Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project

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Abstract:
As part of an International Cooperative Biodiversity Groups (ICBG) program to study Jordan's biodiversity, the relative levels of antioxidant activity and the total phenolic content of aqueous and methanolic extracts of a total of 95 plant species, all of Jordanian origin and those collected at random, have been measured. The total phenolic content of aqueous and methanolic extracts of the investigated plant species ranged from 4.4 to 78.3 mg and from 2.1 to 52.8 mg gallic acid equivalents g^{-1} dry weight, respectively, while the total antioxidant capacity ranged from 20.0 to 916.7 and from 15.1 to 915.6 μmol Trolox equivalents g^{-1} dry weight, respectively. Based on this collection, approximately 5% of assayed plants showed high levels of antioxidant activity. There was a significant linear correlation between antioxidant activity and total phenolic content for aqueous and methanolic extracts, suggesting that phenolic compounds were the predominant antioxidant components in the investigated plant species. Interestingly, a few of the collected plants had high-antioxidant activity yet “low” phenolic content includes *Ceratonia siliqua* and *Viscum cruciatum*. These plants may serve as sources of antioxidants with new chemotypes.
Keywords: Antioxidants; Total phenolic content; Jordanian flora; Free radicals

Article:

1. Introduction

The Hashemite Kingdom of Jordan (Jordan) lies in the heart of the Middle East, about 100 km from the southeastern coast of the Mediterranean Sea, between latitudes 29°-33°N and longitudes 35°-39°E. It covers a wide range in elevation, from the lowest place on earth, 400 m below sea level near the Dead Sea, to plateaus of more than 1700 m above sea level near the Jebel Rum. This dynamic typography and climate places the geopolitical borders of Jordan at the junction of four biogeographical areas: the Mediterranean, the Irano-Turanean, the Saharo-Arabian, and the Tropical or Sudanian (figure 1) 1, 2. As such, Jordan encompasses a unique and rich habitat for a wide variety of plant life, most of which can be collected and studied efficiently in a relatively small land area 3, 4. More than 2500 wild plant species from 700 genera are found in Jordan, and of these, there are approximately 100 endemic species, 250 rare species, and 125 very rare species 5.

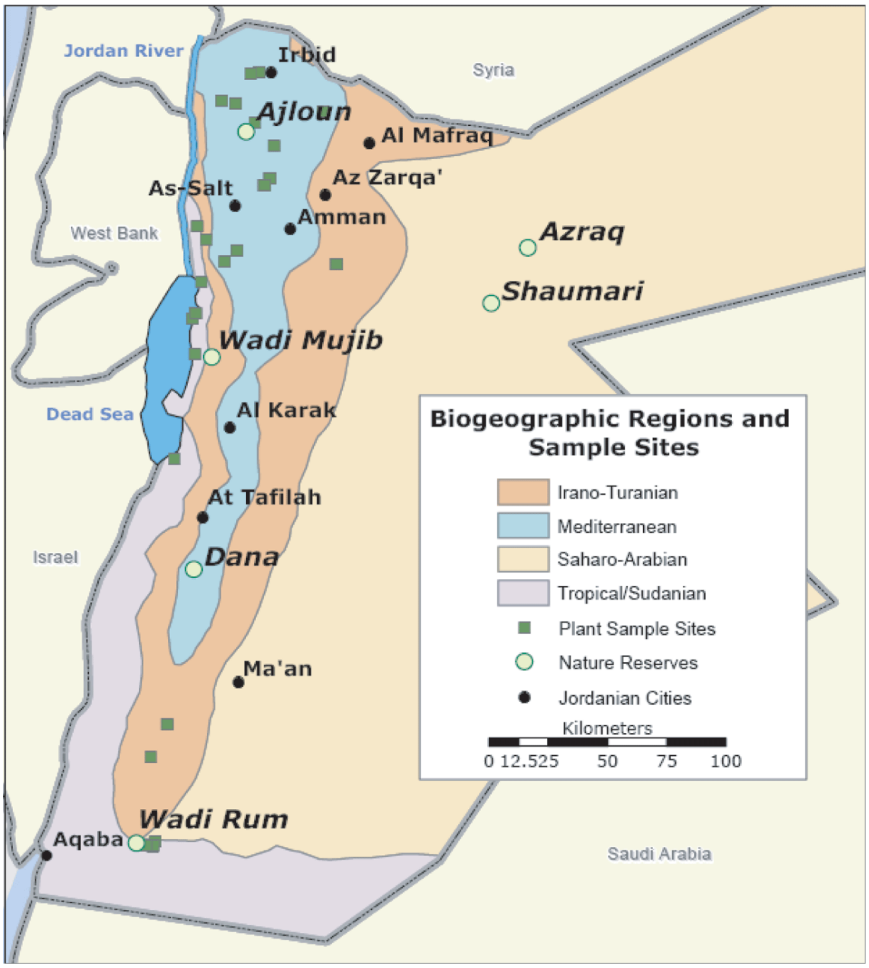

Figure 1. Jordan's biogeographic regions and plant collection sites.

