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Screening for Cytotoxic and Antioxidant Activity of Selected Wild Plants at Shafa Badran, Amman, Jordan

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ABSTRACT

This study determined the cytotoxicity, antioxidant activity, total phenolic and flavonoid contents of hydro-ethanolic and aqueous extracts of aerial parts of *Anchusa strigosa Banks et Sol, Cardaria draba, Marrubium vulgare, Sarcopoterium spinosum and Capparis spinosa*. These medicinal plants were collected from Jordan. Hydro-ethanolic and aqueous extracts displayed strong scavenging capacity for DPPH radical; IC_{50} values ranging from 11 to 57, and from 13 to 464 µg/mL, respectively. Total phenolic contents, expressed as gallic acid equivalent, varied from 3.4 to 16 mg/g dry plant in hydro-ethanolic extract and from 1.8 to 15.1 mg/g in aqueous extracts. Total flavonoids in the same extracts, expressed as quercetin equivalent, ranged from 2.55 to 10.1 and from 0.68 to 9.4 mg/g dry plant, respectively. There was a strong correlation between antioxidant activities and phenolics for aqueous extracts (r= 0.893), but moderate correlation for hydro-ethanolic ones (r=0.581) implying the contribution of other non-phenolic compounds to the antioxidant activity in the latter. Both hydro-ethanolic and aqueous extracts of *Sarcopoterium Spinosum* showed the highest antioxidant activity, reducing power, phenolic, and flavonoid contents. Therefore, *Sarcopoterium Spinosum* is a promising candidate for antioxidants from a natural sourceFurthemore. Hydro-ethanolic extracts of all plants were not toxic to HCT116 or SW480 cell-lines.

Keywords: Jordan, Antioxidant, Cytotoxicity, DPPH, Phenolics, Flavonoids.

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INTRODUCTION

The human body produces reactive oxygen species (ROS) as part of its normal biological activities. However, when their production exceeds the body's ability to eliminate them oxidative stress occurs, leading to damage or alteration of vital regulatory systems in the body. ROS has been reported to contribute to the causation of many diseases including atherosclerosis, arthritis, cardiovascular disorders, Alzheimer, aging, and cancer [1, 2].

Studies have shown that oxidative stress in biological systems can be prevented by dietary antioxidants which serve as reactive oxygen scavenger molecules [3]. Dietary antioxidants are either chemically synthesized or naturally obtained from plants rich in phenolic and flavonoid compounds. The toxic effects of synthetic antioxidants [4] necessitated the search for safer natural alternatives of dietary antioxidants especially from botanical sources.

Medicinal plants have been used for a long time to cure ailments [5, 6] as well as sources of Antioxidants. Antioxidant activity of medicinal plants is attributed to their phenolic and flavonoid contents [7, 8]. Jordan has more than 2500 wild plant species [9], this diversity in plant life motivated researchers to seek antioxidants and potential anti-cancer drugs from natural sources [9, 10].

The objective of this investigation is to contribute to Jordan's medicinal plant literature by investigating the antioxidant activity, phenolic and flavonoid contents of 5 medicinal plants and by evaluating their cytotoxicity against HCT116 and SW480 cell-lines. The plants were collected from Shafa Badran, Amman. They are: *Anchusa strigosa Banks et Sol* of the *Boraginaceae family, Cardaria draba L.* of the *Brossicaceae family, Marrubium vulgare L* of the *Lamiaceae family, Sarcopoterium spinosum L. Spach* of the *Rosaceae family* and *Capparis spinosa* of the *Capparidaceae family.* All these plants are reported by Qasem [5] to have been used as medicinal plants in Jordan and elsewhere. The antioxidant activities and total phenolics of the extracts of *Anchusa strigosa* and *Marrubium vulgare* were reported by Alali *et al.* [9], and that of *Sarcopoterium spinosum* were reported by Al-Mustafa and Al-Thunibat [11] and Al-Farrayeh [12] but under different conditions than the ones we are studying. However, to our best knowledge, there are no reports on the flavonoid contents of any of these five plants, nor on their cytotoxicity against HCT116 or SW480 cell lines. That is why they have been taken as the focus of this study.

