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Antioxidant Activity of Methanolic Extracts of *Aloysia triphylla*, *Ceratonia siliqua*, *Cupressus sempervirens* and *Punica granatum* Selected from Karak Region-Jordan

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Abstract: To determine the antioxidant activity of *Aloysia triphylla*, *Ceratonia siliqua*, *Cupressus sempervirens* and *Punica granatum* the content of total phenolics in the extracts was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as Gallic acid equivalents (GAE). Determined by 2, 2-diphenyl-1, 1-picrylhydrazyl (DPPH) and reducing antioxidant power (FRAP). The results of TPC of plant extracts were ranged between 62.16- 261.8 mg/g. The highest amount was in *Cupressus sempervirens* cones 261.8 mg /g followed by *Punica granatum* peels 243 mg /g. It can concluded *Punica granatum* peels possesses significant antioxidant activity.

Key words: Total phenolic content · DPPH radical scavenging · Reducing antioxidant power (FRAP)

INTRODUCTION

Antioxidants are synthetic or natural substances found in many plants, including fruits and vegetables and they are also available as dietary supplements, this natural donation may prevent or holdup some types of cell damage by slowing or preventing the oxidation of other molecules. Antioxidants are often reducing agents such as thiols or polyphenols. They are considered to play an important role in preventing the development of such chronic diseases as cancer, heart disease, stroke, Alzheimer's disease, Rheumatoid arthritis and cataracts [1]. Plants include minerals and primary metabolites and a diverse group of secondary metabolites with antioxidant activity [2]. The dynamic components are normally extracted from all plant structures, but the concentrations of these components vary depending on genetic, environmental, pre-and post-harvest treatments and analytical methodologies. However, parts which include, leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds known to contain the highest concentration of the bioactive compounds are favored for therapeutic purposes [3]. The cells in our body are exposed to oxygen every day, oxygen is important for our body's health, but exposure to oxygen also causes oxidation. In oxidation, body chemicals are changed and become what are known as free radicals. Exposure to environmental factors, such as sun exposure, cigarette smoke, alcohol and pollution, also creates free radicals [4]. Assessed the antioxidant activity of *Aloysia triphylla*, which shown fast scavenging rate against 2, 2-diphenyl-1-picrylhydrazyl, Trolox equivalent antioxidant capacity and hydroxyl radical scavenging. Also *Achillea fragrantissima*, *Teucrium polium*, *Rosmarinus officinals* and *Alhgi graecorum*, that shown high antioxidant activity of these plant extracts, that are a good sources for medicinal drugs [5]. Crude extracts of these plants rich in phenolics and they are increasingly of interest in the food industry because they delay oxidative degradation of lipids and thereby improve the quality and nutritional value of food [6]. Plants have an almost unlimited ability to synthesis aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12, 000 have been isolated [7].

According to Meagher and Thomson [8] more than 4, 000 phytochemicals have been cataloged and classified as primary or secondary metabolites, depend on their purpose in plant metabolism. Primary metabolites include sugars, amino acids, proteins, purines and pyrimidines for

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nucleic acids etc. Secondary metabolites are the remaining chemicals in plant such as alkaloids, terpenes, flavonoids, lignans, steroids, curcumines, saponins, phenolics, flavonoids and glucosides [9]. Jordanian people believe that medicinal plants can be used for treating infectious as well as chronic diseases [10, 11].

Also according to Afifi *et al*. [12] many plants were used as antidiabetic agent's and evaluated in *vitro* and in *vivo* systems in the form of their crude extracts and isolated pure compounds with varying degrees of hypoglycemic or antihyperglycemic bioactivities.