In 2003, Research Triangle Institute (RTI) in collaboration with Jordan University of Science and Technology, and Virginia Polytechnic Institute and State University, received a planning grant from the Fogarty International Center of the US National Institutes of Health, as part of the International Cooperative Biodiversity Groups (ICBG) program, to promote sustainable economic growth, biodiversity conservation, and training and education in Jordan through natural products-based drug discovery. The team focused on plants and nonobligate predatory soil bacteria as potential sources for new drug leads, in particular, to compounds, acting as anticancer agents, antibiotics, and/or modulators of the central nervous system. These studies are unique, in that very few plants of Middle Eastern origin, particularly those of Jordan, have been explored for pharmaceutical leads, especially from the extreme environments found in the deserts and near the Dead Sea. Since 80% of plants found in Jordan are common with other countries of the Middle East, Jordan's flora may be considered as the representative of the larger region 5.

The team collected over 120 plant samples, roughly 25-30 from each of the four biogeographic regions of Jordan (figure 1). At this early stage in the program, random sampling was chosen (as opposed to targeted collections) to demonstrate proof of concept that Jordan was a viable source of plants with promising biological activities. The sampling strategy included a linear transect of Jordan, focusing on the western portion of Jordan near the Jordan Valley, to assay the biodiversity and pharmaceutical potential of all four biogeographic regions. Several of the collected samples displayed promising biological activities, some of which have been reported previously 6-8 and some of which are currently under investigation. Herein, we present the antioxidant activity and total phenolic content for 95 plant samples, and statistical correlations are drawn between such activities for both aqueous and methanolic extracts.

Superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$) 9-11 are extremely reactive and potentially damaging transient chemical species. Tissue damage, resulting from the imbalance between reactive oxygen species generating and scavenging systems (oxidative stress), has been implicated in the pathology of a number of disorders, such as atherosclerosis, ischemia-reperfusion injury, cancer, malaria, diabetes, inflammatory joint disease, asthma, cardiovascular diseases, cataracts, immune system decline, and could play a role in neurodegenerative diseases and aging processes 10-18.

One of the most common and reliable methods to assay free radical scavenging capacity is the Trolox equivalent antioxidant capacity (TEAC) assay, which was first described by Miller *et al*. (1993) and improved by Re *et al*. 19-20. It is a decolorization assay in which the pre-formed blue/green radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) is generated by oxidation of ABTS with potassium persulfate and reduced in the presence of hydrogen-donating antioxidants. TEAC assay values of many compounds and plant extracts have been reported in the literature 21-23. Since this assay is rather simple, operationally, it can be used in screening studies, especially when $ABTS^+$ reacts rapidly with antioxidants within 30 min. Moreover, $ABTS^+$, which is soluble in both aqueous and organic solvents, is not affected by ionic strength, and is viable over a wide pH range 24. Due to these favorable qualities it can be used with a wide range of matrices to measure both hydrophilic and lipophilic antioxidant capacities of extracts and body fluids, including typical antioxidants, such as phenolic compounds, and more atypical antioxidants, such as glutathione, uric acid, ascorbic acid, α-tocopherol, β-carotene, and lycopene 20.

The Folin-Ciocalteu colorimetric assay has been used for many years as a measure of total phenolics in natural products 25. Its basic mechanism is an oxidation/reduction reaction in which the oxidation of phenols by Folin-Ciocalteu reagent (phosphomolybdic-phosphotungstic acid) yields a colored product with λ_{max} at 765 nm. It is a simple, sensitive, and precise method and can be useful in characterizing and standardizing botanical samples, using gallic acid as reference standard 25.

Many research groups have examined plants as potential sources of natural antioxidants 26-31. Our research team has capitalized upon access to unique, and largely understudied, plant species from diverse, and at times, extreme habitats and coupled this with the use of two well-established assays for antioxidant activity and total phenolic content, to evaluate the antioxidant activity and total phenolic content of a random sampling of the flora of Jordan. Of the hundreds of previous studies on antioxidant activity of higher plants, few researchers have examined the plants that can be found in the Middle East. In doing so, we have identified a suite of plant species with the antioxidant activity that warrants further investigation, especially for a series of plants wherein the activity could not be attributed to compounds with a phenolic moiety. Moreover, we have established a baseline of antioxidant activities for approximately 3% of the flora of Jordan.

