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Chemical Properties, Phenolic Profiles and Antioxidant Activities of Pepper Fruits

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ABSTRACT

This study was conducted to investigate the phytochemical properties of different varieties of pepper fruits, to identify and quantify the phenolic profile of methanolic extracts of the aforementioned plants and to evaluate the antioxidant activity of the crude extracts of the plants under investigation in comparative study. Results revealed that ascorbic acid and total carotenoids contents of pepper fruits ranged between 83.30 to 194.44 (mg/g fw) and 12.19 to 75.72 (µg/g F.W), respectively. Balady green pepper extract contained the highest total polyphenols and total flavonoids contents as 52.65 (mg GAE/g) and 41.69 (mg QE/g), respectively. Hesperidin was the predominant flavonoid in red bell and Balady green peppers extracts with concentrations of 1513.13 and 1065.65 µg/g, respectively. While, pyrogallol was identified as the highest phenolic compound in yellow bell pepper extract as 2175.89 µg/g. This study also confirmed the highest antioxidant activity of Balady green pepper extract using total antioxidant capacity (TAC), DPPH radical scavenging and reducing power assays. This study recommends consumers using the balady green pepper as a rich and inexpensive source of natural antioxidants

Keywords: pepper fruits, methanolic extracts, polyphenols, HPLC, antioxidant activity

INTRODUCTION

Free radicals or reactive oxygen species (ROS) are harmful intermediates to human cells as they undesirably react with macromolecules such as DNA, lipids, protein and cell membrane causing oxidative stress and related disorders (*Alho and Leinonen 1999; Mantena et al., 2008*). In other words, oxidative stress is principally an imbalance between free radicals production and the capability of the human body to neutralize or detoxify their risky effects via antioxidants neutralization (*Lassoued et al., 2015*).

Antioxidants are substances that diminish cellular damage attribute to oxygen, such as that caused by free radicals. They play an important role in activating the defense system of living organisms and protecting against cell damage by their capacity of scavenging the free radicals, forming complexes with pro-oxidant metals that quenchers and reducing factor of singlet oxygen formation (*Cabral de Oliverira et al., 2009; Adedayo et al., 2010*). Additionally, well known antioxidants are considered to have a key role in protection against numerous diseases especially cardiovascular disease, cancer (Dumas *et al*., 2003), and inflammatory disorders (*Chanda and Kaneria2012; Deng et al., 2013*).

Conveniently, all organisms contain small antioxidants molecules such as tocopherols, ascorbic acid and glutathione as anti-free radical defense systems (*Kalaivani and Mathew 2010*). Besides, antioxidant enzymes (superoxide dismutase and peroxidase, etc.) show the large antioxidants molecules that inhibit (ROS) from attacking other essential proteins by absorbing them (*Taher et al., 2018*). Interestingly, dietary fruits and vegetables are rich source of flavonoids and phenolic compounds show strong antioxidant activities and can prevent body cells from injuries which initiated by hydrogen peroxide or lipid peroxides through scavenging of free radicals (*Kaewseenjan et al., 2015*).

Pepper fruits (*Capsicum annum l.)* belonging to solanaceae family, are excellent sources of antioxidants such as polyphenolic compounds, carotenoids, vitamins C and A for that it's an important source of nutrients in human diet (*Guil-Guerrero et al., 2006*). Their solvent extracts showed strong effects as antifungal, antibacterial and chemotherapeutic agents (*Srivastava et al., 2011*). The red and orange pepper fruits are seemed due to the presence of carotene and capsanthin, while β-cryptoxanthin, zeaxanthin, lutein and β-carotene are responsible of the yellowish orange color of peppers (*Ademoyegun et al., 2011*). Green peppers are also documented to be an important source of chlorophylls (*Yamaguchi et al., 2001*). The amount of ascorbic acid decreased or remained unchanged as color of pepper developed in some cultivars of peppers, but increased in others (*Simonne et al., 1997*). Provitamin A in most cultivars of pepper enriched as color developed with the exception of yellow varieties, while the highest activity of provitamin A was exhibited in brown peppers compared with other colours (*Simonne et al., 1997*).

Collectively, no comprehensive data are available in the literature regarding antioxidant capacity assessment of balady green pepper extract in a comparative study with colored bell peppers extracts. Therefore, the present study aims to (I) investigate the phytochemical properties (II) identify and quantify the phenolic constituents and (III) evaluate antioxidant activity of the three coloured pepper fruits using three different methods.

MATERIALS AND METHODS

Plant material and Extraction

Samples were obtained from local market at Mansoura city, Dakahlia governorate, Egypt (2016-2017). Fresh yellow bell (YBP), red bell (RBP), and Balady green pepper fruits (BGP) (*Capsicum annum l.*) were washed, sliced into small pieces and stored until the beginning of the extraction. Samples were extracted by soaking in methanol for 2 days at room temperature then, filtered. The residue was re-extracted with methanol for another 2 times. The collected filtrates were evaporated using rotary evaporator under pressures at 45C°. Crude methanolic extracts for pepper fruits (PEs) were kept in refrigerator at 4C° until starting analysis.