MATERIAL AND METHODS

Chemicals and equipment

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), quercetin, gallic acid, sodium carbonate, Folin-Ciocalteu, dipotassium hydrogen phosphate, potassium dihydrogen phosphate were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade Methanol was from Tedia (OH, USA), sodium nitrite from Scharlau (Barcelona, Spain). Aluminium chloride and ferric chloride from SD fine chemicals (Mumbai, India), trichloroacetic acid from Qualigen Fine chemicals (Mumbai, India). Hydrochloric acid from Biosolve BV (France). Potassium ferricyanide, sodium hydroxide and ascorbic acid were bought from GCC diagnostics (Deeside, UK). Spectrophotometric measurements were performed by T60 U-Visible spectrophotometer from PG Instruments Limited (UK), the incubator and the water bath were from Memmert (Schwabach, Germany). Centrifugation of the samples was performed using Z326 centrifuge from Hermle Labortechnik GmbH (Wehingen, Germany)

Plant material

The wild plants were collected from the same habitat in one day in April 2013 during the flowering season in the Shafa Badran suburb of Amman, Jordan. They were later identified by both Dr. Khaled Abulaila, Researcher Botanist, Herbarium Keeper Plant Genetic Resources/Directorate of Biodiversity at the National Center for Agricultural Research and Dr. Barakat E. Abu-Irmaileh from the Department of Plant Protection, Faculty of Agriculture, the University of Jordan. Voucher specimens were stored in the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Zarqa University. The whole plants except for roots (aerial part of the plant) were air-dried at room temperature in the shade until constant weight was obtained. Plant parts were ground to a fine powder, and were kept at room temperature but protected from exposure to light.

Extraction process

All plant extracts were prepared by infusion in either boiling deionized water or 70% ethanol. The ratio of plant to extraction solvent was 1:10 (mass of plant extracted ranged from 10-20 g). Plant infusions were kept overnight at room temperature, then filtered and centrifuged. The solvent was vacuum dried and the dry extracts were weighed and protected from exposure to light and stored in the refrigerator for subsequent analysis.

Assay methods

Cell lines

Human colorectal cancer (CRC) cell lines (SW480 and HCT116) were a generous gift from Dr. Rick F. Thorne (University of Newcastle, Australia). The cells were cultured in DMEM containing 10% fetal calf serum (Bio Whittaker, Verviers, Belgium).

Cell viability assay

Hydro-ethanolic extracts were tested for their cytotoxic effect against SW480 and HCT116 cell lines. Appropriate masses of dry extracts were dissolved in DMSO then diluted to different concentrations (0-200 µg/mL) with culture buffer. Cytotoxicity of the extracts to colorectal cancer cells was determined by the MTT assay [13]. Briefly, cells were seeded at 5000/well onto flat-bottomed 96-well culture plates and allowed to grow for 24 hours before the desired treatment. Cells were then incubated for 72 hours with 200 µl of different extract concentrations (0 to 200 µg/ml). Cells were then labeled with MTT from the Vybrant MTT Cell Proliferation Assay Kit (Molecular Probes, Eugene, UR) according to the manufacturer's instructions and the resultant formazan was solubilized with DMSO. Absorbance was read in a microplate reader at 540 nm.Then the concentration of extract to cause 50% inhibition (IC_{50}) of biological activity of cancer cells was calculated.