2. Results, discussion, and conclusions

2.1. Antioxidant activity

The total antioxidant capacity of the aqueous and methanolic extracts of the individual plant species ranged from 20.0 to 916.7 μmol and from 15.1 to 915.6 μmol TE g^{-1}dry weight for aqueous and methanolic extracts, respectively, with little variation between replicates (table 1). Highest levels of antioxidant activity were obtained with the aqueous and methanolic extracts of the Carob tree, *C. siliqua* L. (Fabaceae); namely 916.7 and 915.6 μmol TE g^{-1} dry weight, respectively and from the aqueous extract of the Nettle desert, *Forsskaolea tenacissima* L. (Urtiaceae), namely 813.6 μmol TE g^{-1}dry weight. The high-antioxidant activity of *C. siliqua*, also known as St. John's bread, may be attributed to its high content of polyphenolic compounds 32, 33. However, with respect to *F. tenacissima*, we believe this is the first report of high-antioxidant activity for this plant, which was collected from a unique habitat in the Sudanian biogeographic region in the southern part of Jordan at an elevation of 493 m. This species flourishes mainly in Wadis (Arabic for “valley”) of hot deserts, as

typified in the lower Jordan Valley and the Dead Sea areas of Jordan 34. Other plants with high-antioxidant activity were: *Pistacia palaestina* Boiss. (Anacardiaceae), *Viscum cruciatum* Sieb. (Loranthaceae), and *Hypericum triquetrifolium* Turra (Hypericaceae) with values of 549.0/590.9, 460.8/590.5, and 457.4/535.5 TE g^{-1} dry weight for aqueous/methanolic extracts, respectively.

Table 1. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of 95 plant species from Jordan

		Antioxidant activity[a] (μmol TE g^{-1} dry weight)		Total phenolic content[b] (mg GE g^{-1} dry weight)	
Family	**Scientific name**	**Aqueous extracts**	**Methanolic extracts**	**Aqueous extracts**	**Methanolic extracts**
For both assays, all data are shown as mean (% variation) from two extract replicates, each analyzed in duplicate.					
[a]Data are expressed as μmoles of Trolox equivalents per g dry weight.					
[b]Data are expressed as mg of Gallic acid equivalents GAE) per g dry weight.					
[c]Positive control for antioxidant activity.					
[d]Positive control for total phenolic content.					
Acanthaceae	*Acanthus syriacus* Boiss.	77.0 (0.9%)	82.7 (4.0%)	15.1 (5.3%)	15.4 (0%)
Aizoaceae	*Aizoon hispanicum* L.	48.3 (3.7%)	15.1 (2.6%)	5.0 (8.0%)	2.1 (14.3%)
	Aizoon canariense L.	38.1 (9.9%)	15.9 (5.0%)	4.4 (22.7%)	3.6 (2.7%)
Anacardiaceae	*Pistacia palaestina* (Boiss.)	549.0 (0.5%)	590.9 (0.3%)	42.5 (5.2%)	52.3 (1.9%)
Apiaceae	*Daucus carota* L.	75.8 (7.0%)	49.0 (3.5%)	16.9 (4.7%)	11.7 (2.6%)
	Ferula communis (L.)	160.7 (1.7%)	86.7 (5.4%)	24.6 (2.0%)	18.4 (2.2%)
	Malabaila secacul (Mill) Boiss.	75.6 (0.9%)	23.6 (13.1%)	13.8 (1.4%)	4.0 (7.5%)
Asclepiadaceae	*Calotropis procera* (Ait)	90.2 (5.3%)	50.8 (2.4%)	19.4 (3.1%)	11.4 (0%)
	Gomphocarpus sinaicus Boiss.	133.3 (1.2%)	63.9 (5.3%)	25.9 (0.8%)	23.8 (7.6%)
Asteraceae	*Aaronsohnia factorovskyi* Warb &Eig.	189.0 (2.3%)	214.9 (1.9%)	29.1 (2.4%)	22.1 (2.7%)
	Achillea santolina L.	72.6(0.3%)	60.2 (0.3%)	20.5 (2.9%)	19.2 (1.0%)
	Carthamus tenuis (Boiss & Blanche) Bornm.	162.9 (4.1%)	61.8 (4.2%)	27.8 (4.7%)	16.2 (5.6%)
	Centaurea ammocyanus Boiss.	85.0 (7.2%)	50.0 (11.0%)	18.1 (8.3%)	10.9 (22.0%)
	Cichorium pumilum Jacq.	72.7 (3.3%)	53.1 (7.3%)	16.1 (5.0%)	10.6 (0.9%)
	Crepis bulbosa (L.)	55.8 (3.9%)	65.0 (0.9%)	11.2 (2.7%)	11.6 (6.0%)
	Dittrichia viscosa (L.) Greuter	269.8 (1.1%)	247.8 (0.9%)	57.3 (8.7%)	43.9 (3.4%)
	Echinops philistaeus & zohary	100.3 (3.4%)	77.8 (4.1%)	24.0 (9.2%)	19.6 (6.1%)
	Gundelia tournefortii L.	63.6 (8.6%)	53.0 (2.8%)	16.0 (9.4%)	11.0 (7.3%)
	Helichrysum sanguineum (L.) kostel.	232.3 (0.5%)	164.8 (3.3%)	41.3 (9.2%)	34.7 (3.2%)
	Iphiona mucronata (Forskal) Ascherson &	65.2 (0.5%)	46.2 (4.8%)	17.3 (2.9%)	12.7 (0.8%)