Preliminary phytochemical testing of crude plant extracts

Phytochemical screening of methanolic extracts of pepper fruits was done using standard methods as described by *Jayaprakash and Sangeetha (2015).* Glycosides test was done according to *Samatha et al., (2012).*

Estimation of ascorbic acid content

Ascorbic acid content of fresh pepper fruits was measured according to *Casanas et al., (2002)* via titration procedure using the 2, 6-dichlorophenol indophenol solution

[0.25 g 2, 6- dichlorophenol (DCPIP) and 0.2 g $NaHCO_3$/L distilled water]. Four grams of each fresh pepper fruit sample was extracted separately with 10 ml of (4% w/v) oxalic acid. Filtrates were titrated against indophenol solution dye (v_2) until pink color appeared and stable for a few minutes. Two ml of ascorbic acid (0.1% w/v) solution and 10 ml of (4% w/v) oxalic acid were homogenized separately and titrated against DCPIP dye (v_1). Ascorbic acid content (mg/g) was calculated using the next formula:

$$\text{Ascorbic acid content (mg/g)} = \frac{v2 \times amount\ of\ ascorbic\ acid\ (mg)}{v1 \times sample\ weight\ (g)}$$

Where v_2 and v1 represent volumes of DCPIP requested for the titration of the sample and for 0.1 g ascorbic acid, respectively.

Determination of carotenoid contents

Total carotenoid contents of fresh pepper fruits were estimated according to the scheme described by Jaeger de *Carvalho et al., (2012)*. Briefly, 2 g of fresh pepper fruit was homogenized, separately with 5 ml acetone then filtered. Residue was re-homogenized for another 2 times in the same manner. The precipitate of each sample was soaked overnight in 20 ml acetone until become colorless. The resultant extract was collected and transferred to a suitable separatory funnel containing 20 ml petroleum ether 60-80ºC followed by addition of few drops of distilled water and carefully shaken. This step was repeated two times and the pet.ether layer was separated then filtered over anhydrous sodium sulfate to remove any water. Absorbance of carotenoid was recorded at 450 nm. Total carotenoid content of fresh pepper fruits was calculated by the next formula:

$$\text{Total carotenoids (µg/g)} = \frac{A \times v(ml) \times 10000}{A_{1cm}^{1\%} \times P(g)}$$

Where A= Absorbance of the tube sample at 450 nm; V= Total volume of extract; $A_{1cm}^{1\%} = 2592$ (Extinction Coefficient of β-carotene in the solvent); P= weight of sample (g).

Determination of total phenolic contents

Total phenolic content of PE was measured spectrophotometrically using three replicates according to Folin-Ciocalteau method as described by *Farag et al., (2014)*. A volume of about 0.5 ml (containing 500 ppm) of each methanolic extract was mixed with 0.1 ml Folin-Ciocalteau reagent and 0.5 ml of sodium carbonate 7.5% (w/v). Resultant mixture was incubated for 60 min in the dark at room temperature and the absorbance was determined at 740 nm, against distilled water as blank. The standard curve was plotted using gallic acid as a reference for phenolic compound. Total phenolic content in methanol extract was expressed as mg gallic acid equivalent per g dry extract (mg GAE/g dry weight of extract).

Determination of total flavonoid contents

Total flavonoids content were estimated using aluminum chloride assay as proposed by *Munhoz et al., (2014)*. Each methanolic extract (0.5 ml) with concentration of 500 ppm was dissolved in 2 ml of the same solvent. Then, 0.2 ml of 1M potassium acetate, 0.3 ml of (10% w/v) $AlCl_3$ and finally, 2 ml of distilled water was added to the mixture. The obtained mixture was remained at room temperature and allowed to stand for 30 min. Absorbance of samples was measured at 430 nm. In blank sample, aluminum chloride was replaced by the same volume of distilled water. Quercetin was used as a reference for plotting the standard curve. Total flavonoids content in methanol extract were expressed as mg quercetin equivalent per gram extract (mg QE/g dry extract) from triplicates.

Determination of phenolic acid and flavonoids using HPLC-analysis

Determination of flavonoids and phenolic acid compounds were performed using high-performance liquid chromatography (HPLC) in food technology research institute, Doki, Giza, Egypt. Identification of flavonoids was done as described by *Mattila et al., (2000*) using HPLC connected to diode-array (DAD) and electro-array (EC). Phenolic compounds were separated and determined by reverse phase HPLC (RP-HPLC)/diode array detection (DAD) compatible with the technique mentioned by *Goupy et al.,* (1999).