In addition plant extracts play a major role as antimicrobial agents; for example *Aloysia triphylla* essential oil partially inhibited the growth of the fungal strains and pathogenic yeast. No antifungal effect was observed against *Trichoderma viride* [13]. On the other hand, *Cupressus sempervirens* was studied as bacteriostatic and fungistatic activities [14]. And they are found that the essential oil of *C. sempervirens* leaves and fruits showed stronger antimicrobial activity against tested microorganisms, because *C. sempervirens* fruits were moderately rich in tannins. From carob leaf extract (*Ceratonia siliqua*), they makes silver nanoparticles, which can reduce silver ions into silver nanoparticles (AgNPs) within 2 min of reaction time, without using any harsh conditions, the AgNPs showed an effective antibacterial activity toward *Escherichia coli* pathogen [15]. Essential oil compounds of *Punica granatum* peels also tested by Growther *et al*. [16], who observed high antibacterial activity against Shiga toxin producing *E. coli* (STEC), because of exposed to Punicalagin and Ellagic acid. The aim of this study is to evaluate the antioxidant activity of several Jordanian plants using three different methods and to evaluate the relationship between the antioxidant activity and total phenolic content of the plants.

MATERIALS AND METHODS

Sample Collection: Fresh leaves of *Aloysia triphylla*, *Ceratonia siliqua*, *Cupressus sempervirens* cones and *Punica granatum* peels were collected from different regions of Karak city they were taken to the research Lab, Department of biological sciences in Mutah University-Jordan.

Sample Preparation: The chosen parts were washed thoroughly with tap water to remove dirt, dried at room temperature for 28 days. Samples crushed into uniform powder to increase the surface area for extraction. Sample (50g) of each part was weighed, placed into sterile conical flasks each containing 500 ml of 99% Methanol. Each conical was tightly covered, shaken vigorously at 150 rpm and kept for 3 days to enhance proper dissolution of the bioactive compounds in the samples. Each sample solution was filtered using Buchner funnel under vacuum with Whatmann filter paper 125mm (Whatmann Int. Ltd., Maidstone, U.K) at room temperature. Each filtrate (methanol extracts) was then centrifuged at 3000 rpm for 10 minutes. Then evaporated in a rotary (BUCHI Rotavapor BL-710D, Switzerland) at 45°C until the extracts became completely dry. The extracts were stored at 4°C in a refrigerator until required for further analyses [17].

Determination of Extraction Yield (%): The yield (%, w/w) from all the dried extracts was calculated as:

$$\text{Yield (\%)} = (W1 \times 100)/W2$$

where W1 is the weight of the extract after evaporation of solvent and W2 is the weight of the plant powder [18].

Evaluation of Antioxidant Activity of the Extracts

Determination of Total Phenolic Contents (TPC): Total phenolic contents were determined using Folin-Ciocalteu reagent [19]. Crude extract (500µg/mL) was combined with Folin-Ciocalteu reagent (0.5 ml) and distilled water (7.5 ml). After 10 min, 1.5 ml of 20% sodium carbonate (w/v) was added. Then mixture was heated in a water bath at 40°C for 20 min after that cooled in an ice bath. The absorbance of blue colored solution was measured at 755 nm using a spectrophotometer. Amount of TPC were expressed as Gallic acid equivalents (GAE) mg/g of dry weight.

DPPH Radical Scavenging Assay: The hydrogen atom or electrons donating ability of the corresponding extract were measured from the bleaching of purple color methanol solution of DPPH. The spectrometric assay uses stable radical 2, 2-diphenyl picryhydraxzyl (DPPH) reagent [20]. DPPH is one of the chemical compounds that possess a proton free radical and it shows a maximum absorption at λ 517nm because of its bright purple color. When DPPH encounters proton radical, its purple color fades rapidly and this s cavenging action forms the basic mechanism for measuring antioxidant activity [21]. 1000 µl of various concentrations of the extracts in methanol were added to 4 ml of (0.004%) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated in following way:

$$I\ \% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{Sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate. Trolox [(6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid, was used as standard for the construction of the calibration curve and the final concentration ranged from (0-1.5 µg/mL). Finally DPPH radical scavenging activities were expressed as mg Trolox equivalents per gram of plant extract [22].