Table 1. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of 95 plant species from Jordan

Family	Scientific name	Antioxidant activity[a] (µmol TE g^{-1} dry weight)		Total phenolic content[b] (mg GE g^{-1} dry weight)	
		Aqueous extracts	Methanolic extracts	Aqueous extracts	Methanolic extracts
	Schweinf.				
	Matricaria recutita L.	147.6 (0.9%)	142.6 (1.0%)	20.6 (6.8%)	15.1 (3.3%)
	Notobasis syriaca (L.)	64.0 (7.5%)	43.8 (5.5%)	13.7 (5.8%)	8.6 (2.3%)
	Pallenis spinosa (L.) Cass.	50.3 (5.2%)	33.8 (0.3%)	11.5 (8.7%)	7.3 (5.5%)
	Pallenis spinosa (L.) Cass.	55.7 (2.9%)	57.1 (6.0%)	13.5 (3.7%)	10.5 (4.8%)
	Scolymus maculatus L.	70.1 (6.1%)	35.8 (1.4%)	16.6 (10.8%)	9.0 (3.3%)
	Scorzonera syriaca Boiss & Blanche.	69.5 (4.0%)	44.6 (0.2%)	15.1 (9.3%)	11.2 (9.8%)
	Varthemia iphionoides Boiss. & BL.	176.7 (0.6%)	85.3 (1.2%)	33.9 (4.7%)	20.5 (2.4%)
	Varthemia montana (Vahl) Boiss.	56.9 (0.7%)	51.6 (6.2%)	10.2 (5.9%)	11.1 (0%)
Boraginaceae	*Alkanna strigosa* Boiss. & Hohen.	157.9 (2.1%)	104.2 (5.6%)	16.7 (5.4%)	11.0 (5.5%)
	Anchusa italica Retz.	83.3 (1.3%)	88.2 (3.3%)	12.3 (24.4%)	16.2 (0%)
	Anchusa strigosa Banks & Sol.	66.7 (2.5%)	43.6 (1.4%)	10.5 (9.5%)	6.1 (1.6%)
	Asperugo procumbens L.	189.8 (0.6%)	87.5 (2.3%)	26.0 (5.0%)	15.7 (1.3%)
	Echium glomeratum Poiret	148.9 (0.9%)	108.2 (4.5%)	12.8 (19.5%)	13.3 (9.0%)
	Echium judaeum Lacaita	70.2 (8.4%)	71.3 (0.7%)	11.7 (0.9%)	11.5 (6.1%)
	Lappula spinocarpos (Forssk.) O. Kuntze	227.5 (0.5%)	124.4 (1.4%)	34.0 (1.8%)	25.0 (2.4%)
	Nonea melanocarpa Boiss.	180.3 (0.8%)	129.7 (0.7%)	25.1 (6.4%)	22.2 (0.9%)
	Paracaryum rugulosum (D.C) Boiss.	140.6 (1.0%)	98.0 (2.4%)	22.8 (6.6%)	15.4 (5.8%)
	Podonosma oriantalis (L) Feinbrun.	176.1 (2.7%)	96.6 (3.0%)	20.1 (2.5%)	19.4 (1.5%)
Brassicaceae	*Eruca sativa* Mill	64.6 (2.5%)	34.6 (11.6)	19.0 (3.2%)	9.5 (4.2%)
	Sinapis alba L.	49.7 (1.6%)	26.5 (1.1%)	14.5 (0.7%)	8.4 (4.8%)
	Zilla spinosa (L.) Prantl	42.0 (1.0%)	23.3 (6.0%)	7.3 (6.8%)	4.1 (0%)
Caesalpiniaceae	*Cassia italica* (Mill)	79.0 (1.5%)	53.3 (4.7%)	24.0 (0.4%)	16.3 (1.2%)
Capparaceae	*Capparis cartilaginea* Decne	91.8 (4.6%)	81.1 (4.7%)	28.3 (2.8%)	20.8 (2.9%)
Chenopodiaceae	*Hammada eigii* lljin	49.5 (7.9%)	30.9 (5.2%)	11.3 (6.2%)	4.9 (0%)
	Salsola jordanicola Eig	58.9 (1.9%)	34.2 (2.0%)	9.9 (2.0%)	7.2 (0%)
	Salsola vermiculata L.	41.5 (2.7%)	27.7 (4.3%)	7.0 (1.4%)	5.5 (5.5%)
	Salsola vermiculata L.	40.8 (1.2%)	32.6 (8.0%)	7.8 (3.8%)	7.0 (8.6%)
Cistaceae	*Helianthemum lippii* L.	274.2 (2.5%)	176.1 (1.8%)	30.5 (11.5%)	25.0 (6.4%)

Table 1. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of 95 plant species from Jordan

