Developing a database for total phenolic content, total flavonoid content, and antioxidant activity of Jordanian crops

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ABSTRACT
This research aims at analyzing the antioxidant concentration and activity of 60 Jordanian crops including fruits, vegetables, and wild plants. Antioxidant quantities were analyzed using two analytical methods: total flavonoids and Folin Ciocalteu procedures, while antioxidant activities were analyzed using cupric ion reducing capacity (CUPRAC) as well as the free radical 2,2-dipryl-hydrazyl (DPPH) methods. The highest polyphenol content (M catechin/100 g fresh crop) was observed in rosemary (101.339 ± 0.501). In addition, the highest total flavonoid content (mg rutin/100 g fresh crop) of the analyzed was 5.397 ± 0.044 for parsley curly. Furthermore, sage showed the highest antioxidant activity (mg trolox/100 g fresh crop) (2.906 ± 0.019). On the other hand, DPPH radical scavenging activity % ranged from 1.567 ± 1.193 for green beans to 92.300 ± 1.015 for yellow sweet pepper. Also, DPPH radical scavenging activity (mg ascorbic acid/100 g fresh crop) was the highest (622.562 ± 9.093) for jujube. The analyzed Jordanian crops are considered sources of antioxidants. Values found by our research team are comparable with those found by other teams for some crops and different for others.

Introduction
Oxidative stress is well known to be a key player in the etiology and pathogenesis of many chronic ailments such as cancer, diabetes, and heart diseases. The onset of the oxidative stress status is due to the cascade events of cellular molecules’ oxidation. This cascade of events starts with the interaction of highly reactive species with cellular molecules that leads to the loss of one or more electrons from the last orbit, leaving the cellular molecules highly unstable till their orbits are refilled with the missing electrons. These actions result in the formation of a chain of secondary reactive species that attack and damage other vital biological components including proteins, lipids, and DNA. Stopping these adverse oxidation reactions can be achieved either by neutralizing the oxidized compounds or preventing the overproduction of the deleterious reactive species. Helpful compounds and enzymes termed antioxidants own such abilities in combating the reactive species and minimizing the onset of oxidative stress status and its harmful consequences. Antioxidants can be classified in several ways. They can be either exogenous (dietary or supplements) or endogenous antioxidants, non-enzymatic or enzymatic antioxidants, natural or manmade antioxidants, water-soluble or lipid-soluble antioxidants.
Many studies have shown an inverse relationship between the consumption of antioxidants and mortality, as well as morbidity risks of chronic diseases\textsuperscript{[2,5]} such as cancer, diabetes, heart diseases,\textsuperscript{[2]} and obesity.\textsuperscript{[1]} In addition, several reports showed that antioxidants promote beneficial gut microbiota.\textsuperscript{[1]} Furthermore, they promote healthier aging\textsuperscript{[6]} and longevity.\textsuperscript{[7]}

Among the ample examples of dietary antioxidants are the well-recognized potent antioxidants polyphenols. Polyphenols are well-known, natural exogenous antioxidants present abundantly in plants\textsuperscript{[3]} They consist of numerous heterogeneous structural subclasses with various classifications. However, the most used classification is based on their chemical structure,\textsuperscript{[3,7]} where polyphenols are classified into phenolic acids, flavonoids, stilbenes, and lignans. These four classes have been reported to possess many health benefits. Catechins, flavan-3-ol from the flavonoid class, were found by many investigators to promote skin health and reduce its aging upon exposure to UV light,\textsuperscript{[8]} protect against neurodegenerative diseases such as Alzheimer’s disease,\textsuperscript{[9]} antihypertensive, anti-COVID-19,\textsuperscript{[10]} and immunostimulatory.\textsuperscript{[11,12]} Likewise, flavonoids are thought to have anti-obesity, anti-infection, anti-COVID-19,\textsuperscript{[13]} antihypertensive,\textsuperscript{[14]} and skin protecting properties.\textsuperscript{[15]} Explicitly, rutin is being used as an anticancer agent owing that to its anti-cholesterol,\textsuperscript{[16]} anti-inflammatory, apoptotic, and autophagy stimulating characteristics\textsuperscript{[17]}. On the other hand, trolox, a water-soluble analog to the most common form of vitamin E; 