Free radical scavenging ability by DPPH radical (1,1-diphenyl-2-picrilhydrazyl)

The radical scavenging assay was carried out quantitatively in accordance with Blois [14], with minor modification. Stock solutions of dry plant extracts were prepared in methanol. DPPH methanol solution (2.5 ml of 54 µM concentration) was placed in screw-cap test tubes wrapped with aluminum foil, then diluted plant extracts (2.5 ml) of known concentrations (5–2500 µg/ml) were added. The mixtures were vortexed for a few seconds then the tubes were incubated in a water bath at 37 °C for 30 min. At the end of the incubation period absorbance was measured at 517 nm. Ascorbic acid was used as positive control. Methanol was used as DPPH solution blank. To account for any inherent absorbance from the extract at the working wavelength, blank extract solutions similar in concentrations to those tested for their scavenging activity against DPPH solutions were also incubated in the water bath and measured at the same wavelength. The absorbance values of these controls were subtracted from the corresponding absorbance of the DPPH/extract concentration. All measurements were performed in triplicates. DPPH scavenging effect was calculated from the following equation:

$$\% \text{ DPPH scavenging effect} = \frac{(A_o - A_1)x100\%}{A_o}$$

Where A_o is the absorbance of DPPH solution, A_1 is the absorbance of DPPH after the reaction with the plant extract. The 50% scavenging effect values (IC_{50}) were calculated, from a plot of % DPPH inhibition against the concentrations of extracts, utilizing ED50 plus v1.0 software. Results were reported as average values ± SD.

Reducing power assay

The reducing powers of the aqueous and hydro-ethanol extracts of the plants were determined as described by Yen and Chen [15]. Plant extracts (2.5 mL) having concentrations ranging from 0.25 to 50 mg/mL were mixed with 2.5 ml of 0.2 M phosphate buffer at pH 6.6, and 2.5 ml potassium ferrocyanide (1% w/v). The mixture was incubated in a water bath at 50ºC for 20 minutes, then 2.5 mL of trichloroacetic acid (10% w/v)

were added and the mixture was centrifuged at 3000 rpm for 10 minutes. A portion of the supernatant (0.5 ml) was mixed with 1 ml of distilled water and 0.5 ml of ferric chloride (90.1% w/v). The absorbance was measured at 700 nm against a blank which lacks the extract. Ascorbic acid was used as standard. All assays were carried out in triplicates.

The extract concentration providing 0.5 absorbance unit was calculated from a plot of the absorbance against extract concentrations.

Determination of total phenolic contents

The total phenolic contents in the plant extracts were measured as described by Singleton *et al*. [16] with minor modification. Stock solutions of the plant extracts were prepared in methanol then working solutions were diluted with deionized water (to avoid precipitation of sodium carbonate). Hydro-ethanolic or aqueous extracts (0.5 mL having concentrations ranging from 0.210 to 1.340 mg/mL and from 0.246 to 2.720 mg/mL, respectively) were mixed with 2.5 mL of Folin-Ciocalteu (10% v/v) reagent and allowed to react for 6 min at room temperature, protected from light, then sodium carbonate (2.0 ml of 7.5% w/v) was added and the reaction was allowed to proceed for 40 minutes. During the course of the reaction a blue color developed. Its absorbance was measured at 760 nm. Experiments were carried out in triplicates to ensure reproducibility. For each run a plant extract having the same concentration as that analyzed for total phenolics but lacking Folin-Ciocalteu reagent served as control. A calibration curve was constructed for gallic acid under similar conditions using concentrations ranging from 4.1 to 32.6 μg/mL. A plot of absorbance vs concentration produced a straight line. The concentrations of total phenolic compounds in each extract were calculated from the gallic acid equation, and the results were expressed as mg gallic acid equivalent per gram dry plant (mg GE/g DW).