Family	Scientific name	Antioxidant activity[a] (µmol TE g^{-1} dry weight)		Total phenolic content[b] (mg GE g^{-1} dry weight)	
		Aqueous extracts	Methanolic extracts	Aqueous extracts	Methanolic extracts
Crassulaceae	*Umbilicus intermedius* Boiss.	141.8 (4.2%)	74.0 (2.7%)	18.4 (4.3%)	11.9 (0.9%)
Cucurbitaceae	*Bryonia cretica* L.	120.8 (1.3%)	65.0 (6.5%)	22.4 (2.7%)	13.3 (4.5%)
	Bryonia cretica L	170.5 (2.1%)	22.0 (10.5%)	19.6 (2.6%)	5.6 (3.6%)
Dipsacaceae	*Knautia bidens* (sm.) Lindley	143.1 (2.4%)	78.6 (2.4%)	25.7 (5.8%)	17.9 (6.1%)
Ephedraceae	*Ephedra alata* Decne.	46.6 (6.9%)	60.2 (2.3%)	16.2 (2.5%)	11.9 (0.8%)
Fabaceae	*Ceratonia siliqua* L.	916.7 (0.2%)	915.6 (0.2%)	54.2 (3.3%)	52.8 (11.4%)
	Ononis natrix L.	48.0 (5.4%)	64.6 (5.6%)	9.3 (6.5%)	12.3 (1.6%)
	Ononis spinosa L.subsp. antiquorum (L.) Arcangeli	85.9 (4.3%)	47.1 (4.9%)	18.5 (3.2%)	10.6 (15.1%)
	Retama raetam (Forssk).	131.3 (1.8%)	67.9 (4.4%)	26.4 (18.2%)	24.6 (4.1%)
Geraniaceae	*Erodium bryoniifolium* Boiss	45.3 (2.6%)	25.4 (13.0%)	15.1 (3.3%)	10.8 (3.7%)
Ginkgoaceae	*Ginkgo biloba* L.[c]	311.5 (0.4%)	275.6 (0.5%)	39.0(9.7%)	35.3 (2.8%)
Hypericaceae	*Hypericum triquetrifolium* Turra	457.4 (1.7%)	535.5 (0.7%)	44.6 (4.0%)	40.6 (6.4%)
Lamiaceae	*Eremostachys laciniata* (L)Bunge.	85.6 (5.7%)	86.8 (7.0%)	16.2 (42.6%)	19.4 (2.6%)
	Marrubium vulgare L.	76.5 (0.1%)	53.9 (9.0%)	14.0 (2.9%)	11.8 (1.7%)
	Phlomis brachyodon (Boiss.) Zohary	59.6 (3.2%)	60.4 (3.6%)	12.7 (7.9%)	15.2 (4.6%)
	Phlomis brachyodon Boiss.	54.2 (0.2%)	46.2 (13.9%)	9.6 (4.2%)	10.9 (23.0%)
	Salvia dominica L.	29.9 (6.4%)	29.4 (5.8%)	6.7 (3.0%)	6.6 (1.5%)
	Salvia hierosolymitana Boiss.	223.9 (0.6%)	155.3 (0.8%)	33.8 (0.6%)	24.4 (0.8%)
	Salvia spinosa L.[d]	90.4 (4.8%)	74.6 (5.1%)	20.8 (6.3%)	21.0 (2.4%)
	Scutellaria galericulata L.	85.0 (2.2%)	77.5 (6.3%)	18.8 (11.7%)	16.7 (9.0%)
Liliaceae	*Asparagus aphyllus* L.	65.3 (3.2%)	37.8 (5.6%)	15.6 (3.8%)	9.8 (4.1%)
	Scilla hanburyi Baker	81.0 (2.7%)	44.5 (5.2%)	17.3 (0.6%)	6.9 (7.2%)
Loranthaceae	*Viscum cruciatum* Sieb.	460.8 (0.4%)	590.5 (0.2%)	39.7 (6.3%)	36.5 (4.1%)
Malvaceae	*Alcea acaulis* (Cav.) Alef.	20.0 (16.0%)	24.2 (1.7%)	12.3 (10.6%)	5.3 (0%)
Papaveraceae	*Astragalus beershabensis* Eig. et Sam.	65.3 (0.9%)	29.2 (5.5%)	16.3 (1.8%)	11.1 (7.2%)
	Glaucium aleppicum Boiss. et Hausskn. ex Boiss.	161.9 (1.1%)	81.2 (3.6%)	20.6 (9.2%)	18.8 (2.1%)
	Lupinus varius L.	77.0 (0.6%)	69.5 (2.2%)	16.8 (0%)	13.7 (3.6%)
	Roemeria hybrida (L.) DC.	70.5 (2.6%)	58.4 (0.7%)	14.9 (22.8%)	12.6 (0.8%)
Plantaginaceae	*Plantago lanceolata* L.	29.7 (14.1%)	45.3 (8.4%)	7.6 (3.9%)	9.0 (2.2%)

