

Antioxidant Properties of Selected Wild Plants

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Abstract: The antioxidant properties of 4 Jordanian plant extracts were studied by chemical assays of total antioxidant capacity and ferric reducing power. The extracts total phenols and flavonoids contents were also determined. The plant methanolic extracts were found to have different levels of antioxidant activities in the assays tested. Considering the overall antioxidant activities, the studied plants were arranged in the following decreasing order of their antioxidant strength; *Pictacia palaestina* > *Hypericum triquetrifolium* > *Arbutus andrachne* > *Ceratonia siliqua*. The antioxidant activities of studied plants were explained by their content of total phenols & flavonoids.

Keywords: Total antioxidant capacity, reducing power, flavonoids, phenols, Jordan.

1. Introduction

Free radical reactions occur in the human body and food systems. Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology.

An over-production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the body antioxidant defense system and free radical accumulation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as those involve the cardio- and cerebro-vascular systems and cancers. The consumption of fruits and vegetables containing antioxidants has been found to offer protection against these diseases. Dietary antioxidants can augment cellular defenses and help to prevent oxidative damage to cellular components (Halliwell, 1989).

The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone have been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity (Kahl, & Kappus, 1993). The search for antioxidants from natural sources has received much attention, and efforts have been put into identify compounds that can act as suitable antioxidants to replace synthetic ones.

Plants are rich in phenolic compounds and flavonoids which have been reported to exert multiple biological effects, such as antioxidant activities, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic (Miller, 1996). The antioxidant activity of phenols is mainly due to their redox properties, which allow them to act as reducing agents, electron/hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential (Rice-Evans, *et al.*, 1995). Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly support the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao, 1996; Block, & Patterson, 1992; Ness, & Powles, 1997). Crude extracts of herbs and other

plant materials are rich in phenols and flavonoids and several studies reported a positive linear correlation between the total phenolic compounds and the antioxidant activities of aqueous and methanolic extracts of different plant species (Cai, *et al.*, 2004; Tawaha, *et al.*, 2007). The present study was directed towards the identification of antioxidant abilities of methanolic extracts from the following Jordanian plants: *Pictacia palaestina* (leaves), *Hypericum triquetrifolium* (whole plant), *Arbutus andrachne* (leaves) and *Ceratonia siliqua* (fruits) using different *in vitro* antioxidant testing assays.

2. Materials and Methods

2.1. Chemicals

Folin-Ciocalteu phenol reagent, Catechol, Rutin were obtained from Sigma Chemicals Co. (St. Louis, USA). All other chemicals were of analytical grade.

2.2. Plant Material

The following dried parts of plants: *Pictacia palaestina* (leaves), *Hypericum triquetrifolium* (whole plant), *Arbutus andrachne* (leaves), *Ceratonia siliqua* (fruits) were collected from different locations in Jordan. The choice of plant material used were dependent on reports of antioxidant activity and total phenolic content of selected Jordanian plant species (Tawaha, *et al.*, 2007; Alali, *et al.*, 2007).

2.3. Extraction Method

All plant parts were dried under the same conditions (at room temperature, in a dark and clean place). The air dried plant parts were grounded in a blender with a particular size to ensure the plant powders in identical size, and then 100 g of each plant powder were soaked for 5-7 days with 1000 ml of 80 % methanol at 25 °C. After filtration, the filtrate was evaporated with a rotary evaporator to remove the methanol under reduced pressure at 50 °C. The dry crude extracts of the plants were stored in refrigerator in glass bottles until use. Determination of antioxidant activities of the dry crude extracts of the plants were evaluated using *in vitro* model assays, for all experiments the same stock solution of

1mg/ml from each crud extracts were prepared by dissolving 0.1g of dry crude extract/ standard and diluted in 100 ml of 98% methanol.

2.4. Chemical Screening

2.4.1. Determination of total phenolic content

The amount of phenolic compounds present in the extracts was determined by Folin Ciocalteu reagent (Duh and Yen, 1997). Catechol was used as the standard for the calibration curve. One milliliter of each plant extract (1mg/ml) was transferred into a 20 ml volumetric flask, and diluted with 10 ml of distilled water. One milliliter of 0.2 N Folin-Ciocalteu phenol reagent (Sigma-Aldrich) was then added, followed by mixing the mixture thoroughly. After 3 minutes, 2 ml sodium carbonate (Na_2CO_3) (100 g/l) was added, and the absorbance was measured at 760 nm with single beam spectrophptometer (Novaspec 11, LKB Biochrome, England) against blank. The total amount of phenolic compounds (mg/g) was determined from the calibration curve, and expressed as catechol equivalent (CE).

