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# محتوى المركبات النشطة حيويا والأنشطة المضادة للأكسدة ليعض توليفات الأجزاء النباتية المنتشرة فى الأسواق المحلية المصرية

# The bioactive compounds content and antioxidant activities of some plant parts formulae distributed in Egyptian local markets

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## المستخلص:

**تعد الأجزاء النباتية مصدرًا مهمًا للمركبات النشطة بيولوجيًا وقد استخدمت في الطب الشعبي لعدة قرون .لذلك هدفت الدراسة الحالية إلى تحديد المركبات النشطة بيولوجيا وأنشطة مضادات الأكسدة لبعض تركيبات أجزاء النبات المنتشرة في الأسواق المحلية المصرية. وكذلك دراسة مدى تأثيرها فى الوقاية من أمراض القلب والأوعية الدموية وتصلب الشرايين . أظهرت النتائج أن المجموعة النباتية المختارة تحتوى على مضادات أكسدة قوية تعمل على ايقاف كلا من أكسدة بيروكسيد الدهون والبروتينات الدهنية منخفضة الكثافة . أظهرت المجموعة النباتية المختارة فروقًا طفيفة في درجة النشاط المضاد للاكسدة عندما تم حسابها والتى تراوحت بين ٩٢.٧٤ – ٩٤.٥٦ %. يرجع وجود مضادات الأكسدة العالية في المجموعات النباتية التى تم اختيارها إلى وجود مركبات نشطة بيولوجية عالية فى محتواها من الفينولات والتى سجلت ٢٢٧٥ و ٣٨٤٨٤ ملجرام /١٠٠ جم للمجموعتين الأولى والثانية على التوالي.اظهرت مستخلصات الأجزاء النباتية المستخدمه زياده معنويه كبيرة بين الفينولات الكليه ونشاط مضادات الاكسده – 0.9179 =r2) ( 0.9462, p< 0.05. وفى النهاية نوصى باستخدام المستخلصات التى تم اختبارها بنجاح كاوسيلة في الوقاية من تصلب الشرايين عن طريق تثبيط أكسدة البروتين الدهني المنخفض الكثافة (LDL)الذي يستحق المزيد من الدراسات المكثفة.**

## الكلمات المفتاحية:

**أجزاء النبات، منتجات معالجة الأغذية، مجموع الفينول، أكسدة البروتين الدهني منخفض الكثافة، تصلب الشرايين**

# The bioactive compounds content and antioxidant activities of some plant parts formulae distributed in Egyptian local markets

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## Abstract:

Plant parts are important source of bioactive compounds and have been used for folk medicine for many centuries. The present study aimed to determine the bioactive compounds and antioxidant activities of some plant parts formulae distributed in the Egyptian local markets. Also, the potential effect of the tested formulae as a promising tool in the prevention of cardiovascular diseases (CVD) including atherosclerosis will be in the scope of this investigation. The results showed that selected formulae possessed stronger antioxidant properties in inhibiting both lipid peroxidation ($\beta$-carotene bleaching assay) and CuSO4-induced LDL oxidation. The tested formulae showed slightly differences in antioxidant activity (AA= 92.74-94.56%). The high antioxidant properties of the tested formulae appeared to be attributed to its high bioactive compounds in particular phenolics content were 2275 and 38484 mg.100g$^{-1}$ for formula I and II, respectively. The values of two tested formulae absorbances throught 120 min are coming well i.e. closing the line of 50 mg α-tocopherol and up to the line of 50 mg /L of butyhydroxy toluene (BHT). When all selected formulae were included in the statistical analysis, there was a positive and highly significant ($r^2$= 0.9179 - 0.9462, $p< 0.05$) relationship between total phenolics and antioxidant activity. Finally, the tested formulae could be used successfully as a promising tool in the prevention of atherosclerosis by inhibiting LDL oxidation which merits further intensive studies.