Determination of antioxidant activity

Total antioxidant capacity

The total antioxidant capacity of pepper methanolic extracts was determined using phosphomolybdate method, as mentioned by *Arefin et al., (2015)*. Exactly, 0.3 ml of each methanol extract with a concentration of 500 ppm was added to one ml of reagent mixture (0.6 M sulphuric acid, 28 Mm Na_2HPO_4, and 4 mM ammonium molybdate) then incubated at 35C˚ for 90 min in water bath. After the solution mixture was cooled at 25C˚, absorbance value was recorded at 765 nm. Blank was prepared by substituting the sample with 0.3 ml of methanol. Ascorbic acid served as a standard. The experiment was performed in three replicates.

DPPH free radical scavenging activity

Evaluation of the antioxidant activity was determined in three replicates using 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging according to *Dasgupta et al., (2015)*. About 0.3 ml of each concentration of methanol extracts was treated with 0.9 ml of methanolic DPPH solution (0.06 mM). The absorbance of mixture was measured at 517 nm, after incubation in dark for 30 min at 25C˚. The control was prepared by adding 0.3 ml methanol instead of sample extract. Different concentrations of BHT were also tested as reference antioxidant. The inhibition percentage of DPPH scavenging activity was calculated as follows:

$$\text{\% inhibition of DPPH assay} = \frac{A_\circ - A}{A_\circ} \times 100$$

Where $A_\circ$ = the absorbance of the control; A = the absorbance of the sample.

Reducing power assay

Reducing capacity assessment was determined using triplicates by the method of *Wang et al., (2015)*. Briefly, One ml of different concentration ranged between (62.5 to 1500 µg/ml) of various extracts was added to 3 ml sodium phosphate buffer (0.2 mM, pH= 6.6) and 3 ml of (1.25 % w/v) potassium ferricyanide. After that, the reaction mixture was incubated in water bath at 50 Cº for 25 min. Then, 3 ml of trichloroacetic acid (10% w/v) was added, followed by shaking and the tubes were rest for 10 min. After wards, 2.5 ml of distilled water was mixed with 2.5 ml of upper layer and 1ml of ferric chloride (0.1% w/v) and left for 10 min. The absorbance of the mixture was determined at 700nm.EC_{50} values were calculated.

Statistical analysis

All analyses were performed in three replicates using the SPSS 17.0 software (SPSS Inc, Chicago, USA). The mean values were compared with averages based on the analysis of variance using on-way ANOVA at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 lists the results of phytochemical screening of pepper fruits extracts. The mother methanolic extracts of pepper fruits exposed the occurrence of flavonoids, glycosides, saponins, terpenoids, alkaloids and phenols. Tannins were absent in all peppers extracts. Likewise, our results belonging to the phytochemical constituents of peppers extracts agreed to a large extent with those described by *(Saidu and Garba 2011*; *Jyothiprabha and Venkatachalam 2016*), for different solvent extracts of pepper fruits.

Table 1. The preliminary phytochemical test of pepper fruits extracts.

Phytochemical test	RBP	YBP	BGP
Flavonoids	+++	++	+++
Tannins	-	-	-
Glycosides	++	+++	++
Saponins	+	+	+++
Terpenoids	+++	+++	+++
Alkaloids	++	+++	+++
Phenols	+	++	+

YBP= yellow bell pepper; RBP=red bell pepper; BGP= balady green pepper

Ascorbic acid content

Vitamin C plays an important role in plants and human cells *Pacier and Martirosyan (2015*). Pepper fruits like most vegetables are low in calories but high in vitamins especially in vitamin C. However, different levels of ascorbic acid could be detected, this may be due to varieties, post-harvest (processing and storage) ,maturity stage and growth conditions such as, soil, temperature and fertilizer (*Lee and Kader, 2000; Njoku et al., 2011*). It could be seen from table (2) that ascorbic acid content of pepper fruits ranged between 83.3 to 194.44 mg/g fw. The ascorbic acid content in yellow pepper (194.44 mg/g fw) and in red pepper (180.56 mg/g fw) are significantly higher when compared with that of green pepper (83.30 mg/g fw). Similar evidence is available to support the current results. *Nerdy (2018)* recorded a wide variation in vitamin C content of different colored peppers with superiority of yellow bell pepper.

Total carotenoids

Carotenoids are plant pigments that are common in fruits and vegetables and have many functions as a biological antioxidant. Table 2 showed the total carotenoids content of pepper fruits with various colors. The results presented that red pepper contained the highest amount of total carotenoids (75.72 µg/g), followed by yellow peppers (28.34 µg/g) and lastly for balady green pepper (12.19 µg/g). Based on the obtained results, the variation in carotenoids content of different colored peppers agreed with those stated by *Kevrešan et al., (2009)*. They established that the total carotenoid content in fresh red pepper was higher than that of yellow pepper.

Table 2. Some chemical contents of fresh peppers fruits.