\alpha-tocopherol,\textsuperscript{[18]} caused the reduction of the metastasis of human lung\textsuperscript{[18,19]} and cervical cancer cell lines.\textsuperscript{[18]} Vitamin C is a natural exogenous water soluble\textsuperscript{[20]} antioxidant\textsuperscript{[3]} with reported health activities. Therefore, in biological systems, antioxidants can be expressed in terms of antioxidant concentration as well as their activities.\textsuperscript{[21]}

Based on the importance of the antioxidant actions possessed by many natural agents such as polyphenols in the prevention and treatment of oxidative-induced diseases, the notion of estimating these compounds in local crops seems logic and of vital significance. In addition, planning a sound dietary regimen requires an accurate food composition database. Moreover, such type of database may form the fundamental bases needed for developing potent antioxidant-related nutraceuticals.\textsuperscript{[3,22]} Indeed, there were several attempts to develop antioxidant database internationally and the attempts are ongoing.\textsuperscript{[20,23–33]} To our best knowledge, no databases were developed for the total phenolic content, total flavonoid content, and antioxidant capacity for Jordanian crops. This research aims at analyzing the total phenolic content, total flavonoid content, and antioxidant activity of 60 crops grown in Jordan including fruits, vegetables, and wild plants.

**Materials and methods**

Fresh food crops were bought from the local central market in Amman/the capital city of Jordan and confirmed to be domestic by the seller. Crop collection was performed between March 2016 till December 2017. The crops were collected in their edible stages of ripening. Fresh crop samples were transferred immediately to the laboratory for preparation and chemical analysis. Samples were cleaned with water and dried gently with tissue paper. They were then finely chopped into tiny pieces and 1 g of each sample was extracted with ethanol, methanol, and water at 50°C, 50°C, and 90°C\textsuperscript{[34]}, respectively/2hrs with intermittent shaking. Resultant extracts were subjected to centrifugation at 3000 rpm/15 min, filtered using a Whatman filter paper number 4, kept in 10 ml test tubes, purged with liquid nitrogen, and stored at –20°C until further analysis (no longer than 2 months).

Antioxidant parameters were analyzed in the crops as quantities and activities. Antioxidant quantities were analyzed spectrophotometrically using two analytical methods: total flavonoid methods and Folin Ciocalteu. Rutin and catechin were used as standards for the two methods, respectively. While antioxidant activities were analyzed by two analytical methods: cupric ion reducing capacity (CUPRAC) and 2,2-dipicrylhydrazyl (DPPH) free radical assays. Trolox standard was used to express the results of CUPRAC assay, while DPPH assay results were expressed in two ways: radical scavenging activity percentage (%) and as mg ascorbic acid. All results were calculated to be per 100 g
fresh crop. All standards and solutions used for the analyses were of analytical grade (Sigma*, Fischer* (China), Labscan* (Thailand), LabChem* (USA), and GCC* (UK)) and extracts were prepared using deionized water.

**Folin Ciocalteu analytical method**

Details of the antioxidant content analysis via Folin Ciocalteu method were followed according to Agbor et al.[35] Fresh Folin Ciocalteu reagent was added to 10 µl sample to complete the volume up to 1000 µl within 15 min. Antioxidant quantities in crop samples were measured at wavelength of 750 nm against catechin standard which was dissolved in methanol.

**Total flavonoid analytical method**

Analysis of total flavonoids was followed in details as published by Pyrzynska[36] method. For a 1 ml sample, 0.5 ml methanolic AlCl₃ (2% wt/v) solution. Thereafter, a mixture of 0.5 ml of deionized water and 0.5 ml of 1 M HCl was added and the mixture was shaken vigorously to complete the reaction within 10 min. The total flavonoid content of the crop samples was measured at a wavelength of 400 nm against rutin standard solutions which were dissolved in either in ethanol or in methanol.

**CUPRAC method of assay**

Details of analysis for the antioxidant activity analysis by CUPRAC procedure were followed according to Apak et al.[34] Sample extracts (0.5–5 ml) were mixed with 10.21 ml concentrated HCl. Then, the mixture volume was completed to 100 ml by 50% methanol. The mixture was then refluxed for 120 min at 80 °C. This mixture was then neutralized to PH 7 by 1 M NaOH. The mixture volume was completed to 4.1 ml by adding 1 ml CuCl₂, 1 ml neucoprine, and 1 ml acetate buffer consecutively. The reaction mixture was then incubated under heat (50°C) for 20 min, cooled to room temperature, and finally centrifuged at 5000 rpm/7 min. CUPRAC antioxidant activity was measured at a wavelength of 450 nm, while the standard Trolox was dissolved either in methanol or according to the sample extraction solvent.