Determination of total flavonoid contents

The total flavonoids were measured as described by Sahu and Saxena [17] with minor modification. Briefly, plant extract (0.5 ml of known concentration) was added to 2 ml of deionized water followed by the addition of 0.15 ml of $NaNO_2$ (5% w/v), then after 6 min 0.15 ml of $AlCl_3$ (10% w/v) was added and allowed to react for another 6 min at room temperature. Finally, 2 ml of NaOH (4% w/v) and 0.2 ml distilled water were added. The absorbance was measured at 510 nm. For each flavonoids determination analysis, a control was also analyzed. The control consisted of similar concentration of plant extract, but the volumes of $NaNO_2$, $AlCl_3$ and NaOH were replaced with equal volumes of distilled water. Concentrations within the linear range of quercetin absorbance were used to construct the calibration curve. Flavonoid contents were estimated as mg of quercetin equivalent/g dry plant (mg QE/g DW).

Statistical analysis

The direction and magnitude of correlation between variables was achieved by analysis of variance (ANOVA) and quantified by the Pearson correlation factor ''r'' using Excel software. P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Cytotoxicity

The cytotoxicity of hydro-ethanolic plant extracts against human colorectal cell lines SW480 and HCT116 were evaluated by micro-culture tetrazolium assay (MTT). The effective doses were calculated from dose-response curves; IC_{50} data are presented in Table (1). The highest toxicity value was shown by *Anchusa strigosa* against HTC116 cell line. However, according to the U.S. National Cancer Institute a crude extract is considered cytotoxic if it has an IC_{50} value <30 μg/mL after 72-hour exposure [18]. The data in Table (1) show that all of the extracts have higher IC_{50} than 30 μg/mL, hence they are non-toxic to either of the two cell lines. Other researchers from Jordan and elsewhere in the world studied the cytotoxic effects of some of these plants against different cell lines and found them to be non toxic as well. For example Abu-Dahab and Afifi [10] reported high IC_{50} value (111.05 μg/mL) of ethanolic extract of *Cardaria draba* against MCF-7cell line. Al-Khatib [19] found the leaf and root extracts of *Anchusa strigosa* not toxic to MCF-7, MDA-MB-231 or T-47D cell lines.

Belayachi *et al.* [20] found *Marrubium vulgare* from morocco not toxic (IC_{50}> 50 µg/mL) to SW480 cells; they all concur with our findings. Aljaiyash *et al.* [21], however, reported that ethanolic extract of *Capparis spinosa* collected from Libya is toxic (IC_{50} = 18.8 µg/mL) to HCT116 cell line.

Table (1): IC_{50} of hydro-ethanolic extracts against HCT116 and SW480 colorectal cancer cells.

Plant extract	HTC116	SW480
	IC_{50} (µg/ml)	
Anchusa strigosa	186	252
Cardaria draba	237	412
Marrubium vulgare	257	R
Sarcopoterium spinosum	279	275
Capparis spinosa	*>500*	-

Antioxidant activities of extracts

The antioxidant activity of the extracts was investigated by two different methods having different mechanisms. The DPPH free radical (DPPH•) method measures the scavenging ability of the extract while the Ferric-reducing power method measures the total reducing capacity of the extracts.

DPPH method

A plant extract rich in antioxidant components provides a hydrogen radical (H•) to DPPH• radical thus terminating the chain reaction and protecting vital molecules from being damaged. The stoichiometric decrease in the absorbance of DPPH• upon its reaction with a hydrogen radical provided by the extract is measured by a UV-Vis spectrophotometer at 517 nm. In this investigation, the percentage inhibitions of DPPH at different extract concentrations is calculated and plotted, then from the plot a linear regression curve is established to calculate the amount of sample needed to cause a 50% decrease in the absorbance of DPPH (IC_{50}). Table (2) displays the calculated IC_{50} values for the DPPH scavenging ability of both hydro-ethanolic and aqueous extracts. The extracts display a wide range of antioxidant capabilities, with hydro-ethanolic extracts having better antioxidant activity than the aqueous extracts. This is most likely due to the different polarities of the two solvents which resulted in extracting different constituents of the plants. *Sarcopoterium spinosum* displayed the highest antioxidant activities in both hydro-ethanolic (11±2 µg/ml) and aqueous extracts (13±1 µg/ml). All of the plants possess lower antioxidant activity in comparison to ascorbic acid (2.6 µg/mL).