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Family	Scientific name	Antioxidant activity[a] (μmol TE g^{-1} dry weight)		Total phenolic content[b] (mg GE g^{-1} dry weight)	
		Aqueous extracts	Methanolic extracts	Aqueous extracts	Methanolic extracts
Polygonaceae	*Rumex pictus* Forssk	161.2 (3.4%)	60.6 (6.9%)	21.1 (2.8%)	10.9 (9.2%)
Resedaceae	*Ochradenus baccatus* Del.	78.2 (3.6%)	47.8 (4.0%)	20.2 (0.5%)	8.8 (2.3%)
Rhamnaceae	*Ziziphus lotus* (L.) Lam	230.2 (0.7%)	197.7 (2.3%)	30.4 (4.9%)	25.0 (11.2%)
Rosaceae	*Amygdalus arabica* Oliv.	234.9 (3.0%)	197.6 (0.8%)	31.1 (9.0%)	31.8 (0.6%)
	Amygdalus communis L.	140.5 (2.3%)	179.7 (1.5%)	22.7 (2.6%)	21.8 (3.2%)
Rutaceae	*Ruta chalepensis* L.	83.6 (6.3%)	72.6 (1.5%)	21.1 (1.9%)	19.5 (3.6%)
Salvadoraceae	*Salvadora persica* L.	38.7 (3.1%)	32.0 (1.9%)	12.8 (1.6%)	10.1 (2.0%)
Scrophulariaceae	*Kickxia aegyptiaca* (L.) Nabelek	45.7 (3.5%)	38.1 (0.8%)	16.9 (5.3%)	10.7 (1.9%)
	Scrophularia xanthoglossa Boiss.	79.3 (4.9%)	50.1 (6.4%)	18.1 (0.6%)	12.3 (9.0%)
	Verbascum fruticulosum Post	68.4 (1.6%)	46.6 (6.4%)	13.4(1.5%)	11.9 (11.8%)
	Verbascum jordanicum Murb	38.0 (4.7%)	38.4 (7.6%)	9.0 (7.8%)	8.7 (5.8%)
Solanaceae	*Hyoscyamus aureus* L.	59.6 (0.7%)	52.2 (1.7%)	11.9 (2.5%)	12.0 (4.2%)
Urtiaceae	*Forsskaolea tenacissima* L.	813.6 (0.6%)	364.3 (1.7%)	78.3 (3.1%)	39.3 (5.1%)
	Urtica pilulifera L.	49.1 (10.4%)	20.8 (3.9%)	7.9 (10.1%)	4.8 (14.6%)
Zygophyllaceae	*Fagonia bruguieri* DC.	70.5 (0.9%)	33.1 (1.8%)	14.4 (2.8%)	7.9 (1.3%)
	Fagonia glutinosa Del. Var. grandifflora Boiss.	66.8 (1.2%)	26.1 (1.9%)	11.3 (1.8%)	7.5 (22.7%)
	Fagonia mollis Del.	72.5 (0.6%)	31.4 (4.5%)	24.7 (4.5%)	6.7 (0%)
	Nitraria retusa (Forskal) Ascherson	44.8 (8.7%)	78.0 (4.5%)	12.5 (4.8%)	15.2 (4.0%)
	Nitraria retusa 2(Forskal) Ascherson	290.6 (1.4%)	200.3 (2.8%)	47.8 (3.8%)	37.7 (24.9%)
	Peganum harmala L.	36.2 (10.8%)	28.0 (20.4%)	8.4 (15.5%)	8.0 (25.0%)

Based on this collection, approximately 5% of assayed plants showed high levels of antioxidant activity (namely, above 300 and 275 μmol TE g^{-1}dry weight for aqueous and methanolic extracts, respectively), as determined by using *Ginkgo biloba* L. (Ginkgoaceae) as a positive control (311.5 and 275.6 μmol TE g^{-1} dry weight for aqueous and methanolic extracts, respectively). The difference between the antioxidant activity of aqueous and methanolic extracts was statistically significant (p<0.0001).

2.2. Total phenolic content

As was the case with antioxidant activity measurements, there was a wide variation in the total phenolic content of the individual plant species, ranging from 4.4 to 78.3 mg and from 2.1 to 52.8 mg gallic acid equivalent (GAE) g^{-1} dry weight for the aqueous and methanolic extracts, respectively, with strong reproducibility in the replicate measurements (table 1). Many plant species displayed remarkably high levels of total phenolic content,

often with GAE values >20 mg g^{-1} dry weight 35. This could be compared to *Salvia spinosa*, a genus that is well-known as a rich source of polyphenols with values of 20.8 and 21.0 mg g^{-1} dry weight for aqueous and methanolic extracts, respectively. The highest values obtained were from the aqueous extracts of *F. tenacissima* and *Dittrichia viscosa* of 78.3 and 57.3 mg GAE g^{-1} dry weight respectively, and the methanolic extracts of *C. siliqua* and *P. palaestina* of 52.8 and 52.3 mg GAE g^{-1} dry weight, respectively (table I). Interestingly, neither *D. viscose* nor *F. tenacissima*, or any other species of the same genera, have been investigated previously for polyphenol content. Several *Pistacia* species are known to be rich in gallotannins and related phenolic compounds 36, 37. With the antioxidant activities, the difference between the aqueous and methanolic extracts regarding total phenolic content was also statistically significant (p<0.0001).

