schoenanthus*	*Crithmum maritimum*	*Hammada scoparia*	*Retama raetam*	*Zizyphus lotus*
Total phenolic (mg GAE g DW^{-1})	Ethanolic	63.86±0.01^{d}	62.47±0.05^{e}	93.87±0.03^{c}	112.12±0.11^{b}	139.08±0.17^{a}
	Aqueous	128.63±0.12^{a}	116.13±0.2^{b}	41.90±0.15^{d}	97.27±0.03^{c}	35.17±0.21^{e}
Total flavonoid (mg CE g DW^{-1})	Ethanolic	7.09±0.07^{d}	6.05±0.13^{e}	70.00±0.10^{b}	15.93±0.23^{c}	70.71±0.09^{a}
	Aqueous	16.00±0.09^{c}	21.26±0.08^{b}	14.11±0.20^{d}	14.50±0.12^{d}	29.17±0.03^{a}
Total condensed tannins (mg CE g DW^{-1})	Ethanolic	22.84±0.09^{d}	16.62±0.07^{e}	82.44±0.20^{b}	28.15±0.16^{c}	129.17±0.12^{a}
	Aqueous	59.97±0.16^{a}	58.31±0.19^{b}	35.22±0.11^{d}	40.83±0.08^{c}	22.22±0.06^{e}

Data are the mean of three replicates±SD. Different letters represent values that are significantly different between control and treatments at 5% according to the Duncan's multiple range test.

Table 3. Antioxidant activity of the aqueous and ethanolic extracts of the five studied plants

	Extracts	*Cymbopogon schoenanthus*	*Crithmum maritimum*	*Hammada scoparia*	*Retama raetam*	*Zizyphus lotus*
TAA (mg GAE g DW^{-1})	Ethanolic	87.91±0.06[e]	89.35±0.08[d]	236.00±0.19[b]	248.89±0.31[a]	179.44±0.21[c]
	Aqueous	69.02±0.09[e]	82.35±0.05[b]	80.90±0.11[c]	139.00±0.04[a]	73.47±0.05[d]
Antiradical activity (IC_{50} mg mL^{-1})	Ethanolic	0.6.00±0.02[a]	0.80±0.01[b]	0.60±0.08[b]	0.80±0.01[a]	0.19±0.08[c]
	Aqueous	0.30±0.01[c]	0.60±0.01[a]	0.45±0.08[b]	-	0.24±0.07[d]
Iron reducing power (EC_{50} mg mL^{-1})	Ethanolic	0.38±0.01[c]	0.46±0.02[b]	0.10±0.01[e]	0.90±0.01[a]	0.30±0.02[d]
	Aqueous	0.11±0.01[d]	0.40±0.01[a]	0.33±0.01[c]	0.36±0.01[b]	0.40±0.11[a]
Chelating effect (EC_{50} mg mL^{-1})	Ethanolic	68.00±0.08[b]	84.00±0.09[a]	85.11±0.13[a]	8.40±0.02[c]	0.11±0.01[d]
	Aqueous	-	-	-	5.37±0.01[a]	0.16±0.01[b]

Data are the mean of three replicates±SD. Different letters represent values that are significantly different between control and treatments at 5% according to the Duncan's multiple range tests.

Table 4. Identification and quantification by reversed-phase high performance liquid chromatography of the main phenolic compounds extracted with ethanol 70% from the five examined plants.

Phenolic compounds	Quantity (%)				
	Cymbopogon schoenanthus	*Hammada scoparia*	*Retama raetam*	*Ziziphus lotus*	*Crithmum maritimum*
Resorcinol	5.08	-	-	-	-
Caffeic acid	3.18	-	-	-	-
2.5-Dihydroxybenzoic acid	2.01	-	-	-	-
Quercetine-3-o-rhamnoside	32.82	3.64	-	-	-
Trans-cinnamic acid	9.72	-	-	-	-
Gallic acid	8,80	2.93	-	6.56	-
Ferulic acid	9.83	-	-	-	-
Vanillic acid	-	8.02	-	37.04	10.37
Apigenin-8-C-glucoside	-	31.17	-	-	-
Coumaric acid	-	3.84	-	-	-
Rosmarinic acid	-	1.93	-	4.17	-
Chlorogenic acid	-	7.05	-	-	5.39
Syringic acid	-	-	0.2	-	-
Kaempferol	-	-	2.58	-	-
3.5-dimethoxy-4-hydroxybenzoic acid	-	-	-	5.16	-
Quercetine-3-galactoside	-	-	-	10.46	-
Epigallocatechin		-	-	-	35.89
Total phenolics	**71.44**	**58.58**	**4.36**	**63.39**	**52.82**

- = not detected

nolic extracts it ranged from 0.10 (*H. scoparia*) to 0.90 mg mL^{-1} (*R. raetam*).