Samples	Ascorbic acid (mg/g F.W)	Carotenoids (µg/g F.W)
RBP	180.56	75.72
YBP	194.44	28.34
BGP	83.30	12.19

YBP= yellow bell pepper; RBP=red bell pepper; BGP= balady green pepper

Total phenolic content

Polyphenols are secondary metabolites widely distributed in plants and one of the principal sets of compounds employ as antioxidant. Total polyphenolic content was determined according to the Folin-Ciocalteau method using gallic acid as a standard. According to the data presented in table (3), balady green pepper extract (BGPE) presented the highest polyphenol content i.e. 52.65 mg GAE / g dw extract. On the contrary, yellow and red pepper, their methanolic extracts contained considerably low amounts of total polyphenols i.e. 43.59 and 42.57 mg GAE / g d.w extract, respectively.

The obtained value of yellow pepper extract was noticeably lower than that documented by *Park et al., (2012*), who recorded a value of 91.47 mg GAE/ g dw. Also, total polyphenols values of red and green pepper methanolic extracts in this study were much lower than those reported by *Park et al., (2012*). Noticeably, our results showed a different trend regarding total polyphenolic content in bell peppers as that described in the literature. For instance, *Chuah et al., (2008*) observed that red paprika contained the maximum total phenolic content (TPC), while green paprika had the lowest content. Likewise, *Zhang and Hamauzu (2003)* established that total polyphenols content in different colored bell pepper followed this order: red bell pepper > yellow > green bell pepper. Our results are also in agreement with those reported by *Blanco-Ríos et al., (2013),* who mentioned that the green orion variety contained the maximum concentration in polyphenols, while no variations were noticed between the varieties taranto (yellow), mazurca (red) and simpaty (orange). Overall, polyphenolic content in peppers is affected by agronomic conditions, postharvest handling, type of cultivar and maturity *Zaki et al., (2017*).

Table 3. Total polyphenols and total flavonoids contents of different extracts of pepper fruits.

Samples	Polyphenols (mg GAE/g)	Flavonoids (mg QE/g)
RBPE	42.57	39.19
YBPE	43.59	33.65
BGPE	52.65	41.69

YBPE= yellow bell pepper extract; RBPE=red bell pepper extract; BGPE= balady green pepper extract

Total flavonoids content

Total flavonoid content was determined by using the aluminum chloride method with quercetin as a reference. Total flavonoids of samples are illustrated in table (3) and varied from 33.65 to 41.69 mg QE/g dw. Balady green extract showed the highest amount of total flavonoids (41.69 mg QE/g), followed by red pepper extract (39.19mg QE/g) and lastly for yellow pepper extract (33.65 mg QE/ g). Our results are in agreement with those described by *Marinova et al., (2005),* who recorded that green pepper had a higher flavonoids (27.4 mg QE/100g FW) than that of red pepper(13.7 mg QE/100g FW). Contrarily, *Materska and Perucka (2005*) found that red pepper contained higher flavonoids content that of green ones. It is worthy to mention that, our values were higher than those stated by *Shaimaa et al., (2016*), who stated that total flavonoid contents of some Egyptian sweet and chilli peppers extracts ranged from 10.28 to 15.52 mg QE/g DW. Differences in environmental and/or genetic factors, may explain the discrepancy of total flavonoid contents when compare with those of the previously mentioned range. Finally, differences in flavonoids and polyphenols contents may be related to pepper maturity, species, growing and post-harvest conditions.

HPLC-analysis

Thirty-one phenolic compounds of peppers extracts could be identified and quantified by HPLC (Table4). The phenolic profile of all extracts under investigation involved fifteen compounds as phenolic acids, two simple phenols, six flavonoid aglycones and eight flavonoid glycosides with different quantities.

Interestingly, hesperidin was identified as the predominant phenolic compound in red and green peppers extracts with concentrations of 1513.13 and 1065.65 µg/g, respectively (Table, 4). Other phenolic constituents with high or moderate quantities of red pepper extract were catechin, pyrogallol, luteolin 7-glucose, P-OH-benzoic acid, apig. 6-rhamnose 8- glucose, rutin, naringin, quercetrin, ellagic acid, apig.6-arbinose 8-galactose, salicylic, gallic and benzoic acids with a concentration of 793.50, 757.66, 413.57, 395.16, 314.70, 290.39, 275.00, 241.83, 172.18, 156.42, 143.99, 115.74 and 111.81 µg/g, respectively. Successively, seventeen phenolic compounds were detected in trace amounts in red pepper extract. Pyrogallol was also detected in considerably high quantity in green pepper extract (572.77µg/g, table 4). Other phenolic constituents with moderate amounts in green pepper extract were quercetrin (394.23µg/g), catechin (295.39 µg/g), catechol (279.42 µg/g), naringin (190.19 µg/g), luteolin 7-glucose (181.12 µg/g), apig. 6-rhamnose 8- glucose (170.96 µg/g),apig.6-arbinose 8-galactose (151.66 µg/g) , protocatechuic acid(116.09 µg/g), ellagic acid(106.67 µg/g) and rutin(93.43 µg/g). While nearly nineteen phenolic compounds existed in trace amounts in green pepper extract.