2.4.2. Determination of total flavonoid content

The amount of flavonoids was determined by the Miliuskas method (2004) with slight modifications. Rutin was used as the standard for a calibration curve. One milliliter of each plant extract (1mg/ml) was mixed with 1 ml of 2 % aluminum trichloride (AlCl_3) in ehanol, after the mixture was diluted with ethanol to 25ml, allowed to stand for 40 minutes at 20°C, its absorbance was measured at 415 nm with single beam spectrophptometer (Novaspec 11, LKB Biochrome, England). In the blank, amixture of 1 ml of the plant extract and 1 drop of acetic acid was diluted with ethanol to 25ml, followed by measurement of the absorbance as described above. The total amount of flavonoids was determined from the calibration curve, and expressed as rutin equivalent (RE).

2.5. Antioxidant Testing Assays

2.5.1. Determination of Total Antioxidant Capacity

This assay is based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto, et al., 1999). The tubes containing 0.3ml of extracts (1mg/ml) and 3ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95° C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution is measured at 695 nm with single beam spectrophptometer (Novaspec 11, LKB Biochrome, England) against a blank. The blank tube contained 3.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample (0.3ml methanol/ethanol),and it is incubated under the same conditions as the sample. Total antioxidant capacity of plant extract was measured from the regression equation ($y=0.003x-0.035$) prepared from the concentration versus optical density of ascorbic acid and the antioxidant capacitywas expressed as ascorbic acid equivalent (AAE).

2.5.2. Ferric Reducing Power Assay

A method developed by Oyaizu (1986) was adopted for the determination of reducing power. One ml of plant extract

(1mg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, then rapidly cooled, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant was taken and then 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added to it. Mixed well and allowed to stand for 10 minutes. The absorbance was measured at 700 nm with single beam spectrophptometer (Novaspec 11, LKB Biochrome, England) against blank (Phosphate buffer at pH 6.6). Ascorbic acid was used as a reference standard. An increase in absorbance at 700 nm of the reaction mixture was interpreted as increase in reducing activity of extract.

2.6 Statistical Analysis

The results wereexpressed as mean \pm S.D., using EXCEL 2007 programme.

3. Results and Discussion

3.1 Extractive yield

Extractive yield ([g extract/ 100g dry plant part] x100) for each plant was determined and the most efficient extraction yield by methanol was obtained for *Arbutus andrachne*(31.0%), followed by *Pictacia palaestina*(30.5%), *Hypericum triquetrifolium* (27%), *Ceratonia siliqua* (23.2%).

3.2 Total phenolic & flavonoid contents

The total phenolic content(mg Catechol equivalent/g extract) of the plant extracts are shown in Fig. 1 in decreasing order. As shown in Fig. 1, the highest amount of phenols was observed in *Pictacia palaestina* (149 mg/g), followed by *Hypericum triquetrifolium* (124 mg/g), *Arbutus andrachne* (105 mg/g)&*Ceratonia siliqua* (23mg/g) being the lowest. The total flavonoid content (mg Rutin equivalent/g extracts) of the plant extracts are shown in Fig. 2 in decreasing order. As shown in Fig. 2, the highest amount of flavonoidswas observed in*Hypericum triquetrifolium*(171 mg/g), followed by *Pictacia palaestina*(115 mg/g),*Arbutus andrachne*(112 mg/g)&*Ceratonia siliqua* (43 mg/g) being the lowest.

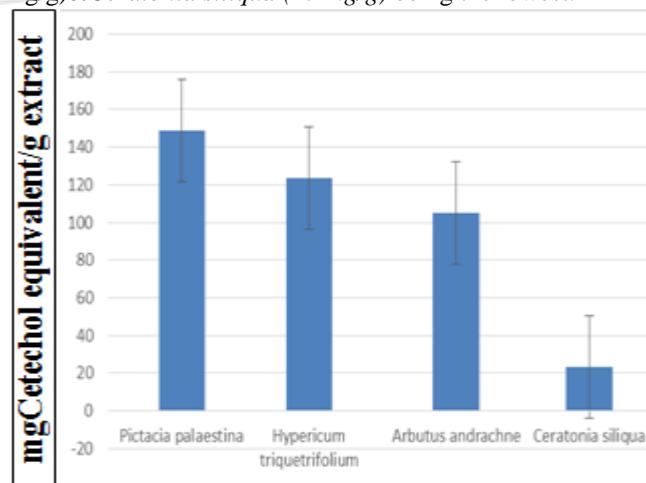


Figure 1: Total phenolic content (mg Catechol equivalent/g extract) of *Pictacia palaestina*,*Hypericum*

triquetrefolium, Arbutus andrachne & Ceratonia siliqua) extracts. Each column represents the mean value and bars represent the S.D., (n = 6).