Phenolic compounds identification

A total of 20 phenolic compounds were identified in the five assessed plants (**Table 4**). Important variability in phenolic acids (11 compounds) and flavonoids (9 compounds) composition among the studied plants was registered. The glycosylated form of quercetine, quercetine-3-o-rhamnoside, which possesses several biological activities (37), was the major compound found in *C. schoenanthus,* while the glycosylated form of apigenin, apigenin-8-C-glucoside was the major compound found in *H. scoparia*. In addition, *Z. lotus* resulted rich in vanillic acid and *C. maritimum* in epigallocatechin.

In a recent study, Rocha-Amador and colleagues (38) have reported that the glycosylation of quecetine enhances its bioaccessibility and then the biological effect. The major compound in *H. scoparia*, apigenin-8-C-glucoside, also called vitexin, has recently received an increased attention due to its wide range of pharmacological effects, including but not limited to anti-inflammatory, anti-oxidant, anti-cancer, anti-hyperalgesic and neuroprotective effects (40). Vanillic acid, the major compounds of *Z. lotus* extract, showed in a recent study a powerful antioxidant effect, counteracting the oxidative stress induced by amyloid beta protein involved in Alzheimer disease (41). The major compound of *C. maritimum*, epigallocatechine, is a powerful antioxidant molecule known for its neuroprotective and antidiabetic potentials (42, 43).

Antiproliferative activity

The anticancer activity of TMP has not been declared in literature and the high antioxidant activities in these species prompted us to investigate their anti-proliferative capacity. For this reason, the cytotoxicity of each extract was assessed against the human breast cancer cell line MCF-7 using the MTT assay. The results showed that after 48 h of treatment with TMP aqueous and ethanolic extracts, cancer cell proliferation was inhibited in a concentration, solvent and plant tested-dependant manner (**Fig. 2**). At the concentration of 1 mg mL^{-1}, all the plant extracts showed the highest cytotoxic effects on the treated cancer cells. All the TMP ethanolic extracts at the same concentration induced cell death in a range from 16.52 to 66.37% of treated cells, except for the *C. maritimum* ethanolic extracts; in this case, only a minor effect was observed on the MCF-7 cell line. In particular, *H. Scoparia* (**Fig 2, B**), *R. Raetam* (**Fig 2, F**) and *C. Schoenantus* (**Fig 2, D**) ethanolic extract showed the highest antiproliferative effect (66.37%, 34.5% and 56.19% of cell viability, respectively). Interestingly, among the examined plant extracts, the aqueous extract of *C. schoenanthus* (**Fig. 2, C**) showed the highest efficacy to inhibition the cell proliferation (around 40% of cell viability) especially in the range of concentration from 0.1 to 1.0 mg mL^{-1}, where no significant differences were registered (**Fig. 2**). Moreover, aqueous extracts of *H. scoparia* (**Fig. 2, A**) and *Z. Lotus* (**Fig. 2, G** *)* at a concentration of 1 mg mL^{-1}, showed an important effect on cell vibility reduction (around 70% and 60% of reduction, respectively).

Discussion

Since most of the Tunisian medicinal plants are generally used for consumption of herbal tea or soup or medicinal bolus to treat/prevent different pathologies, the aqueous extraction performed in this study resulted necessary. In order to test the efficacy of the extraction using only water, a comparison with the alcoholic solvent extraction, largely used in chemical industry, was carried on. Aqueous ethanol mixtures are generally used for the extraction of several phenolic compounds from aromatic and medicinal plants. This is mainly due to the diverse range of polyphenols that the aqueous ethanol mixtures can successfully dissolve. Besides, ethanolic mixtures, up to certain percentage, have an high degree of acceptability for human consumption models (26).

Several published studies showed that different solvents, due to their polarity, have significantly various extraction capacities for phenolic molecules in plants (27, 28). Plant materials may contain different percentages of phenolic acids, phenylpropanoids, anthocyanins, and tannins, among others, which can interact with other plant molecules such as carbohydrates and proteins that may lead to the formation of complexes, often insoluble. Furthermore, the phenolics solubility is affected by the polarity of solvent(s) used. For these reasons, it is very difficult to set up an extraction method suitable for all phenolics present in plant kingdom (29). Thus, there is no uniform or completely satisfactory procedure for the extraction of all phenolics or, at least, a specific class of phenolic compounds in plant materials (27,28). For this reason, it is important to properly analyse the composition of phenolic compounds in plants so that their health-promoting properties can be adequately studied.