Ferric Reducing Antioxidant Power (FRAP) Assay: FRAP assay was performed according to the methods of [23]. With minor modifications, an amount of 50 µl extracted samples were mixed with 3 mL FRAP reagent in test tubes, to get the final concentration of plant extract in the solution (100 µg/mL).The test tubes were done in triplicate. Blank samples were prepared of methanol and extracted samples also. Both samples and blank were incubated for 8 minutes at 37°C and the absorbance of the samples was determined against blank at 593 nm. The FRAP assay was based on the reducing power of antioxidants in which a potential antioxidant will reduce the ferric ions to ferrous ions, which form a blue colored ferrous-tripyridyl triazine complex. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing.

Acetate Buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) $FeCl_3$. 6 H_2O: (M.W. 270.30), 20 mM. Trolox final concentration (0-1 µg/mL) was used as standard for the construction of the calibration curve. The antioxidant capacity based on the ability to reduce ferric ions of extract was expressed as mg Trolox equivalents per gram of plant extract.

Statistical Analysis: All data analysis were performed using Microsoft excel 2007. Results were done as mean± standard deviation (SD).

RESULTS AND DISCUSSION

Yield (%) of Plant Extract: The highest yield was obtained with methanolic extract (28.32%) of *Punica granatum* peels, while the lowest was that of *Cupressus sempervirens* cones (4.74%). The yield of plant extracts was in the order of *Punica granatum* peels (28.32%) > *Ceratonia siliqua* leaves (26.52%) > *Aloysia triphylla* leaves (7.04%) > *Cupressus sempervirens* cones (4.74%) as shown in Table 1.

The total phenolic contents of plant extracts were determined spectrophotometrically at 760nm, by comparing with standard curve of gallic acid. TPC values calculated of all MeOH plant extracts by using the equation obtained from standard curve Fig. 1. TPC results were ranged between (62.16- 261.8 mg GAE/gm of plant extract). The highest amount was in *Cupressus sempervirens* cones (261.8 mg GAE/gm) followed by *Punica granatum* peels (243 mg GAE/gm) and (68.83, 62.16) for *A. triphylla* and *C. siliqua* leaves respectively. As shown in Fig. 2, the variation in the percentage yield between plant extracts may be related to many reasons: the part of plant which was used, time of harvesting and age of the plant.

MeOH extracts of selective plants, were evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radicals can be observed by the decrease in the absorbance at λ 517nm. These MeOH extracts showed antioxidant activity with different concentration providing 50% inhibition (IC_{50}) values as shown in Fig. 3. The best free radical scavenging activities reflected by the smallest IC_{50} values. A Trolox standard curve was established to evaluate TEAC (Trolox equivalent antioxidant capacity) and then TEAC expressed mg of Trolox per gm of plant extract. Trolox calibration curve Fig. 4, Also the results of $TEAC_{DPPH}$ are shown in Table 2, the lowest value of $IC_{50}$was (0.047mg/ml) found in *P. granatum* peels which has the highest activity (15.96 mg Trolox/ gm of plant extract), the other values found in the following order (*C. sempervirens* cones 0.062 mg/ ml, *C. siliqua* leaves 0.32 mg/ ml and *A. triphylla* leaves 0.8 mg/ ml). There is an inverse relationship between TPC and IC_{50} values by comparing with values in Fig. 2.

FRAP methods also used to determine the total antioxidant capacity. The principle of this method is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe^{+3}-TPTZ) to ferrous, colored form (Fe^{+2}-TPTZ) in the presence of antioxidants and measuring the absorbance at 593 nm. The FRAP values have been calculated by comparing the absorbance values of test samples containing serially diluted Trolox at 593 nm Fig. 5. The antioxidant activities of MeOH extracts by FRAP assay are shown in Table 3. As shown from the table the Trolox equivalent antioxidant capacities

Table 1: Weight and percentage yield of crude plant extracts

Plant species	Dry weight of plant(gm)	Weight of Methanolic extract (g)	% of methanolic extract
Aloysia triphylla	50	3.52	7.04%
Ceratonia siliqua	50	13.26	26.52%
Cupressus sempervirens	50	2.37	4.74%
Punica granatum	50	14.16	28.32%