In case of yellow pepper extract, pyrogallol was identified as the largest phenolic compound as 2175.89 µg/g. The order of richness of other phenolic compounds with significant amounts was catechin > catechol > hespirdin > salicylic acid > benzoic acid> ellagic acid > P-OH-benzoic acid> gallic acid > chlorogenic acid > protocatechuic acid.

Accumulated polyphenol content in different peppers is essentially found to be influenced with ripening stage and genotype *Shaha et al., (2013).* So, obtained data relating the identification of phenolic constituents of peppers extracts were not easy to compare with literature data. However, our data slightly in agreement with those of *Shaimaa et al., (2016),* who stated that hesperidin, quercetrin, rutin, catechol, oleuropein, chlorogenic, pyrogallol and 3-hydroxy tyrosol, were presented at higher levels than other phenolics in of some Egyptian sweet and chilli peppers. While, *Nagy et al., (2015)* summarized that the major phenolic componds in New Hybrids of Chili peppers were naringenin-diglucoside, catechin, vanillic acid-derivative and luteolin-glucoside.

Antioxidant Activity

Many procedures have been developed to evaluate the antioxidant capacity in vitro, in order to let rapid screening of examined samples. In the present study, antioxidant activity of pepper fruit extracts was estimated by DPPH radical scavenging scheme in addition to phosphomolybdenum antioxidant and reducing power assays.

Total antioxidant activity

Phosphomolybdenum antioxidant test has been considered on the basis that the reactant antioxidants in sample extract might afford to the reduction of phosphate-molybdenum (VI) to green molybdenum complex (V) *Chaouche et al., (2014*). Total antioxidant activity of extracts was expressed as the number of ascorbic acid equivalents (AAE) per gram. In this study, total antioxidant capacity of the examined extracts ranged between113.03 to 245.75 as mg ascorbic acid equivalent per gram (table 5 and fig 1).

Table 4. Identification and quantification of pepper extracts phenolic contents by HPLC.

Phenolic compounds	RBPE	YBPE	BGPE
(A) Phenolic acids			
Gallic acid	115.74	119.48	89.98
4-Aminobenzoic acid	21.34	50.19	22.09
Protocatechuic acid	97.21	95.37	116.09
Chlorogenic acid	60.47	103.78	60.84
P-OH-benzoic acid	395.16	123.19	65.85
Caffeic acid	41.33	62.96	18.09
Vanillic acid	17.70	31.62	43.85
P-Coumaric acid	26.07	18.14	46.69
Ferulic acid	11.88	35.14	48.42
Ellagic acid	172.18	144.52	106.67
Alpha-coumaric acid	7.65	6.41	3.36
Benzoic acid	111.81	173.04	66.55
Salycillic acid	143.99	203.48	86.78
3,4,5-methoxy-cinnamic acid	13.82	13.61	14.69
Cinnamic acid	8.11	4.65	3.51
(B) Simple Phenols			
Pyrogallol	757.66	2175.89	572.77
Catechol	89.77	225.73	279.42
(C) Free flavonoids			
Catechin	793.50	745.53	295.39
Quercetin	46.36	9.66	16.24
Apegnin	36.28	6.59	32.39
Naringenin	13.64	1.54	2.12
Hespirtin	37.00	7.07	38.05
Kampferol	31.15	9.53	22.48
(D) Bound flavonoids			
Apig. 6-arbinose 8- glactose	156.42	67.88	151.66
Apig. 6-rhamnose 8- glucose	314.70	77.31	170.96
Luteolin 7-glucose	413.57	92.21	181.12
Naringin	275.00	50.13	190.19
Rutin	290.39	49.51	93.43
Hespirdin	1513.13	213.06	1065.65
Apigenin. 7-O-neohespiroside	40.27	4.51	33.55
Quercetrin	241.83	62.34	394.23

YBPE= yellow bell pepper extract; RBPE=red bell pepper extract; BGPE= balady green pepper extract; GSE = grape seed extract

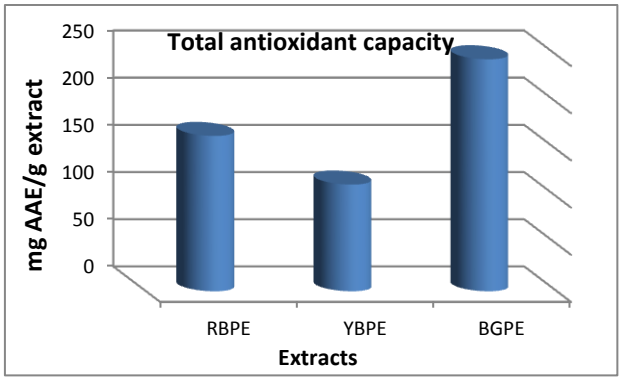

Fig. 1. Total antioxidant capacity of tested extracts

YBPE= yellow bell pepper extract; RBPE=red bell pepper extract; BGPE= balady green pepper extract