Table (2): IC50 of DPPH scavenging effect of hydro-ethanolic and aqueous extracts

Plant extract	DPPH Scavenging IC_{50} (µg/ml)	
	Hydro-ethanolic extract	*Aqueous extract*
Anchusa strigosa	57± 1	464 ± 11
Cardaria draba	88 ± 2	191±7
Marrubium vulgare	121± 8	160± 1
Sarcopoterium spinosum	11 ± 2	13 ±1
Capparis spinosa	76± 2	209±11
Ascorbic acid	2.60± 0.02	

Several researchers investigated the DPPH scavenging activity of similar plants collected from Jordan as well as from other countries. However, many of them prepared extracts from different parts of the plants, used different extraction solvents and expressed their values in different units. Therefore, for a meaningful comparison of results we quoted the results of those researchers who conducted their experiments and analysis under comparable conditions to ours. Baghiani *et al.* [22] reported IC_{50} value higher than 75 mg/mL from ethanolic extract of Algerian *Capparis spinosa*, while Alsabri *et al.* [23] reported a value of 57.75 mg/mL for Libyan *Capparis spinosa*. Both plants have much lower antioxidant activity than *Capparis spinosa* (0.076 mg/mL) of this investigation. Younes *et al.* [24] observed that the IC_{50} of methanolic extract of Algerian *Cardaria draba* was achieved at 0.53 mg/mL, while in this investigation it was achieved at a much better value of 0.088 mg/mL. The methanolic extract of Tunisian *Marrubium Vulgare* reported by Aouadhi *et al.* [25] has an IC_{50} of 35 mg/mL, while those reported by Boulila *et al.* [26] differ by orders of magnitude (0.093 to 0.131

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mg/mL). We obtained an IC_{50} value of 0.121 mg/mL for the hydro-ethanolic extract of *Marrubium vulgare* which is comparable to that obtained by Boulila *et al.* [26]. As for the *Sarcopoterium spinosum*, Loizzo *et al.* [27] reported IC_{50} values of 78.9 µg/mL and 85.6 µg/mL from ethanolic extracts of the plants collected from Italy and Lebanon, respectively. Al-Farrayeh [12] reported an IC_{50} value of (300 µg/mL) for methanolic extract of *Sarcopoterium spinosum* that was collected from a different area in Jordan. This IC_{50} value is an order of magnitude higher than the one we found in this investigation for the hydro-ethanolic extract (11µg/mL). It is clear that there is a large variation in the antioxidant activities of similar plants from different countries as well as from the same country. These differences could be attributed to a number of factors, chief amongst which are: plant habitat, extraction temperature, extraction solvent, age of the plant, storage after drying, time of collection, enzymatic and non-enzymatic process that may occur during the drying process [28].

Ferric reducing power

The reducing power method was used to calculate the ability of plant extracts to reduce Fe (III) to Fe (II). Fe (III) reduction is often used as an indicator of electron-donating activity which is an important mechanism of phenolic antioxidant action [29]. The final reaction product in this method is a colored complex of Fe^{3+}/Fe^{2+} which has maximum absorbance at 700 nm. The higher the absorbance of the complex, the higher the reducing power of the tested compound is and the better antioxidant it is. The results of the reducing power of the hydro-ethanolic plant extracts are shown in Figure (1). It can be seen that the reducing power of extracts increases with increasing concentrations and that *Sarcopoterium spinosum* has the highest reducing power. This same plant also has the highest scavenging ability as evident in the DPPH data. The concentration of each extract that would give 0.5 absorbance unit is calculated from the reducing power graphs, and the results are tabulated in Table (3). It can be witnessed that plants' reducing powers of hydro-ethanolic extracts are much better (0.5 absprbance is achieved at 0.64 mg/mL to 5.32 mg/mL) than those of aqueous extracts (0.5 absprbance is achieved at 1.02-90 mg/mL). This result is consistent with DPPH data. In comparison with ascorbic acid (16 µg/mL), all plants have much lower reducing power