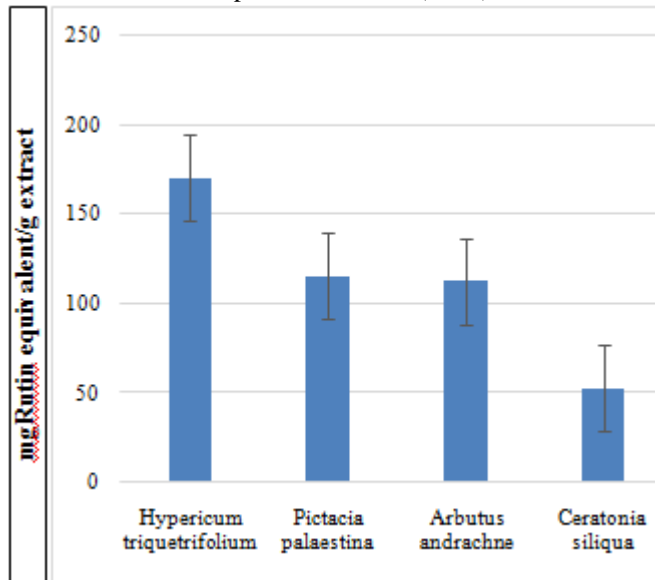


Figure 2: Total flavonoids content (mg rutin equivalent/g extract) of *Hypericum triquetrefolium*, *Pictacia palaestina*, *Arbutus andrachne* & *Ceratonia siliqua* extracts. Each column represents the mean value and bars represent the S.D. (n = 6).

3.3 Antioxidant activities

3.3.1. Total antioxidant capacity

Figure 3 illustrates the total antioxidant capacities ($\mu\text{g AA equivalent/mg extract}$) of plant extracts in decreasing order. As shown in Fig. 3 the highest activity was found in, *Pictacia palaestina* (232 $\mu\text{g/mg}$), followed by *Arbutus andrachne* (197 $\mu\text{g/mg}$), *Hypericum triquetrefolium* (186 $\mu\text{g/mg}$), & *Ceratonia siliqua* (69 $\mu\text{g/mg}$) being the lowest.

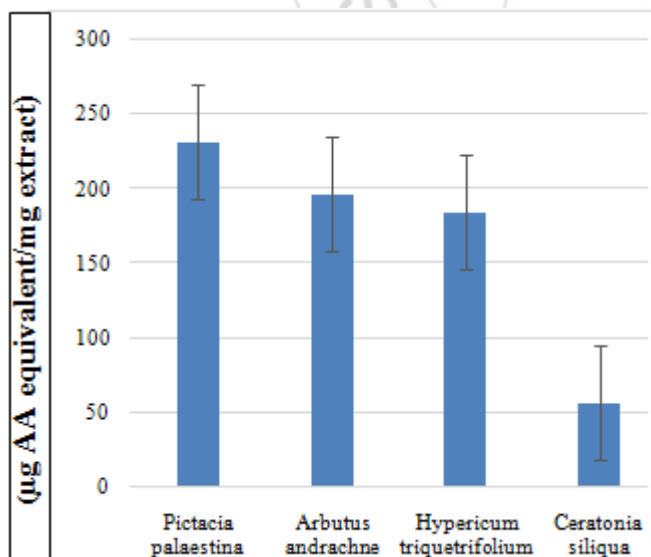


Figure 3: Total antioxidant capacity ($\mu\text{g AA equivalent/mg extract}$) of *Pictacia palaestina*, *Arbutus andrachne*, *Hypericum triquetrefolium* & *Ceratonia siliqua* extracts. Each column represents the mean value and bars represent the S.D. (n = 6).