## Key words:

Plant parts, food prcessing by-products, total phenolics, LDL oxidation**,** atherosclerosis

## Introduction

The modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Thus there is a need to explore alternative therapies particularly from nutritional/plant sources as these are cost effective and possess minimal side effects. Plants produce an amazing amount of complex chemicals we can use as medicines to "curb and cure" disease. Many of authorities and academic centers of research pay more attention towards the area of cancer chemoprevention compounds. One of the most impressive findings in the field of chemoprevention is the very large number of compounds that have been demonstrated to prevent the occurrence of cancer. Many of these classes are lies in an enlarged group of compounds called phytochemicals (*phyto* is Greek for plant). Phytochemicals are bioactive non-nutrient chemical compounds found in plant foods such as fruits, vegetables, grains, and other plant foods. Phenolic compounds can be further divided into flavonoids (including flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavones), phenolic acids, stilbenes, coumarins, and tannins. Many phytochemicals are potent effectors of biologic processes and have the capacity to influence disease risk via several complementary and overlapping mechanisms (**Steinmetz and Potter, 1991**). On the other hand, **WHO, IUCN and WWF, (1993)** defined phytochemicals as non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in medicinal plant leaves and each works differently. Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO) more than 80% of the world's population, mostly in developing countries depend on traditional plant based medicines for their primary healthcare needs.

Biologically active plant chemicals other than traditional nutrients that have a beneficial effect on human health have been termed phytochemicals (**Hasler, 1998**). Phytochemicals are naturally occurring, biologically active chemical compounds in plants. In plants, phytochemicals act as a natural defense system for host plants and provide colour, aroma and flavour. Phytochemicals are protective and disease-preventing particularly for some forms of cancer and heart diseases. The most important action of these chemicals with respect to human beings is somewhat similar in that their function as antioxidants that react with the free oxygen molecules or free radicals in our bodies. Free radicals can damage the cells of our bodies and must be removed (**Percival *et al.*, 2006; Aly *et al.*, 2017 and ElAbasy,**

**2019**). Therfore, the present study aimed to determined the bioactive compounds and antioxidant activities of some plant parts formulae distributed in the Egyptian local markets. Also, the potential effect of the tested formulae as a promising tool in the prevention of cardiovascular diseases (CVD) including atherosclerosis will be in the scope of this investigation.

## Materials and Methods

**Plant parts:** Plant parts used in food combinations will obtained purchased from the herbs merchandize, local markets, Egypt. Plant parts include: Marjoram "*Origanum majorana* ", Molokheiya "*Corchorus capsularis* Linn", Sweet fennel "*Foeniculum vulgare* ", Ginseng "*Panax ginseng* ", Black seeds "*Nigella sativa* L. ", Eucalyptus "*Eucalyptus globules* ", Cinnamon "*Cinnamomum verum* ", Spearmint leaves "*Mentha spicata* ", Lemon peels "*Citrus limon*", Potato peels "*Solanum tuberosum*", Orange peels "*Citrus sinensis*", Onion skin "*Allium Cepa* L", Apricot kernels "*Prunus Armeniaca*" and Gum arabic "*Sengalia Senegal*".

## Chemicals and reagents

**Standards:** Gallic acid, butylhydroxytoluene and α-tocopherol were obtained from Sigma chemical Co, St Louis, MO, Sodium tungstate and phosphomolybdic acid were obtained from Fischer, UK.

**Wily mill:** A wily mill (Tecator, Boulder, Co, USA) fitted with a 60 mesh screen sieve was used for grounding and sieving the all tested dried plant parts.

**Folin-Ciocalteu reagent:** One gram of sodium tungstate, 20 g of phosphomolybdic acid, and 750 ml of distilled water are transferred to a 1000 ml flask fitted with a reflux condenser. The solution is refluxed for 10 h; transferred to a graduated flask of 1000 ml capacity and made up to the mark with distilled water.

**Chemicals, solvents and buffers:** All chemicals, reagents and solvents were of analytical grade and purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

## Methods

### Tested Formulae preparation

Each formula was prepared by mixing its herbs content by the listed ratio in blend mixture (Toshiba ElAraby Company, Benha, Egypt) for 5 min. The resulted mixtures were packed in tied polyerhylene bags and kept in refrigerator at 4°C until analysis.