Table 2: DPPH radical scavenging activity of plant extracts expressed by a TEAC [mg Trolox /gm of plant]. Data expressed by mean ± SD, n= 3

Plant species	$TEAC_{DPPH}$ [mg Trolox /g of plant]
A. triphylla	00.94 ± 0.19
C. siliqua	02.34 ± 0.02
C. sempervirens	12.09 ± 0.02
P. granatum	15.96 ± 0.30

Table 3: FRAP values and TEAC of plant extracts by MeOH. Data expressed by mean ± SD, n= 3

Plant	FRAP Value [µg/ml]	$TEAC_{FRAP}$ [mg of Trolox/g of plant extract]
C. siliqua	0.78±0.06	7.23±0.01
P. granatum	0.89± 0.01	8.24±0.03
A. triphylla	0.77±0.01	7.08±0.04
C. sempervirens	0.91± 0.01	8.38±0.01

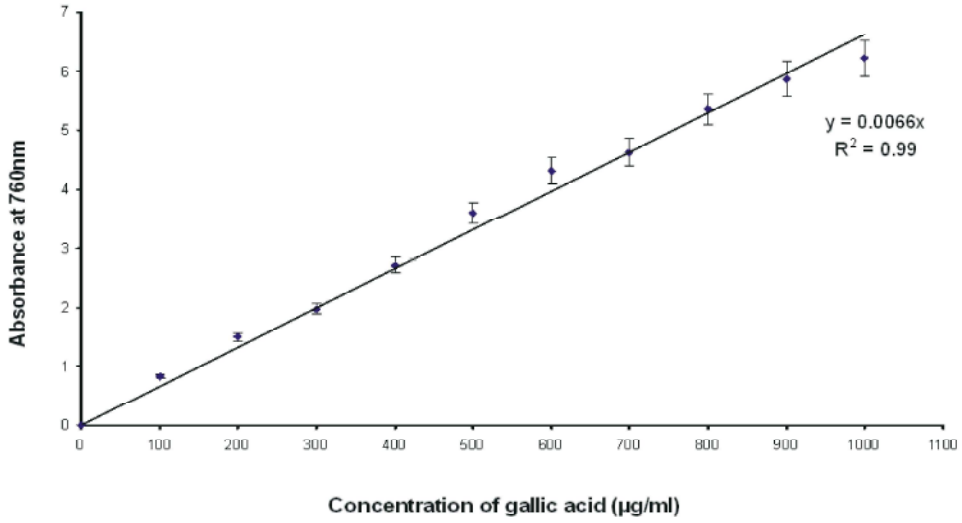

Fig. 1: Gallic acid standard curve and data were expressed by mean ± SD, n= 3

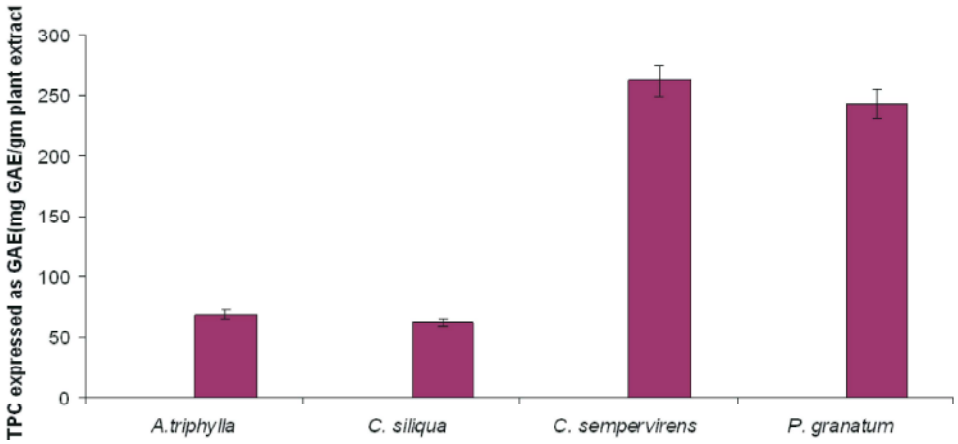

Fig. 2: TPC of MeOH plant extracts expresses as mg GAE/ gm plant extract, data were expressed by mean ± SD, n= 3