2.3. Relationship between antioxidant activity and phenolic content

There was a significant linear correlation between antioxidant activity and total phenolic content for aqueous and methanolic extracts (coefficient r = 0.863 and 0.850, respectively) (figure 1). These results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. These results were consistent with the findings of various research groups, who reported positive correlations between total phenolic content and antioxidant activity 26-38.

In addition to the statistical correlation, figure 2 proves to be useful for identification of plants as potential sources for new chemotype antioxidants. A plant with high-antioxidant activity, for which phenolic content *versus* antioxidant activity falls above the regression line (figure 1), is a plant to investigate for novel antioxidants, such as, *C. siliqua* and *V. cruciatum*.

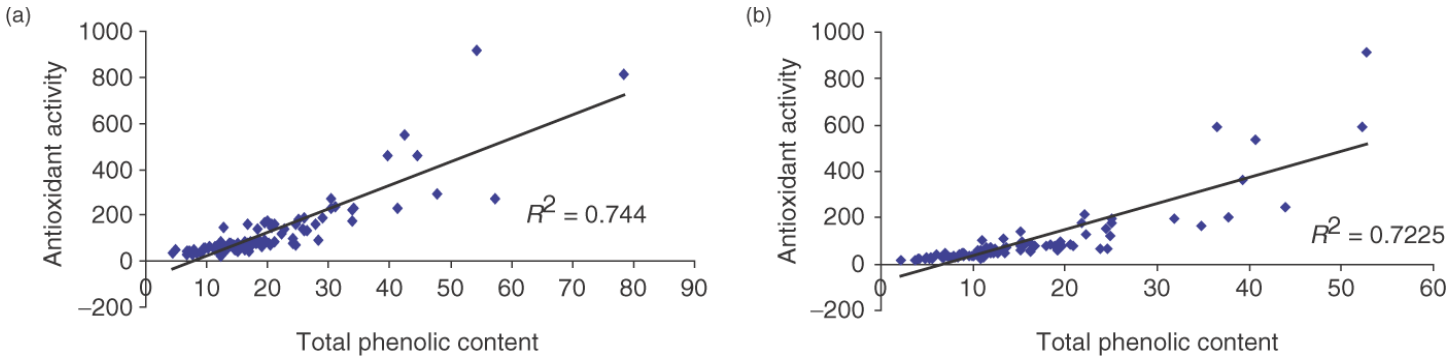

Figure 2. Linear correlation between the total phenolic content and antioxidant activity TEAC. (a) For aqueous extracts: correlation coefficient r = 0.863, coefficient of determination r^2 = 0.744. (b) For methanolic extracts: correlation coefficient r = 0.850, coefficient of determination r^2 = 0.723.

Moreover, while the total phenolic content of the methanolic extract of *C. siliqua* (52.8 mg GAE g^{-1} dry weight) and *P. palaestina* (52.3 mg GAE g^{-1} dry weight) is approximately the same, the antioxidant activity of *C. siliqua* (915.6 µmol TE g^{-1} dry weight) is 1.5 times higher than that of *P. palaestina* (590.9 µmol TE g^{-1} dry weight). We are encouraged by the concept that new, nonphenolic chemotypes with strong antioxidant activity, may be uncovered from those plants where low phenolic content was associated with high-antioxidant activity; such studies are ongoing, in particular, with *V. cruciatum* Sieb., which comes from the high lands in the northern part of Jordan.

The Folin-Ciocalteu assay gives only a crude estimate of the total phenolic compounds in an extract 24. Hence, this may explain, at least in part, the unequivocal correlation between total phenolic content and antioxidant activity of several plant species (table I). For example, although the total phenolic content of aqueous extract of *D. viscosa* (57.3 mg GAE g^{-1} dry weight) was higher than those of *C. siliqua* and *P. palaestina* (54.2 and 42.5 mg GAE g^{-1} dry weight), respectively; the corresponding antioxidant activity of *D. viscosa* (269.8 µmol TE g^{-1}

dry weight) was less than a third with those of *C. siliqua* (916.7 μmol TE g^{-1} dry weight) and *P. palaestina* (549.0 μmol TE g^{-1} dry weight). Similarly, while the total phenolic content of the methanolic extract of *D. viscosa* (43.9 mg GAE g^{-1} dry weight) is higher than that of *V. cruciatum* (36.5 mg GAE g^{-1} dry weight), its corresponding antioxidant activity is less than half (247.8 μmol TE g^{-1} dry weight in comparison with 590.5 μmol TE gμ dry weight). We hope that the ongoing and future research will pursue the identification of the antioxidant compounds from plant species with relatively high-antioxidant activity. A particular focus should be on the plants with high-antioxidant activity and low-phenolic concentrations, as they may prove to yield novel antioxidants. The goal should be to identify and describe new structural classes of natural antioxidant compounds.