**Table (1):** Plant parts include of formula I

| Plant parts | Percentage (%) |
|---|---|
| Black seeds | 8.70 |
| Ginseng | 8.70 |
| Cinnamon | 15.22 |
| Eucalyptus (Cavour) | 10.87 |
| Marjoram | 17.39 |
| Molokhia | 26.09 |
| Sweet fennel | 13.04 |
| **Total** | 100.00 |

**Table (2):** Plant parts include of formula II

| Plant parts | Percentage (%) |
|---|---|
| Black seeds | 15.22 |
| Spearmint leaves | 8.70 |
| Marjoram leaves | 13.04 |
| Sweet fennel | 8.70 |
| Lemon peels | 6.52 |
| Potato peels | 6.52 |
| Orange peels | 6.52 |
| Onion skin | 6.52 |
| Apricot kernels | 6.52 |
| Gum arabic | 21.74 |
| Total | 100.00 |

**Preparation of tested formulae extracts**

Extracts of the tested formulae were prepared according to the method of **Amin *et al.*, (2004)** with some modifications. In brief, the homogenized tested formulae were dissolved in aqueous solvent (deionized water) as the following: In aqueous extract: (20 g from dried plant +180 ml deionized water). The homogenized sample was weighed and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, and Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through what man No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of aqueous extract was removed under reduced pressure at 40°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The extracts were assayed for total phenolics contents and antioxidant activities

**Determination of antioxidant activities**

***Antioxidant activity (AA)***

Antioxidant activity of formulae extracts and standards (α-tocopherol, BHA, ans BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the *β*-carotene bleaching method following a modification of the procedure described by **Marco (1968).** Various concentrations of BHT, BHA, and α-tocopherol in 80% methanol was used as the control. Antioxidant activity was calculated in four different ways. In the first, absorbance was plotted against time, as a kint curve, and the absolute value of slope was expressedas antioxidant value (AOX). Antioxidant activity (AA) was all calculated as percent inhibition ralative to control using the following equation (**Al-Saikhan et al., 1995**).

$AA= (R_{control} - R_{sample}) / R_{control} \times 100$

Where: $R_{control}$ and $R_{sample}$ were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively.

The third method of expression based on the oxidation rate ratio (ORR) was calculated according to the method of **Marinova et al., (1994)** using the equation:

$ORR = R_{sample} / R_{control}$

Where: $R_{control}$ and $R_{sample}$ are the same in the previous equation.

In the fourth method, the antioxidant activity coefficient (AAC) was calculated as described by **Mallett et al., (1994).**

$(AAC) = (Abs_{S\ 120} - Abs_{C\ 120}) / Abs_{C\ 0} - Abs_{C\ 120}) \times 1000$

where: $Abs_{S\ 120}$ was the absorbance of the antioxidant mixture at time 120 min, $Abs_{C\ 120}$ was the absorbance of the control at time 120 min, $Abs_{C\ 0}$ was the absorbance of the control at zero time.

***β-carotene bleaching (BCB) assay***

For *β*-carotene bleaching (BCB) assay, antioxidant activity (AA) against time (every 10 min thereafter for 120 min) for the all tested vegetables processing by-product extracts was measured/constructed according to **Marco, (1968)**. The AA was all calculated as percent inhibition (bleaching rates of *β*-carotene in reactant mixture of plant part extracts) relative to control (bleaching rates of *β*-carotene in reactant mixture of without plant part extracts) such as described by Al-Saikhan *et al.,* (1995).

**Determination of total phenolics**

Total phenolics were determined using Folin-Ciocalteu reagent (**Singleton and Rossi, 1965**). Two hundred milligrams of sample was extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid

at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 0C for 5 min; 0.75 ml of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 0C, absorbance was measured at 725 nm. Results are expressed as ferulic and equivalents.

**Inhibition of low density lipoprotein (LDL) oxidation**

Inhibition of LDL oxidation was determined according to the method of **Princen *et al.*, (1992)**. Adult male white albino rat, Sprague Dawley strain, serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl DMSO containing various concentrations of the all tested vegetables processing by-product extracts. A 20 µl of CuSO4 solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculation the net area under the curve.