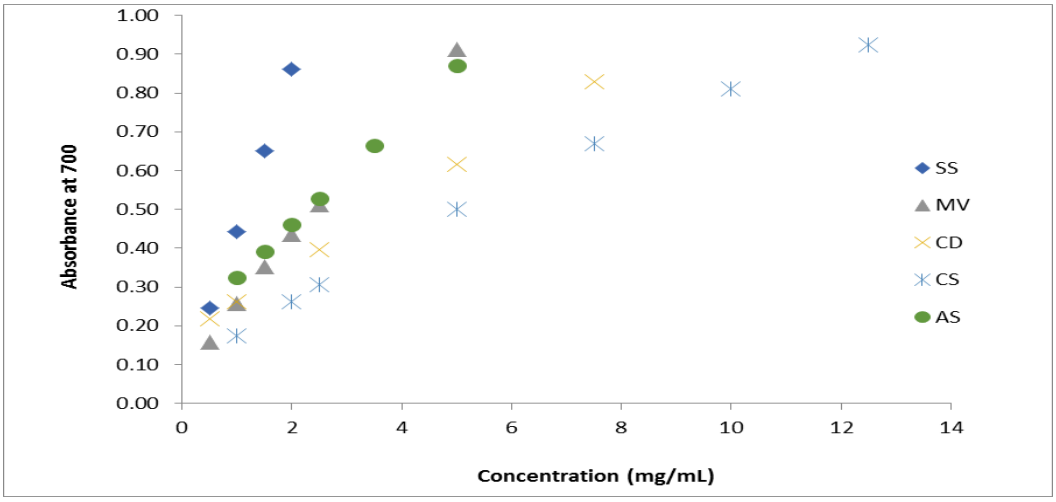

Figure (1): Ferric reducing power of hydro-ethanolic extracts of *Sarcopoterium Spinosum (SS), Marrubium Vulgare (MV), Cardaria Draba (CD),Capparis Spinosa and Anchusa Strigosa (AS)*

Table (3): Concentrations of extracts at 0.5 absorbance unit vs. ascorbic acid

Plant	Hydro- ethanolic extract	Aqueous extract
	Concentration (mg/mL) at 0.5 absorbance unit	
Anchusa strigosa Banks et Sol.	2.29±0.07	90±10
Cardaria draba	3.7±0.2	7.2±0.1
Marrubium vulgare	2.5±0.1	340±50
Sarcopoterium spinosum	0.34±0.02	1.02±0.03
Capparis spinosa	5.59±0.04	13.0±0.4
Ascorbic acid	0.0163±0.0005	

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Total phenolic contents (TPC)

Phenolic compounds are produced in plants as secondary metabolites and they play an important role as antioxidants in many biological activities [30]. Therefore, in order to evaluate the extracts' antioxidant activities their total phenolic contents were measured. The values are calculated from gallic acid calibration curve (Figure 2), expressed as mg of gallic acid equivalent (GE) per g of dry plant (DW), and presented in Table (4). Total phenolics range from 4.6 to 20.6 mg GE/g in hydro-ethanolic extracts, but from 1.8 to 15.1 mg GE/g in aqueous extracts. The differences between total phenolics in hydroethanolic and aqueous extracts are not significant ($p>0.05$). Both types of extracts of *Sarcopoterium spinosum* contain the highest phenolic compounds. The extracts of this plant also displayed the highest scavenging activity and the highest reducing power as well, a strong indication that its antioxidant activity is due to its phenolic contents. However, despite the fact that hydro-ethanolic extract of *Marrubium vulgare* has higher total phenolics (9.8 mg GE/g) than *Anchusa strigosa, Cardaria draba and Capparis spinosa*, it showed lower antioxidant activity (IC_{50} = 121μg/mL) than either of them. This may be attributed to the fact that Folin-Ciocalteu method measures the number of oxidizable phenolic groups, which vary among the different classes of phenolic compounds present in plant extracts. A similar discrepancy between TPC content and antioxidant activity was reported by Alali *et al.* [9] and Tawaha *et al.* [31].