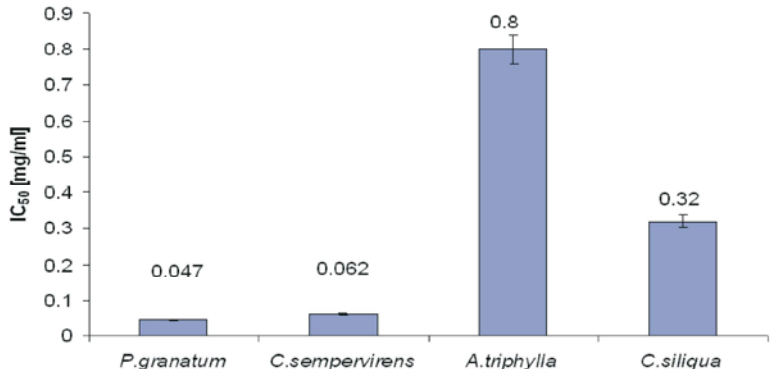

Fig. 3: IC_{50} values of plant extracts expressed by mean ± SD, n= 3

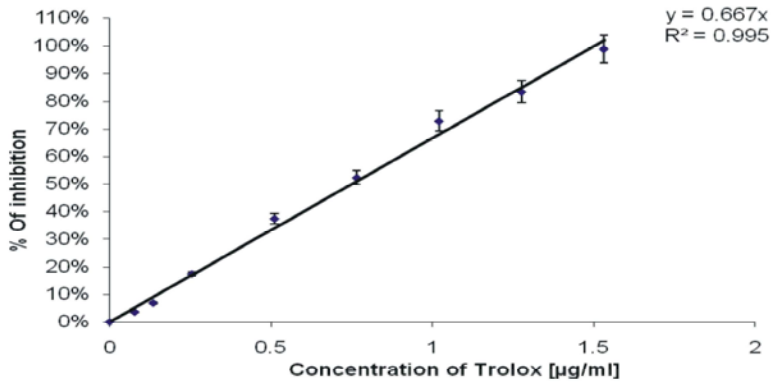

Fig. 4: Trolox standard curve for the TEAC measurement of plant extracts in the DPPH method, data expressed by mean ± SD, n= 3

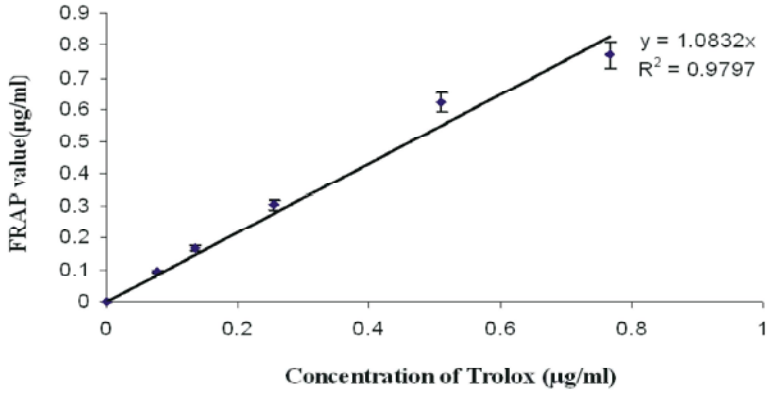

Fig. 5: Trolox standard curve for Trolox equivalent antioxidant capacity measurement of plant extracts in the FRAP assay, data expressed by mean ± SD, n= 3

approximately are the same, but the highest activity was for *C. sempervirens* cones (8.38 mg Trolox/gm of plant extract and the lowest activity was for *A. triphylla* leaves (7.08 mg Trolox/gm plant extract). Antioxidants activities of plant extract by DPPH and FRAP assays, demonstrated that extracts had good antioxidant behavior in both the DPPH and FRAP assays; this may be related to the highly phenolic contents. The two assays shared in a similar mechanistic basis, via transfer of electrons from the antioxidant to reduce an oxidant, as proposed by Huang and Prior [24].