3. Materials and methods

3.1. General

The total antioxidant capacity assay was performed on a MultiSpec-1501, SHIMADZU® photodiode diode array spectrophotometer (Kyoto, Japan), fitted with Julabo F40, Ultratemp 2000 temperature control. The total phenolic content assay was carried out using a Spectronic 601 spectrophotometer (Milton Roy Company, USA). Aqueous and methanolic extracts were prepared by using a KARL KOLB water bath shaker (Scientific Technical Supplies, Dreieich, Germany). The incubator B 28 #04-68155 was obtained from Binder GmbH (Bergstr., Tuttlingen, Germany). HPLC grade MeOH and EtOH were obtained from Scharlau Chemie S.A. (Barcelona, Spain) and Fisher Scientific UK limited, (Loughborough, Leicestershire UK), respectively. ABTS® was obtained from AppliChem GmbH (Ottoweg, Darmstadt, Germany), and potassium persulfate and Trolox® from Acros Organics (New Jersey, USA). Folin-Ciocalteu reagent (2N) was from S.D. Fine-Chem Ltd. (Mumbai, India). Gallic acid monohydrate and sodium carbonate were from Janssen Chemica (Geel, Belgium) and Frutarom Ltd. (Berkhamsted, UK), respectively.

3.2. Plant material

The complete range of wild plant samples were collected throughout Jordan, at random, from each of the four biogeographic regions, (figure 1). At each collection point a global positioning system (etrex; Garmin Ltd., Kansas, USA) was used to determine the longitude, latitude, and elevation of the sampling point. These plant samples were identified taxonomically by two of the authors (MS and KAL), and voucher specimens were deposited at the Herbarium Museum of the Faculty of Pharmacy, Jordan University of Science and Technology. The plant raw materials were cleaned of residual soil and air-dried at room temperature. Plants were ground to a fine powder using a laboratory mill, RetschMühle, (RETSCH GmbH, Haan, Germany), passed through a 24 mesh sieve to generate a homogeneous powder, stored at room temperature (22-23°C), and protected from light until required for analyses.

3.3. Extraction process

For aqueous extractions, a 250 mg aliquot of each grounded plant material was weighed into a test tube and extracted with 10 mL de-ionized water at 80°C for 1 h in a water bath shaker. After cooling, the extract was centrifuged at 1507 ×*g* for 10 min at 25°C, and the supernatant was recovered without collection of the pellet and stored at 4°C until used for the TEAC and total phenolic content assays. Methanolic extractions were conducted in a similar manner using 250 mg aliquot of each ground plant material in 10 mL of 80% methanol at 37°C for 3 h in a shaking water bath. After cooling, the extract was centrifuged at 1507 ×*g* for 10 min, and the supernatant was recovered and stored at 4°C until used for the TEAC and total phenolic content assays. For each plant sample, two replicates of the aqueous and methanolic extracts were prepared 26.

3.4. Measurement of totalantioxidant activity

The antioxidant capacity assay was carried out using the improved $ABTS^{+}$ method as described by Re *et al*. 20, using Trolox as a standard. Total antioxidant activity was expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µ mol Trolox equivalents per g dry weight of plant) 20.

3.5. Measurement of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method based on the procedure of Singleton and Rossi, using gallic acid as a standard 25. Briefly, 50 µL aliquots from each of the replicates were mixed with 450 µL of DI H_2O and 2.5 mL of 0.2 N Folin-Ciocalteu reagent. After 5 min, 2 mL of saturated sodium carbonate (75 g L^{-1}) was added. The absorbance of the resulting blue solution was measured at 765 nm after incubation at 30°C for 1.5 h with intermittent shaking. Quantitative measurements were performed based on a six point standard calibration curve of 20, 100, 200, 300, 400, 500 mg L^{-1} of gallic acid in 80% methanol. The total phenolic content is expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

3.6. Statistical analysis

For both assays, the entire data in table 1 is shown as mean (% variation) from two extract replicates, each analyzed in duplicate. Correlation and regression analysis of antioxidant activity (*Y*) *versus* the total phenolic content (*X*) was carried out by using Microsoft Office Excel 2003, and the quality control was checked and matched with correlation and regression analyses in SAS 9.1 by using the linear regression command, proc reg. Paired *t*-test was applied to test for significant differences between aqueous and methanolic extracts for antioxidant activity and total phenolic content.

3.7. Geographic information systems

The data for figure 1 was created and generated by importing the latitude/longitude locations of plant sample sites and nature preserves from the GPS device into a GIS layer by using ArcGIS 9.1 technology (ESRI; Redlands, CA). Identifiers were assigned to each point and the attributes describing each location were assigned from field data sheets. The GIS layer of biogeographical regions was derived from the work of Al-Eisawi 1. The standard GIS sources for country administrative boundaries, major hydrography, and major cities were used for the remaining map content.

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