Our results using phosphomolybdenum antioxidant assay demonstrated that green pepper extract had the highest antioxidant potential (245.75 AAE/g), followed by red pepper extract (164.54 AAE/g), and lastly for yellow pepper extract as (113.03 AAE/g). Interestingly, the antioxidant activity of balady green pepper extract was 1.49 fold greater than that of red pepper extract and 2.17

fold higher than that of yellow pepper extract. There is no data in the literature of the examined plants to compare with current findings of antioxidant activity using total antioxidant activity assay.

DPPH assay

The DPPH radical is commonly used to determine the antioxidant activity as a substrate. The DPPH is a stable free radical to become stable molecule that can accept a hydrogen radical or electron from antioxidant substances found in examined samples *Taher et al., (2018*). The scavenging of DPPH radical was evaluated by decreasing in the absorbance resulted by antioxidant at 517nm. In this study, pepper extracts were tested in concentrations ranged between (500 to 2000 µg/ml). All of the tested samples had concentration-dependent increases in scavenging potential. For instance, at a concentration of (500 µg/mL), the scavenging action of red, yellow and green peppers reached 31.08, 5.69 and 34.54 %, respectively. While at a concentration of 1500 µg/mL, the scavenging action of these extracts were 47.89, 10.00 and 63.76 %, respectively.

IC_{50} can be defined as the concentration of the examined sample needed to inhibit fifty percent of known radical *Taher (2019)*. The lower calculated IC_{50} value is considered as better antioxidant capacity. Results tabulated in Table 5 and illustrated in fig 2 indicated that the IC_{50} value of samples extracts using DPPH assay ranged between (1114.17 to 3267.68 µg/mL).

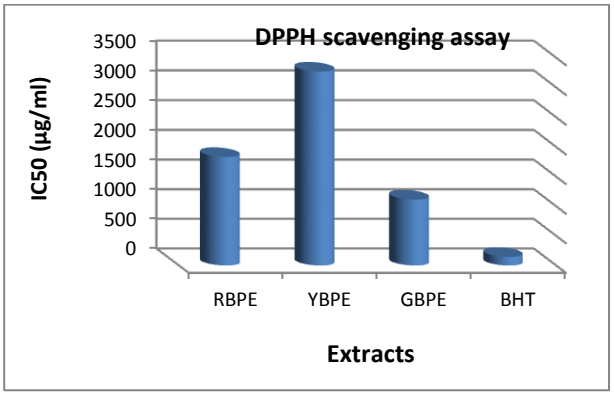

Fig. 2. IC_{50} values of tested extracts using DPPH assay
YBPE= yellow bell pepper extract; RBPE=red bell pepper extract; BGPE= balady green pepper extract; ; BHT = butylated hydroxyl toluene

Balady green pepper extract showed the lowest IC_{50} value (1114.17µg/mL, table 5), indicating considerable antioxidant capacity. While, red and yellow peppers extracts exhibited higher values of IC_{50} as 1832.25 and 3267.68 µg/mL, respectively. Based on the results achieved, IC_{50} value of green pepper extract was little lower than that obtained by *Park et al., (2012)* for green bell pepper methanolic extract (1,153.63 µg/mL). The above results indicated that the scavenging antioxidant activity of pepper extracts is in the following order: green> red > yellow. This finding conflict with that previously reported by *Park et al., (2012)*, who found that yellow pepper extract had the highest antioxidant potential(IC_{50} = 811.09 µg/mL), followed by red pepper extract (IC_{50} = 882.31µg/mL), and lastly for green pepper extract. It could be suggested that environmental factors could be affected on the antioxidant activity of different colored peppers particularly yellow type variety.

Table 5. Total antioxidant capacity and the IC_{50} value of DPPH.

Sample	Total antioxidant capacity (mg AAE /g)	IC_{50} of DPPH assay (µg/ml)	EC_{50} of Reducing power assay (µg/ml)
RBPE	164.54	1832.25	3813.67
YBPE	113.03	3267.68	451.11
BGPE	245.75	1114.17	2039.17
BHT		145.23	
AA			196.81

YBPE= yellow bell pepper extract; RBPE=red bell pepper extract; BGPE= balady green pepper extract; BHT = butylated hydroxyl toluene; AA= Ascorbic acid

Reducing power assay

In this assay, the yellow colored solution of tested sample alters to various shades of green and blue colours. The reducing power assay was performed by the transformation of Fe^{3+} (ferricyanide complex) to Fe^{2+} (ferrous form) in the presence of antioxidant components in examined extracts *Taher et al., (2018)*. Higher absorbance of the reaction mixture indicated greater reducing power.