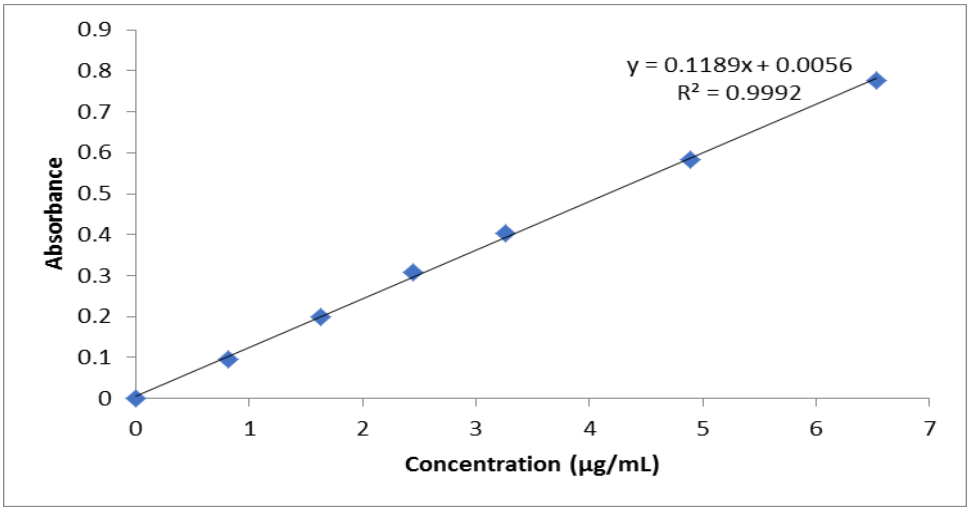

Figure (2): Gallic acid calibration curve

It appears that these plants have low total phenolics regardless of which part of the plant was extracted and which type of extraction solvent was used. For example, Alali *et al.* [9] found that total phenolics extracted by methanol or water from aerial parts of *Anchusa strigosa* was 6.1 and 10.5 mg GE/g DW, respectively. They also found total phenolics of *Marrubium Vulgare* to be 11.8 and 14.0 mg GE/g DW in methanol and water extracts respectively, as well. These values are higher than the ones we obtained as can be seen in Table (4). Al-Mustafa and Al-Thunibat [11] studied methanolic and aqueous extracts from the roots of *Sarcopoterium spinosum* and reported TPC values of 13.2 and 8.7 mg GE/g DW, respectively. Hydro-methanolic extract of aerial parts of the Algerian *Capparis spinosa* [22] contains a higher amount of TPC (14.86 mg GE/g DW) than what we obtained in this investigation (4.6 mg GE/g DW).

Flavonoid contents

Flavonoids are a sub-class of plant phenolics, therefore, they play an important role in antioxidant activities of plants. Since quercetin is a common flavonoid found in plants, quantification of flavonoid contents was carried out in this investigation on the basis of a standard curve of quercetin ($y=0.008x+0.003$, $R^2 = 0.993$, where y is absorbance and x is concentration in μg/mL). The results are expressed as mg quercetin equivalent per g of dry plant (QE/g DW) and presented in Table (4). Flavonoid concentrations in hydro-ethanolic extracts have been found to range from 2.55 to 5.7 mg QE/g DW and from 0.68 to 9 mg QE/g DW in aqueous extracts. The difference between flavonoid contents in hydro-ethanolic and aqueous extracts is not statistically significant ($p>0.05$). *Sarcopoterium spinosum* contains the highest amount of flavonoids in both extracts, a fact

consistent with their high phenolic contents. To our best knowledge, there are no reported flavonoid values of any of these plants form Jordan. Flavonoid values in similar plants from other parts of the world vary greatly depending on the sampling location and extraction solvent. For example, a study conducted by Bhoyar *et al.* [32] on the methanolic leaf extracts of *Capparis spinosa* collected from cold arid desert of trans-Himalayas reported flavonoid values that range from 2.69 to 6.96 mg QE/g DW. However, hydro-ethanolic extract of *Capparis spinosa* leaves collected from Tunisia were reported [33] to have much higher value (59.73 QE/g DW).