**DPPH method of assay**

Analysis of the antioxidant activity via DPPH assay was followed in details as published by Molyneux.[37] To 50 µl sample, 2.95 ml of the DPPH free radical (0.1 mM, prepared in 80% ethanol). The mixture was then incubated in dark for 30 min at room temperature. The DPPH radical scavenging was measured using a wavelength of 517 nm and calculated according to the following equation:

\[
\text{Scavenging effect (\%) = } \left( \frac{\Delta A}{A_0} \right) \times 100 \%
\]

where \( \Delta A \): The difference between the sample and blank absorbance, \( A_0 \): blank absorbance.

**Statistical analysis**

Data of the crop’s antioxidant quantity and activities were analyzed by the statistical package for social sciences (SPSS)* version 26. Data were analyzed to estimate the mixed effect of crop type as well as the extraction solvent by factorial mixed analysis of variance (ANOVA).[38] Multiple regression coefficients were also calculated between different variables. Significance was set at \( P < 0.05 \). Results of the antioxidant content and activity in the tables are presented as average mean ± standard error.
Results

Table 1 displays the common and scientific names with the Arabic pronunciations of the analyzed crops. [39] Crops were classified according to Pellett and Shadervan [39] into fruits, vegetables, and wild plants. The research team analyzed 60 crops (25 fruits, 32 vegetables, and 3 wild plants).

Figure 1 presents the antioxidant quantities of the analyzed crops using Folin Ciocalteu procedure (M catechin/100 g fresh crop) for ethanol, methanol, and water extracts. Significant differences (P < .001**) were shown between the crops as well as their solvent extracts (the interaction effect of crop and solvent) regarding their total polyphenol content. In addition, different solvent extracts of the crop exhibited significant differences (P < .001**). Furthermore, water as a solvent extracted the highest amounts of polyphenols from 35 crops, while ethanol and methanol solvents extracted the highest amounts of polyphenols from 16 to 9 crops, respectively. The highest Polyphenol content of the analyzed crops was 101.339 ± 0.501 M catechin/100 g fresh crop in the water extract of (Table 3; supplementary material).

Figure 2 displays the quantities of the antioxidants of the analyzed crops using total flavonoid method (expressed as mg rutin/100 g fresh crop) ordered in a descending manner. Significant differences (P < 0.001**) were found between different crops (mixed effect of solvents and crop type). Also, different solvents showed significant (P < 0.001**) differences in the amounts of extracted flavonoids. Ethanol solvent extracted the highest amounts of flavonoids in 41 crops, while water and methanol solvents extracted the highest amounts of flavonoids in 10 and 9 crops, respectively. The highest flavonoid content of the analyzed crops was 5.397 ± 0.044 mg/100 g fresh crop for ethanol extract of parsley curly. In addition, water did not extract detectable amounts of flavonoids from loquat, cauliflower, green beans, carrots, bottle gourd calabash, cabbage, cucumis, Indian figs, sweet melon, and watermelon. Similarly, methanol solvent did not extract detectable amounts of flavonoids from banana, cucumis, sweet melon, and watermelon (Table 4; supplementary material).

Figure 3 represents the activity of the antioxidants of the analyzed crops using CUPRAC procedure (mg Trolox/100 g fresh crop) ordered in descending manner. Different crops and solvent extracts revealed significant (P < 0.001**) differences, where methanol extract of sage showed the highest antioxidant activity (2.906 ± 0.019 mg/100 g fresh crop). Water extracts exhibited the highest CUPRAC antioxidant activities in 26 of the analyzed crops. Ethanol and methanol extracts, on the other hand, showed the highest CUPRAC antioxidant activities in 19 and 15 crops, respectively (Table 5; supplementary material).

Figure 4 displays the activity of the antioxidants of the analyzed crops by DPPH method (expressed as percentage). Significant (P < 0.001**) differences were shown for the mixed effect of crop as well as the extract type. DPPH radical scavenging activity percentage ranged from 1.567 ± 1.193% for green beans to 92.300 ± 1.015% for yellow sweet pepper. Water extracts showed the highest DPPH radical scavenging activity for 35 analyzed crops, while methanol and ethanol extracts showed the highest DPPH radical scavenging activity for 16 and 9 crops, respectively (Table 6; supplementary material).