The reducing power of pepper extracts, relatively increased with graded concentrations (62.50 to 1500 µg/ml; fig, 3), which offers that the electron-donating capability of all of the examined samples is dose dependent. For instance, red, yellow and green pepper extracts exhibited absorbance values of 0.146, 0.196 and 0.138 at a concentration of (250 µg/ml), respectively. While at higher concentration (750 µg/ml), aforementioned extracts give absorbances of 0.216, 0.633 and 0.243, respectively.

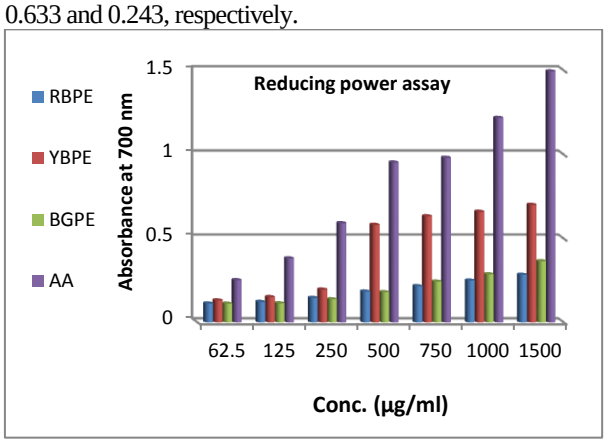

Fig. 3. Reducing power assay of pepper extracts
YBPE= yellow bell pepper extract; RBPE=red bell pepper extract; BGPE= balady green pepper extract; AA= Ascorbic acid

EC_{50} value is defined as the extract concentration that giving 0.500 of absorbance. Unexpectedly, YBPE showed the lowest EC_{50} value (451.11 µg/mL, table 5), followed by BGPE (2039.17µg/mL) and lastly for RBPE (3813.67 µg/mL). Overall, reducing power capacity of peppers extracts followed the order: yellow pepper extract > green pepper extract > red pepper extract.

Correlation between the examinations of total antioxidant ability by DPPH, total antioxidant capacity (TAC) and reducing power (RP) assays.

To evaluate the appropriateness and reliability of the procedures, used to estimate the antioxidant ability of pepper extracts, correlation study was undertaken between DPPH, RP and TAC assays. Regression analysis using the Pearson correlation coefficients (R) is presented in table (6). R value had negative significance level (R= -0.949, $p < 0.001$),

indicating that the estimation of antioxidant activity by DPPH and TAC assays were very correlative. Likewise, it can be established that DPPH and TAC assays were proper and dependable for evaluating antioxidant activity of pepper extracts. Similar findings have been recently reported by *Taher (2019),* who found some strong correlations (R= 0.957-0.995) between the assay techniques used in weighing of total antioxidant activity of different extracts of *Pea peels.* Whereas, non-significant correlation between reducing power assay with DPPH and TAC was noted in this study. The variation in correlation coefficient between different assays of antioxidant, indicate the fact that single method may not be used to assess the total antioxidant capacity *Silva et al.,* (2006)

Table 6. Correlation R between different antioxidant activities estimated by DPPH, TAC and RP assays and those between antioxidant assays and TP and TF of pepper extracts.

	TPC	TFC	DPPH	TAC	RP
TPC	1	-	-	-	-
TFC	0.653 (0.0567)	1	-	-	-
DPPH	-0.668* (0.0492)	-0.995*** (0.0000)	1	-	-
TAC	0.847** (0.0039)	0.937*** (0.0002)	-0.949*** (0.0001)	1	-
RP	- 0.116 (0.7668)	0.651 (0.0575)	-0.63 (0.0687)	0.355 (0.3484)	1

TPC= total polyphenolic content; TFC= total flavonoid content; TAC =total antioxidant capacity; RP= reducing power

R , correlation coefficient. The values in parenthesis represent the P values .* significant level at p<0.05 , ** significant level at p<0.01, * significant level at p<0.001**

Correlation between total antioxidant ability, TPs and TFs

Total antioxidant activity of tested plant extracts is regularly attributed to phenolic contents (*Pandey et al., 2017; Taher et al., 2018*). So, correlation study of total polyphenols and flavonoids contents of the tested extracts in the present study with their antioxidant capacity was done and the results are presented in table (6). The Pearson's correlation coefficient between total flavonoids content (TFC) of the tested extracts with IC_{50} value of DPPH (R= - 0.995 p < 0.0000) and the values of total antioxidant capacity TAC (AAE /g, R= 0.937 p < 0.0002) offered significant correlation. It means that total flavonoids content of the examined extracts have a direct correlation on total antioxidant activity.