If we are correlating between total phenolic content and antioxidant activity by DPPH and FRAP methods, which is commonly used in conjunction with either or

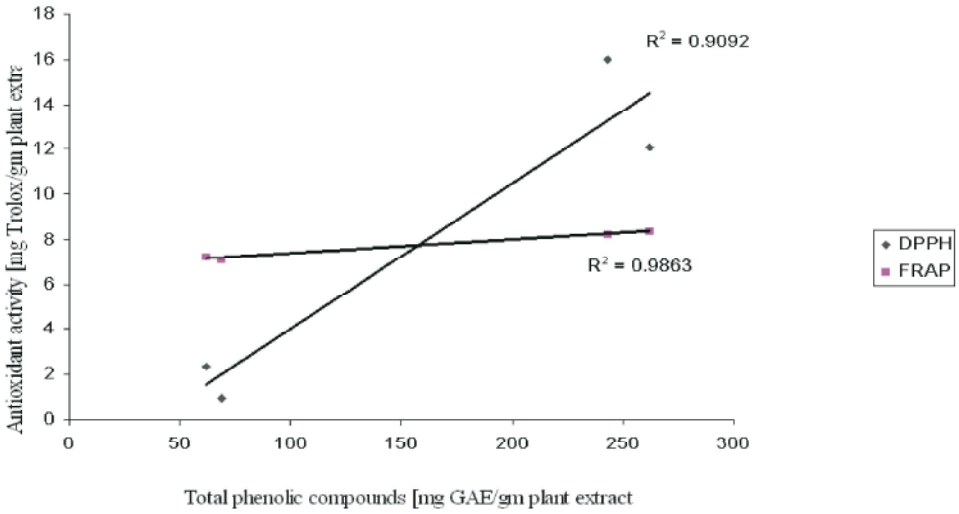

Fig. 6: The relationship between TPC and antioxidant activity obtained by DPPH and FRAP methods

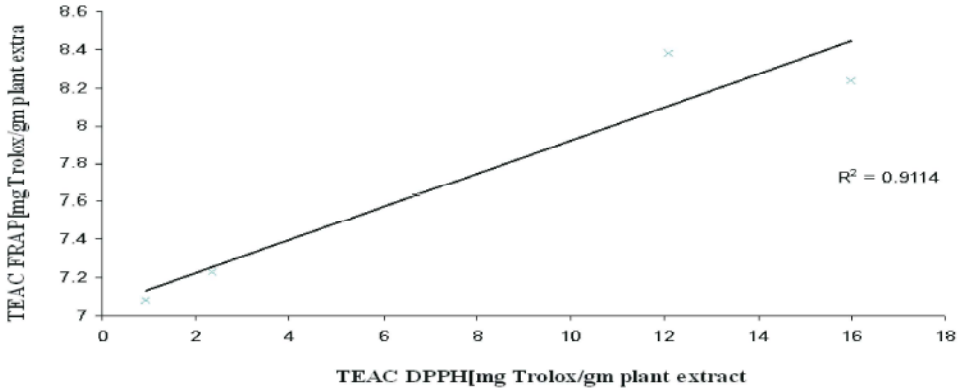

Fig. 7: Correlation between the mean values of ($TEAC_{DPPH}$) and ($TEAC_{FRAP}$)