Figure 5 represents the antioxidant activity of the analyzed crops by DPPH method (expressed as mg ascorbic acid/100 g fresh crop). Significant (P < 0.001**) differences were shown for the mixed effect of crop as well as the extract type. DPPH radical scavenging activity (mg ascorbic acid/100 g fresh crop) was the highest for methanol extract of jujube. Water extracts showed the highest antioxidant activity for about half (27) of the analyzed crops. Methanol and ethanol extracts showed the highest antioxidant activity for 19 and 14 crops, respectively. In addition, methanol extract for eggplant (Aubergine) did not exhibit detectable DPPH antioxidant activity (Table 6; supplementary material).

The correlation coefficient R as well as significance values is presented in Table 2. A highly significant correlation (<0.01**) was found between crop type and flavonoid content of all extracts and crop type and polyphenol content of the ethanol extracts. Similarly, crop type was significantly (<0.05*) correlated with the antioxidant activities measured by DPPH radical scavenging
| Fruits | Scientific names | Arabic names |
|--------|-----------------|--------------|
| Apple, green | Malus sylvestris | Tuffah akhdar |
| Apple, red | Malus sylvestris | Tuffah ahmar |
| Apricot | Prunus armeniaca | Mishmish |
| Banana | Musa nana var. ravnendishi | Moz |
| Clementine | Citrus reticulata | Kalamantina |
| Dates, unripe | Phoenix dactylifera | Rutab |
| Dates, ripe | Phoenix dactylifera | Tamer |
| Fig, fresh ripe | Ficus carica | Tin |
| Figs, Indian | Opuntia ficus-indica | Tin hindi |
| Grapes, white | Vitis vinifera | 'Enab abyad |
| Grapes, black | Vitis vinifera | 'Enab aswād |
| Greengage | Prunus domestica italica | Barkouk akhdar |
| Guava, whole | Psidium guajava | Guiwâfah Safrâ’ |
| Jujube | Ziziphus jujuba | Umzbâb |
| Lemon | Citrus limon | Laymân |
| Loquat | Eriobotrya japonica | Aṣkidinya |
| Melon, sweet | Cucumis melo | Shammâm |
| Nectarine | Prunus nectarina var. nectarina | Nakareen |
| Orange, sweet | Citrus sinensis | Burtukal |
| Peach | Prunus persica | Durrâk |
| Plum | Prunus domestica | Khawkh |
| Pomegranate | Punica granatum | Rummâm helou |
| Pomelo, shaddock | Citrus decumana | Bulâmî |
| Strawberry | Fragaria chiloensis | Frawlah, Shulayq |
| Watermelon | Citrullus vulgaris | Battîkh |

| Vegetables | Scientific names | Arabic names |
|------------|-----------------|--------------|
| Beans, green | Phaseolus vulgaris | Lubya khadra |
| Cabbage | Brassica oleracea var. capitata | Malfûf |
| Cabbage, red | Brassica oleracea var. capitata | Malfûf ahmar |
| Calabash, bottle gourd | Lagenaria siceraria var. L. vulgaris | Qara‘e akhdar |
| Carrots | Daucus carota | Gazar |
| Cauliflower | Brassica oleracea var. botrytis | Qarnabit |
| Celery | Apium graveolens var. dulce | Karafs |
| Corn | Zea mays | Dhurah shamiyeh |
| Cucumber | Cucumis sativus | Khîyar |
| Cucumis | Cucumis melo chate | Fakkous |
| Eggplant (Aubergine) | Solanum melongena | Badhinjân |
| Garden rocket, arugula | Eruca sativa | Jarîr |
| Garlic, Chinese | Allium chinense | Thîm seeni |
| Garlic, local | Allium sativum | Thîm baladi |
| Grape leaves | Vitis vinifera | Warak ‘enab |
| Jew’s mallow | Corchorus olitorius | Mulîkhiyâh |
| Lettuce | Lactuca sativa var. longifolia | Khass |
| Mint | Mentha spp. | Na‘ na’ |
| Okra | Hibiscus esculentus | Bamyah |
| Onion, green, immature | Allium cepa | Bassal akhdar |
| Onion, mature | Allium cepa | Bassal |
| Parsley curly | Petroselinum crispum | Bakdûnes |
| Pepper, hot | Capsicum frutescens | Fûlful har |
| Pepper, sweet, green | Capsicum annuum | Fûlful helou |
| Pepper, sweet, red | Capsicum annuum | Fûlful helou |
| Pepper, sweet, yellow | Capsicum annuum | Fûlful helou |
| Potato, white | Solanum tuberosum | Bataṭah |
| Purslane, common | Portulaca oleracea | Bakleh |
| Squash, summer, zucchini | Cucurbita pepo var. | Kûsā |
| Sweet potato, pale | Ipomoea batatas | Bataṭah helwah |
| Thyme | Origanum syriacum | Za’tar baladi |
| Tomato | Lycopersicum esculentum | Banâdâra |
| Wild plants | | |
| Fennel | Foeniculum vulgare | Shûmarah |
| Rosemary | Rosmarinus officinalis | Ekleel aljabal |
| Sage | Salvia officinalis | Meremeh |
% of ethanol and methanol extracts, as well as CUPRAC assay of ethanol and water extracts. In addition, total flavonoid content and antioxidant activity measured by DPPH assay were significantly (P < 0.05*) correlated.
Figure 3. Antioxidant activity analyzed by CUPRAC method (expressed as mg trolox/100 g fresh sample) of the studied crops ordered in descending order (P-value represents the mixed effect of crop type as well as the extracting solvent; values expressed are the average values of the 3 extracting solvents for each plant).