Table (4): Phenolic and Falvonoid contents of hydro-ethanolic and aqueous extracts

Plant	Hydro-ethanolic extract		Aqueous extract	
	Phenolics mg GE/g DW	*Flavonoids mg QE/g DW*	*Phenolics mg GE/g DW*	*Flavonoids mg QE/g DW*
Anchusa strigosa	7.0±0.1	3.4±0.6	1.8±0.2	0.68±0.04
Cardaria draba	5.87±0.05	3.3±0.2	5.2±0.1	5.5±0.2
Marrubium vulgare	9.8±0.3	4.6±0.4	6.6±0.2	3.17±0.09
Sarcopoterium Spinosum	16.0±0.4	5.7±0.2	15.1±0.6	9±1
Capparis spinosa	4.6±0.1	2.55±0.04	6.11±0.09	3.35±0.05

Relationship between antioxidant activity, phenolics, and flavonoids

The results in Table (5) show a moderately significant linear inverse correlation (r = -0.58) between scavenging activities ($DPPH_{IC50}$) and total phenolics on the one hand, but weak correlation with flavonoid contents (r = -0.411) in hydro-ethanolic extracts. This is a good indication that other non-phenolic compounds may have contributed to the antioxidant activity in these extracts. Other researchers [34, 35] have reported moderate correlation between radical scavenging activity and total phenolics, but weak correlation with flavonoids in plant extracts. They entertained the possibility that the radical scavenging activity of an extract cannot be totally predicted on the basis of its total phenolic contents; other natural compounds contribute to the scavenging activity. By contrast, the aqueous extracts produced strong inverse correlation (r = -0.893) between scavenging activities and total phenolics. A similarly strong inverse correlation (r = -0.892) was found between antioxidant scavenging activities and flavonoids. This reflects that phenolic compounds play a major role in the scavenging activity of aqueous extracts. We also analyzed the correlation between phenolic compounds and flavonoids in both hydro-ethanolic and aqueous extracts. The correlation proved to be highly significant (r = 0.977 and 0.914, respectively).

Table (5): Correlations between scavenging activities, total phenolics and flavonoids in hydro-ethanolic and aqueous extracts

Relationship between Variables	Hydro-ethanolic extract		Aqueous extract	
	Correlation Coefficient (r)	*Coefficient of determination (r^2)*	*Correlation Coefficient (r)*	*Coefficient of determination (r^2)*
Phenolics/$DPPH_{IC50}$	-0.581	0.337	-0.893	0.796
Flavonoids/$DPPH_{IC50}$	-0.411	0.169	-0.892	0.795
Flavonoids/Phenolics	0.977	0.955	0. 914	0.836

CONCLUSIONS

Without a precedent, this investigation reported total flavonoid values (as quercetin equivalents) in the 5 Jordanian plants under investigation. Furthermore, the hydro-ethanolic extracts of these plants displayed high DPPH scavenging activity. In particular, the extract of *Sarcopoterium spinosum* showed very good scavenging capability when compared to standard ascorbic acid. Therefore, the extracts of these plants may play a protective role against oxidative damage caused to cellular macromolecules. Despite their high scavenging activity, their total phenolic and flavonoid contents are low. This may indicate the contribution of other non-phenolic compounds to their antioxidant activity. None of the plants is toxic to HCT 116 or SW 480 cancer cell-lines.

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