both of the DPPH and FRAP assays. The total phenolic compounds were also highly correlated with the antioxidant activities with both of DPPH and FRAP methods. Data of the correlations are summarized in Fig. 6, which shows a strong correlation between total phenolic compounds and the antioxidant activity of MeOH plant extracts which were obtained by FRAP methods (R^2 = 0.9863). On the other hand antioxidant activity obtained by DPPH method, shows a strong correlation, but less than FRAP (R^2 = 0.9092). A satisfactory correlation of TPC with $TEAC_{DPPH}$ and $TEAC_{FRAP}$ suggested that phenolic compounds in the extracts were partly responsible for the antioxidant activities. Earlier workers have reported antioxidant activities of these plant [25-28], some of the results were identical and others different to the present study, maybe due to the solvent extract and the genus of the tree. The antioxidant activity of plant extracts is not limited to phenolics content, but maybe comes from the presence of other antioxidant secondary metabolites, such as flavonoids, proanthocyanidins and anthocyanins as reported by Mraihi *et al*. [29].

Also strong correlation between TEAC values obtained by the DPPH assay ($TEAC_{DPPH}$) and those by the FRAP assay ($TEAC_{FRAP}$), R^2 = 0.9114, as shown in Fig. 7. It was implied that compounds in the extracts were capable of scavenging the DPPH free radical and reducing ferric ions. The difference between the results of this study and previous studies may be explained by several factors; the concentration of extracts, season of plant collection, methods applied, solvent extract and plant parts. In summary, the present study showed that antioxidant activity constituents from *P. granatum* peels followed by *C. sempervirens* cones and these plant extracts need further studies in future to determine their toxicity before applied to humans.

REFERENCES

1. Swati, D., S. Chandrakant, T. Pranav, S. Manas, Deshpande and J. Salvekar, 2012. Antioxidant potential of Morinda Pubescence leaves. International Journal of Chemical Technology Research, 4(4): 1339-1342.
2. Akinmoladun, Ibukun E.O., E. Afor, B.L. Akinrinlola, T.R. Onibon and A.O. Akinboboye, 2007. Chemical constituents and antioxidant activity of *Alstonia boonei*. African Journal of Biotechnology, 6: 1197-1201.
3. Kafaru, E., 1994. Immense help from nature's work shop, guidelines on how to use herbs to achieve healthy living. Nigerian Journal of Pharmaceutical Sciences, 7(1): 79-85.
4. Koby Teresa, 2011. The Antioxidant Activity and Thermal Stability of Lemon Verbena (*Aloysia triphylla*) Infusion. Herb Research Foundation. http://www.herbs.org/herbnews.
5. Tarawneh, K., F. Irshaid, A. Jaran, M. Ezealarab and K. Khleifat, 2010. Evaluation of Antibacterial and Antioxidant Activities of Methanolic Extracts of some Medicinal Plants in Northern Part of Jordan. Journal of Biological Sciences, 10(4): 325-332.
6. Javanmardi, J., C. Stushnoff, E. Locke and J. Vivanco, 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum basilicum* accessions. Food Chemistry Journals, 83: 547-550.
7. Schultes, E., 1978. The Kingdom of Plants (Medicines from the Earth). McGraw-Hill Book, pp: 208.
8. Meagher, E., C. Thomson, G. Morrison, L. Hark and Malden, 1999. Vitamin and Mineral Therapy in Medical Nutrition and Disease. Massachusetts: Blackwell Science Inc., pp: 33-58.
9. Hasler, C.M. and J.B. Blumberg, 1999. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition, 129: 756-757
10. Al Dejawi, A., 1991. Agricultural Technology and Medicinal Therapy, Al Neser Publication, pp: 180.
11. Al Genaidy, M., 1993. Jordan Wild Plants and their Uses, Amman, Jordan, Al-Aseel Publications, pp: 198.
12. Afifi Fatma, Kasabri Violet and Abu-Dahab Rana, 2014. Chemical composition and in vitro studies of the essential oil and aqueous extract of Pelargonium graveolens growing in Jordan for hypoglycaemic and hypolipidemic properties. European Journal of Medicinal Plants, 4(20): 220-233.
13. Hanaa Ali, Hossam El-Beltagi and Nasr, 2011. Evaluation of antioxidant and antimicrobial activity of *Aloysia triphyllia*. Electronic Journal of Environmental, Agricultural and Food Chemistry, 10: 2689-2699.
14. Emamia, S., A. Javad, M. Rahimizadehb, S. Bibi, B. Fazly and H.K. Mohammad, 2006. Chemical and Antimicrobial Studies of *Cupressus sempervirens* L. and *C. horizentalis* Mill. Essential Oils. Iranian Journal of Pharmaceutical Sciences, 2(2): 103-108.
15. Awwd, A., M. Nida and O. Amany, 2013. Green Synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International Journal of Industrial Chemistry, 4: 29.
16. Growther Lali, K. Sukirtha, N. Savitha and S. Niren Andrew, 2012. Antibacterial activity of *Punica granatum* peel extracts against shiga toxin producing *E.coli*. International Journal of Life Science Biotechnology and Pharmaceutical Research, 1(4): 2250-3137.
17. Ifesan, B., C. Hamtasin, W. Mahabusarakam and S. Voravuthikunchai, 2009. Inhibitory effect of *Eleutherine americana* Extract against *Staphylococcus aureus* isolated from food. Journal of Food Science, 74:352-357.
18. Dellavalle, P., A. Cabrera, O.A. Dieg, P. Larranaga, F. Ferreira and M. Rizza, 2011. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria spp*. Chilean Journal of Agriculture Research, 71(2): 231-239.
19. Chaovanalikit, A. and R.E. Wrolstad, 2004. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. Food Science Journal, 69: 67-72.
20. Butrits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotheraphy Research Journal, 14: 323-328.
21. Huwaitat Sajee, Ekbal Al-Khateeb, Samira Finjan and Anwar Maraqa, 2013. Antioxidant and Antimicrobial activities of *Iris nigricans* methanolic extracts containing phenolic compounds. European Scientific Journal, 9(3): 1857-7881.
22. Chung, Y., C.T. Chang, W.W. Chao, C.F. Lin and S.T. Chou, 2002. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis*. Journal of Agricultural and Food Chemistry, 50: 2454-2458.