Figure 4. Antioxidant activity analyzed by DPPH assay (expressed as % DPPH scavenging) of the studied crops ordered in a descending order (P-value represents the mixed effect of crop type as well as the extracting solvent; values expressed are the average values of the 3 extracting solvents for each plant).
Antioxidant activity analyzed by DPPH assay (expressed as mg ascorbic acid/100 g) of the studied crops ordered in a descending order (P-value represents the mixed effect of crop type as well as the extracting solvent; values expressed are the average values of the 3 extracting solvents for each plant).

Table 2. Multiple regression coefficients and significance levels of the studied variables.

| Correlated variables                                      | R-value | P-value |
|-----------------------------------------------------------|---------|---------|
| Crop type, flavonoid content of ethanol extracts          | 0.274   | 0.002** |
| Crop type, flavonoid content of methanol extracts         | 0.288   | 0.001** |
| Crop type, flavonoid content of water extracts            | 0.322   | <0.001**|
| Crop type, polyphenol content, ethanol extracts           | 0.245   | 0.007** |
| Crop type, % DPPH radical scavenging of ethanol extracts  | 0.253   | 0.005** |
| Crop type, % DPPH radical scavenging of methanol extracts | 0.226   | 0.013*  |
| Crop type, antioxidant activity measured by CUPRAC assay of ethanol extracts | 0.168   | 0.042*  |
| Crop type, antioxidant activity measured by CUPRAC assay of water extracts | 0.201   | 0.028*  |
| % DPPH radical scavenging of ethanol extracts, flavonoid content of ethanol extracts | 0.974   | 0.043*  |
| % DPPH radical scavenging of ethanole extracts, flavonoid content of methanol extracts | 0.974   | 0.026*  |
| % DPPH radical scavenging of methanol extracts, flavonoid content of ethanol extracts | 0.974   | 0.030*  |
| Antioxidant activity of ethanol extracts measured by DPPH assay as vitamin C, flavonoid content of ethanol extracts | 0.974   | 0.030*  |

Only significant correlations are presented in this table.
* Represents significant correlation at P<0.05.
** Represents significant correlation at P<0.01.

Discussion

To the best of our knowledge, this is the first 60-crop database showing the antioxidant content and activity of Jordanian crops. The database contains total phenolic content, total flavonoid content, and antioxidant activity measured by two methods. This database included water, ethanol, and methanol extracts of the crops. Tables 3–7 are included as supplementary material where the analyzed crops were arranged in descending manner based on the average value of the three extracts, while the statistical analysis was performed for the mixed effect of the crop as well as its various extracts. Each effect alone showed significant (P < 0.001**) differences for all variables as well.

Results of this investigation showed that rosemary (Rosmarinus officinalis) was the richest crop in total polyphenols. This herb has been reported to be a potent antioxidant herb by virtue of its volatile (carvacrol, pinene, and eucalyptol) as well as nonvolatile (carnosic acids and rosmarinic) phenols. The significant antioxidant potency of rosemary made the European Union recognize the herb as a natural antioxidant allowed to be used in food industry. On the other hand, the highest value for total flavonoid content was extracted from parsley curly...
(Petroselinum crispum). The average value for our three extracts (23 meq/g) is not far from that obtained by El-Sayed et al.\cite{42} (who found a value of 46 meq/g). These variations might be due to differences of extraction solvents used and method, variation in the geographical locations of samples, as well as the growing conditions of the analyzed crops.