In the same route, total polyphenols content in examined extracts had a significant and positive correlation with total antioxidant capacity (AAE /g, R= 0.847, p< 0.0039). Whereas, TPC had a significant and negative correlation with IC50 value of DPPH (R= -0.668, p< 0.0492). On the other hand, there was no significant correlation between EC50 value of reducing power assay with TPC (R= -0.116, p< 0.7668). Our results are different from the data was mentioned by *Loǵanayaki et al., (2013*) who found relationship between TPC and RP.

Overall, the significant correlations found in the present study supports that the phenolic compounds, predominantly flavonoids, contribute to the antioxidant capability of the examined extracts. Our findings are in agreement to the studies which reported the relationship of antioxidant capacity and antiradical activity using DPPH with flavonoid compounds (*Pandey et al., 2017; Taher 2019*). Also, total flavonoids exhibited a good correlation with the most of antioxidant methods *Mccune and Johns (2002).* However, non-significant correlation between total flavonoids and antioxidant activity using reducing power assay was noted in this study. This discrepancy might be explained by the hypothesis that antiradical activity in many cases does not parallel to the antioxidant capacity *Tirzitis and Bartosz (2010*). In addition, *Mccune and Johns (2002),* suggested that the differences in correlation coefficient among antioxidant activity methods with total phenols and total flavonoids may be due to the fact of contribute the antioxidant activity with other compounds such as tocopherol, vitamin C and carotenoids rather than total flavonoids and total phenols.

Although, balady green pepper had the lowest contents of ascorbic acid and total carotenoids, the present study revealed that its methanolic extract had the highest antioxidant capacity in comparison to red bell and yellow bell peppers marketed in Mansoura city. Based on this observation, the variation in antioxidant capacity of colored peppers agreed with those stated by *Shaimaa et al., (2016).* They stated that antiradical potential was generally increased in green immature peppers than red mature and in chilli peppers than sweet. On the contrary, *Tundis et al., 2013)* found that ripe peppers had a higher antioxidant potential than that of immature fruit using β-carotene-linoleate model system. However based on DPPH antiradical assay, the effect was upturned. In our study, the antioxidant activity of mature bell pepper (red) was higher than that the maturing (yellow) pepper using DPPH antiradical and TAC assays. However based on reducing power assay, the effect was reversed. Overall, the antioxidant ability of pepper fruits largely depends on the variety and the status of maturity. As mentioned before, green pepper extract had the highest total flavonoids content (41.69 mg QE/ g, table, 3). So, the great antioxidant activity of BGPE might be due to its high flavonoids content and/or the presence of particular flavonoids rather than those identified by HPLC techique.

In conclusion, the present study confirms the reasonable antioxidant activity of pepper fruit extracts essentially due to its flavonoids content.

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الخواص الكيميائية والتفريدات الفينولية والنشاطات المضادة للاكسدة لثمار الفلفل المختلفه

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تهدف الدراسه الحالية الى تقييم بعض الخواص الكيميائية لثمار الفلفل مختلفه الالوان ولتعريف وتقدير المحتويات الفينولية المتواجده فى المستخلصات الميثانوليه للنباتات محل الدراسة واخيرا لتقييم النشاط المضاد للاكسده لهذه المستخلصات فى دراسة مقارنة . وقد اظهرت النتائج ان محتوى حامض الاسكوربيك والكاروتينات الكلية فى ثمار الفلفل مختلفة الالوان تتراوح بين 83,30 الى 194,44 مليجرام لكل جرام وزن طازج و 7,72 الى 12,19 ميكروجرام لكل جرام وزن طازج على التوالى. وقد اظهرت النتائج ايضا أن مستخلص الفلفل البلدي الاخضر يحتوى على اعلى قدر من الفينولات العديدة الكلية بمستوى 52,65مليجرام مكافئات حامض الجاليك لكل جرام وايضا يحتوي علي اعلى محتوى من الفلافونيدات الكلية بقدر 41,69 مليجرام من مكافئات الكيورستين لكل جرام مستخلص على التوالى . وقد لوحظ ان الهسبريدين هو الفينول الاعلى تواجدا فى مستخلصات الفلفل الحلو فى طور اللون الاحمر والفلفل الاخضر البلدى بتركيزات 1513,13 , 1065,65 ميكروجرام لكل جرام مستخلص على الترتيب . بينما وجد ان البيروجالول هو الفينول الاكثر تواجدا فى مستخلص الفلفل الاصفر بتركيز 2175,89 ميكروجرام لكل جرام مستخلص . وقد أكدت الدراسة ايضا النشاط المضاد للاكسدة المرتفع نوعا لمستخلص الفلفل البلدى اخضر اللون باستخدام اختبارات القدرة المضادة للاكسدة الكلية ، النشاط المضاد لاصل DPPH وبطريقة القدرة الاختزالية . وبالتالى فينصح باستخدام الفلفل البلدى الاخضر اللون رخيص الثمن للحصول على مضادات الاكسدة الطبيعية .