In addition, these wild TMP significantly showed to contain more phenolics compared to many other (not only Tunisian) medicinal and common dietary plants (30, 31). Among TMP extracts, *Z. lotus*, a spontaneous edible plant commonly used in the Mediterranean regions for its beneficial therapeutic impact in nutrition and health care, represents an important source of phenolics such as flavonoids and tannins (9). Phenolic compounds are ubiquitous in plants and have been associated with the healthcare derived from consuming high levels of fruits and vegetable (30). In particular, flavonoids are associated with a broad spectrum of health benefits and are an essential component in a variety nutraceutical, medicinal, pharmaceutical and cosmetic applications. This is due to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic activity associated with their ability to modulate key cellular enzyme functions (32).

The concentration and nature of phenolic compounds in plants are very sensitive to the environmental and climatic conditions (33) and a geographical localization may contribute to increase genetic differentiation between different taxonomic entities (34). The studied saharian plants are very locally distributed in in the ecoregions of Tunisia. Their significant phe-

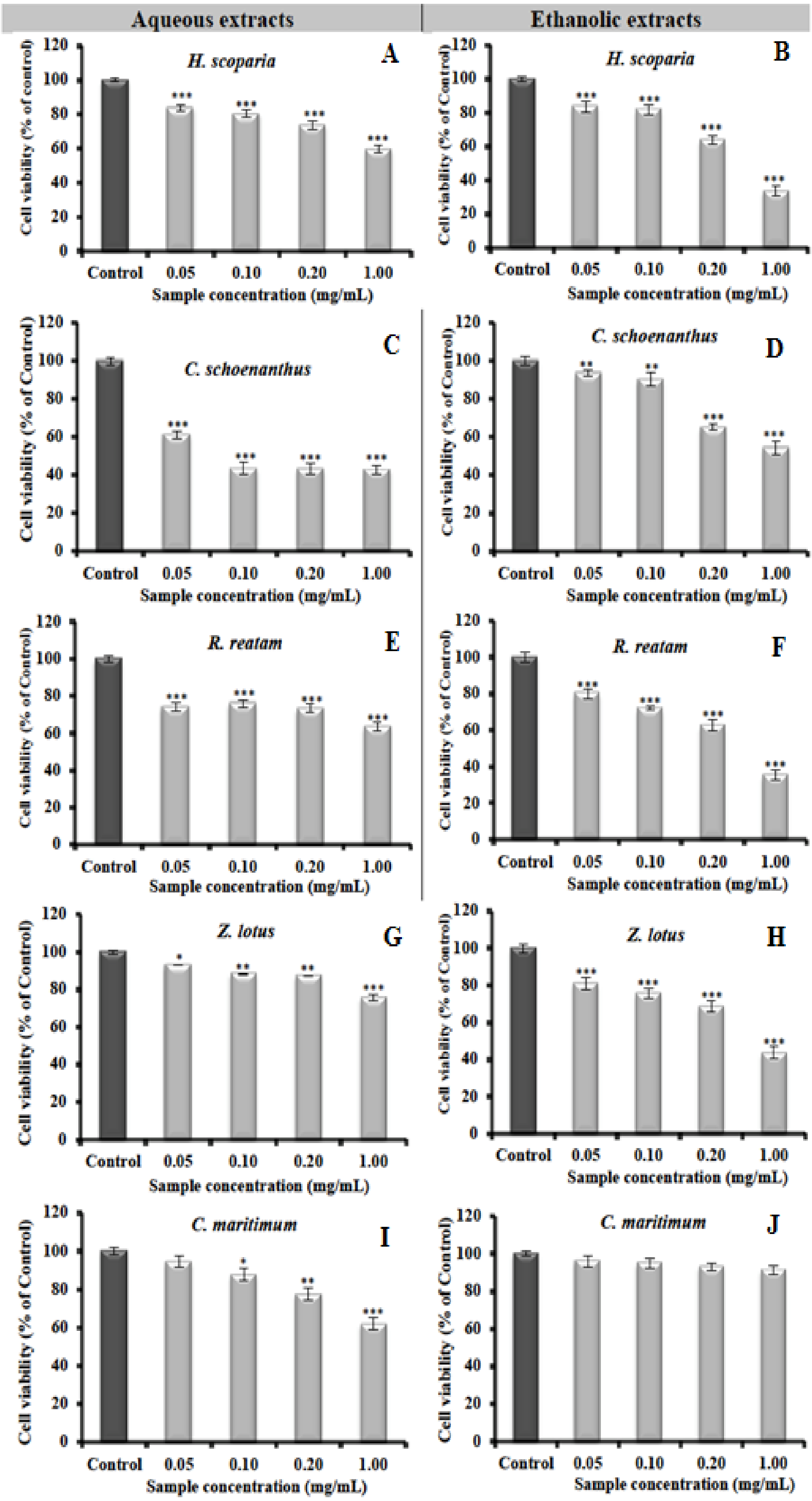

Figure 2. Anti-proliferative activity of the five selected Tunisian medicinal plants against the human breast cancer cell line MCF-7. According to *T-student* test *<0.05, **<0.01, ***<0.001

nolics contents could be an adaptive trait to face the oxidative stress caused by the severe climatic conditions (warm temperatures, dryness, high solar exposure, short growing season) of their habitat. In plants, polyphenol synthesis and accumulation are generally stimulated in response to biotic/abiotic stresses, leading one to think that secondary metabolites may play a role in the adaptation of extremophile species to this constraint (30). Flavonoids, acting as UV filters, protect plants from different biotic and abiotic stresses; moreover they include signal molecules, allopathic compounds, phytoalexins, detoxifying agents and antimicrobial defensive compounds.

Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimatisation and freezing tolerance (32). Moreover, data showed that the *H. scoparia* ethanol extract and the *C. schoenanthus* aqueous extracts depicted an important reducing power compared to other halophytes plants with medicinal properties such as the previously cited *Tamarix gallica* (205 µg mL^{-1}) and *Salsola kali* (L.) (165 µg mL^{-1}) (28).The significant chelating effect showed by *Z. lotus* ethanol extract (0.11 mg mL^{-1}) can be attributed to the high content in tannins. Tannins have several controversial effects on biological systems since they are biological antioxidants and potential metal ion chelators, but they are also responsible of the protein precipitation (30).

The high total phenolic content found in *H. scoparia, R. raetam* and *Z. lotus* ethanolic extracts and in *C. schoenanthus* aqueous extracts seems, therefore, to be related to their important antioxidant capacities. In accordance with this statement, previous published studies confirmed the direct relationship between antioxidant activity and total phenolic content in selected herbs (30,3). In the human body, these phytochemicals are thought to provide health benefits by different mechanisms, including: (1) free-radical scavenging; (2) protection and regeneration of other dietary antioxidants (i.e. vitamin E); and (3) chelating of pro-oxidant metal ions (29). Flavonoids are oxidised by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilise the reactive oxygen species by reacting with the reactive compound of the radical (32).

However, in *C. shoenanthus* and *C. maritimum,* no relationship between their antioxidant activities and total phenolics was found. It is known that different structures or levels of glycosylation of phenolic compounds determinate different functional properties (29); hence, the total phenolics content in plants is not very informative indicator of their possible antioxidant capacities (31). These results agreed with those ones observed by Shahidi and Naczk (37) who reported that differences in antioxidant activities of plants extracts could be attibuted to different chemical structures of the phenolic compounds as well as on their derivatives. In general, phenolics free radical scavenging and antioxidant potency depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules, and is too affected by other factors, such as glycosylation level, or the presence of H-donating groups (-NH, -SH), to name a few.

Oxidative stress is amongst the principal causes of cancer-related death, and antioxidants such as flavonoids have emerged as potential chemoprevention candidates for cancer treatment (44). With this respect, the highest accumulation of phenolic compounds in *H. scoparia, R. raetam*, *Z. lotus* and *C. schoenanthus* extracts (ethanolic or aqueous) and their important antioxidant activity confer to these species a strong anti-proliferative activity. These results are in good agreement with previously studies confirming that phenolic compounds possess antioxidant and anticarcinogenic activities, particularly by inhibiting cancer cell proliferation (32,44). For instance, Zhang and colalborators (45) reported that the polyphenols isolated in strawberry (*Fragaria* sp.) including kaempferol, quercetin, anthocyanins, coumaric and ellagic acids, were demonstrated to inhibit the growth of human breast (MCF-7), colon (HT-29), and prostate (DU-145) tumour cell lines. A case-control study involving women from Klang Valley and Selangor, Malaysia, shown that good antioxidant consumption can reduce oxidative stress and consequently prevent breast cancer risk. On the other hand, it should be noticed that some cancer deseases develop in a highly oxidative state due to the decrease in enzymatic antioxidant defence. Ultimately, phenolic compounds, as potent antioxidant agents, are proven to prevent cancer disease (44).

Final Remarks

Based on their anti-proliferative activities against breast cancer cell lines, TMP such as *H. scoparia, R. raetam, Z. lotus* and *C. schoenanthus* have constituted a rich source of natural secondary metabolites, useful in preventing the onset of different cancer deseases. In conclusion, the present work showed variations in the antioxidant and anti-proliferative capacities and phenolic composition among the five investigated Tunisian ethnomedicinal plants.These plants demonstrated important biological activities and contained much more phenolic compounds than many common vegetables and fruits. These findings may confirm that traditional TMP might represent an interesting natural source of powerful antioxidants and chemo-preventive agents. The use of these TMP can emphasizes disease prevention and whole-body homeostasis instead of disease-oriented therapy.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Compliance

This article does not contain any studies involving human participants or animals performed by any of the authors.

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