The highest antioxidant activity measured by CUPRAC assay was found for sage (Salvia officinalis). The average antioxidant activity found in this research is much lower than that found by Sadowska et al.\cite{43} In terms of antioxidant activity analyzed by DPPH assay and expressed as % of scavenging, our values for green sweet pepper are much higher than those found by Sadowska et al.\cite{44} Similarly, our values for antioxidant activity analyzed by DPPH assay and expressed as vitamin C for jujube (Ziziphus jujuba) are 2–3 times higher than those found by Kumar et al.\cite{45}

The figures of antioxidant activity of any natural plant do not merely depend on its composition, but also on the extraction solvent, conditions of the assay used, and the various action modes of the antioxidants present in the plant.\cite{46,47} Lower values of variables do not necessarily indicate low antioxidant potential, rather, it might be due to the efficacy of solvent extraction, test conditions, or different antioxidant mode exhibited. Therefore, it is not surprising that many investigators reported different values for the same crops despite the fact that the analysis procedures were the same. Joan and Monica\cite{48} found polyphenols in cucumber. Likewise, Choudhary et al.\cite{49,50} found flavonoids in watermelon, while Rolim et al. found flavonoids in melon seeds and peels. In addition to the above-mentioned reasons behind the various results, differences in the values found by our team and other investigators might be due to the different parts of crop used for the analysis and the crop planting area.

Despite the significant differences for the mixed effect of crop and the extraction solvents in all of the studied variables, crop type was only significantly correlated with the flavonoid (of all extracts), polyphenol content (of only ethanol extracts), % DPPH radical scavenging (of ethanol and methanol extracts), and CUPRAC values (of ethanol and water extracts). Also, DPPH radical scavenging activity (measured as % scavenging and vitamin C) was significantly correlated with the total flavonoid content. These results indicate that all solvents used were optimum for the extraction of the total flavonoid content from most of the crops analyzed, while water was the best solvent for the extraction of the total phenolic content. The various results in the antioxidant activities may be due to the various antioxidant modes exhibited by flavonoids and other classes of polyphenols found in the analyzed crops. The correlation indicates the relationship and the model fit between two variables (dependent and independent).\cite{38} R-values for the correlations ranged between 0.168 and 0.974. R-value represents the Pearson’s correlation coefficient between the predicted scores by the regression model and the real values of the dependent variable representing the strength of the association.\cite{38}

**Conclusion**

The analyzed Jordanian crops in this study are considered sources of natural antioxidants. Values found by our research team are comparable with those found by other teams for some crops (using the same methods of analysis) and different for others. Antioxidant content and activity correlated well in the presented results. Future research is recommended to further test the analyzed crops with the highest antioxidant activities in the prevention and/or treatment of oxidative stress-induced chronic ailments at both *in vitro* and *in vivo* levels. In addition, other crops, whether fresh or synthesized, should be analyzed for total phenolic content, total flavonoid content, and antioxidant activity to enrich the existing database. Moreover, these natural crops rich in antioxidants should be included in foods as well as manufactured as pharmaceutical supplements for natural safe antioxidants. Therefore, the knowledge gained from this investigation enriches the database of total phenolic content, total flavonoid content, and antioxidant activity of Jordanian crops both locally and internationally and lays the basis for further related research in nutritional and pharmaceutical industries.
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Author contributions

Conceptualization, Hiba F. Al-Sayyed; Salma M. AbdelQader, Dimas H. Sweidan and Lana T. Rizeq; software, Tawfiq A. Arafat, and Marwan M. Mwalla; validation, Hiba F. Al-Sayyed, Dimas H. Sweidan and Lana T. Rizeq; formal analysis, Salma M. AbdelQader; investigation, Hiba F. Al-Sayyed, Dimas H. Sweidan and Lana T. Rizeq; data curation, Hiba F. Al-Sayyed; writing—original draft preparation, Refat A. Al-Kurd, and Iman F. Mahmoud; writing—review and editing, Hiba F. Al-Sayyed; supervision, Hiba F. Al-Sayyed; project administration, Hiba F. Al-Sayyed, Tawfiq Arafat, Marwan Mwalla; funding acquisition. All authors have read and agreed to the published version of the manuscript.”

Disclosure statement

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