3.3.2. Ferric reducing capacity

Figure 4 shows the ferric reducing capacities of Plant extracts in decreasing order compared to ascorbic acid. As shown in Fig. 5, compared to ascorbic acid (O.D=3), the highest activity was observed in *Pictacia palaestina* (O.D=2.7), followed by *Hypericum triquetrefolium* (O.D=2.1), *Arbutus andrachne* (O.D=2) & *Ceratonia siliqua* (O.D=0.20) being the lowest.

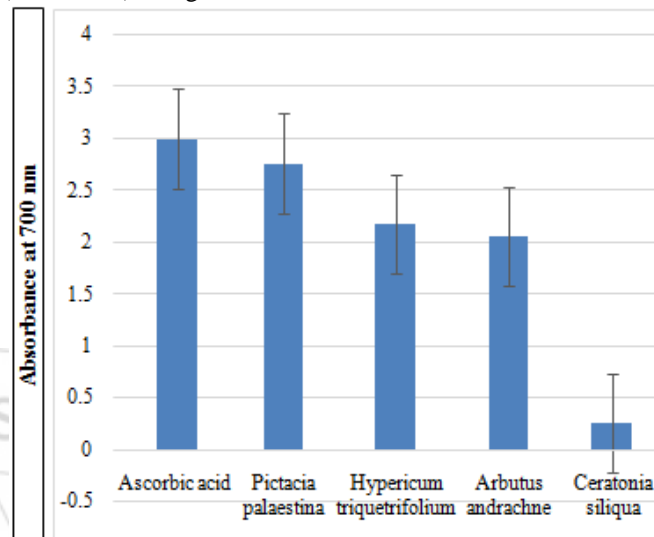


Figure 4: Reducing power (Absorbance at 700 nm) of Ascorbic acid, *Pictacia palaestina*, *Hypericum triquetrefolium*, *Arbutus andrachne* & *Ceratonia siliqua* extracts. Each column represents the mean value and bars represent the S.D. (n = 6).

The results of the present study demonstrated that out of the studied plants, *Pictacia palaestina*, *Arbutus andrachne* and *Hypericum triquetrefolium* extracts possess the highest total antioxidant capacity (Fig.3), probably because these extracts contained the highest amount of phenols (Fig.1) and flavonoids (Fig.2). However, *Ceratonia siliqua* extract, it showed the lowest total antioxidant capacity (Fig.3), & the lowest reducing power (Fig.4), probably because this extract contained the lowest total phenols (Fig.1) and flavonoids (Fig.2).

This result coincides with other studies (Luo, *et al.*, 2002; Conforti, *et al.*, 2002 & Liu, *et al.*, 2007) that showed the phenolic & flavonoid contents of extracts of many plants contribute significantly to their total antioxidant activity.

In regard to the antioxidant activity, based on the ferric reducing power test, it seems that *Pictacia palaestina*, *Arbutus andrachne* and *Hypericum triquetrefolium* extracts had a highest reducing power between the screened plants (Fig. 4). Similar result was also found by Tawaha, 2007 who identified *Arbutus andrachne*, *Hypericum triquetrefolium* and *Rosmarinus officinalis* as being the best sources of free radical scavenging compounds among the Jordanian plants.

4. Conclusions

The extraction yields (g extract/ 100g dry plant part) for each plant species were obtained and the most efficient

extraction yield by methanol was obtained for *Arbutus andrachne* (31.0%), followed by *Pistacia palaestina* (30.5%), *Hypericum triquetrifolium* (27%), *Ceratonia siliqua* (23.2%),

The studied plant extracts were arranged in decreasing order of their total phenol content (mg Catechol equivalent/g extract) as follows: *Pistacia palaestina*>*Hypericum triquetrifolium*>*Arbutus andrachne*>*Ceratonia siliqua*.

The studied plant extracts were arranged in decreasing order of their total flavonoid content (mg Rutin equivalent/g extract) as follows: *Hypericum triquetrifolium*>*Pistacia palaestina*>*Arbutus andrachne*>*Ceratonia siliqua*.

The studied plant extracts were arranged in decreasing order of their total antioxidant capacity (μg AA equivalent/mg extract) as follows: *Pistacia palaestina*>*Arbutus andrachne* > *Hypericum triquetrifolium*>*Ceratonia siliqua*.

The studied plant extracts were arranged in decreasing order of their ferric reducing activity as follows: *Pistacia palaestina* > *Arbutus andrachne* > *Hypericum triquetrifolium* > *Ceratonia siliqua*. Further studies are therefore recommended to evaluate the antioxidant properties of these Jordanian plant extracts on biological models such as erythrocytes.

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