23. Benzie, I. and J. Strain, 1999. Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of antioxidant power and ascorbic acid concentration. National Center for Biotechnology Information, 299: 15-27.
24. Huang, D. and R. Prior, 2005. The chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry, 54: 1841-1856.
25. Vinha, F., O. Marta, C. Ana, S. António, O. Maria and M. Marisa, 2012. Phytochemical characterization and radical scavenging activity of aqueous extracts of medicinal plants from Portugal. European Journal of Medicinal Plants, 2(4): 2231-0894.
26. El Hajaji, H., L. Nadya, A. Katim, C. Yahya, F. Abdellah, E. Abdesslam, Brahim El Bali and L. Mohammed, 2010. Antioxidant Properties and Total Phenolic Content of Three Varieties of Carob Tree Leaves from Morocco.Australian Center for Geomechanics Publications, 4(4): 193-204.
27. Shiban S. Mutahar, Mutlag Al-Otaibi and Najeeb Al-zoreky, 2012. Antioxidant Activity of Pomegranate (*Punica granatum* L.) Fruit Peels. Food and Nutrition Sciences Journal, 10: 4236.
28. Amri Ismail, Hamrouni Lamia, Hanana Mohsen, Gargouri Samia and Jamoussi Bassem, 2013. Chemical composition, bio-herbicidal and antifungal activities of essential oils isolated from Tunisian common cypress (*Cupressus sempervirens* L.). Journal of Medicinal Plants Research, 7(16): 1070-1080.
29. Mraihi Farouk, Jamila Kalthoum and Munevver Sokmen, 2013. Phenolic contents and antioxidant potential of Crataegus fruits grown in Tunisia determined by DPPH, FRAP and β- Carotene/Linoleic Acid Assay. Journal of Chemistry, 6: 378264.