**Statistical Analysis**

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB-12 computer program (Minitab Inc., State College, PA).

**Results and Discussion**

**Physical properties of tested formula**

The water (WHO) and oil (OHC) holding capacity of tested formulae were illustrated in Table (3). From such data it could be noticed that tested formula II recorded WHO and OHC being 10.23 g $H_2O.g^{-1}$ and 3.41 g $oil.g^{-1}$, than formula I being 9.65 g $H_2O.g^{-1}$ and 3.11 g $oil.g^{-1}$, indicating that the higher fiber content in tested formulae hold more water. In similar study, **Ashoush and Gadallah (2011)** found that plant parts such mango peel was higher than that of mango kernel powder being 5.08 and 2.08 g water/g, respectively indicating that the higher fiber content in mangp peel hold more water compared to mango kernel. This observation is agreed with those reported by **Marcos *et al.*, (2006**), **Abdalla *et al.*, (2007), Ajila *et al.*, (2011) and Ahmed *et al.*, (2014).** Also, the present data are in accordance with that observed by **Ahmed, (2016)** who found that higher fiber content in red onion skin hold more water compared to other food by-products including

cauliflower leaves and potato peel powder. Regarding OHC, the all formulae were recorded values close to each other's. Such data are in accordance with that recorded by **Sayed-Ahmed, (2016) and EL-Harbi, (2019)** who subjected many plant by-products (onion skin, mango peel, cauliflower leaves, potato peel and eggplant peel) to OHC parameter.

**Table 3.** Physical properties of tested formulae

| Parameters | Formula I | Formula II |
|---|---|---|
| Water holding capacity (WHC, g $H_2O.g^{-1}$) | $9.65 \pm 0.50^{a}$ | $10.23 \pm 1.43^{a}$ |
| Oil holding capacity (OHC, g $oil.g^{-1}$) | $3.11 \pm 0.33^{a}$ | $3.41 \pm 0.22^{a}$ |

Each value represents the mean of ten replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level $p \leq 0.05$.

**Total phenolics contents of tested formulae**

Total phenolics contents (TPC) of tested formulae is shown in Table (4). The results showed that the total phenolics content was 2275 and 38484 $mg.100g^{-1}$ for formula I and II, respectively. The formulae ii was recorded higher TPC than the formula I due to the high total phenolic content in different components containing in formula II. In similar studies, four (red, violet, white and green) varieties of onion were studied for their TPC. The TPC varied from 4.6 to 74.1 mg/g GAE **(Prakash *et al*., 2007).** Specific phenolic composition performed through HPLC and LC–MS/MS showed the presence of gallic acid, ferulic acid, protocatechuic acid, quercetin, and kaempferol. The unutilised outer layers of the red variety were a rich source of quercetin (5110 µg/g) with high AOA, FRSA and also showed significant protection of DNA damage caused by free radicals. Also, Potato peels are waste by-product of the potato processing industry. They are reportedly rich in polyphenols. Studies have shown that extracts derived from potato peel (PPE) possess strong antioxidant activity in chemical and biological model systems *in vitro*, attributable to its polyphenolic content. It was investigated the ability of PPE to protect erythrocytes against oxidative damage, *in vitro*. The protection rendered by PPE in erythrocytes was studied in terms of resistance to oxidative damage. The total polyphenolic content in PPE was found to be. 93.3 mg/g powder. The major phenolic acids present inPPEwere predominantly: gallic acid, caffeic acid, chlorogenic acid and protocatechuic acid **(Singh *et al*., 2004)**. Oil from fresh flowering plants of marjoram was analysed by capillary GC, GC-MS and GC-FTIR for essential oil composition. Hydrodistillation of plants produced a colourless oil with a

yield of approx. 1 ml/100 g. 43 of the 45 compounds detected were identified, predominant components being terpinen-4-ol (38%), *cis*-sabinene hydrate (15%), *p*-cymene (7%) and gamma-

**Table 4.** Total phenolics contents in selected formulae

| Parameter | Formula I | Formula II |
|---|---|---|
| Total phenolics (mg GAE.100 $g^{-1}$) | 2275± 322 $^{b}$ | 3484± 264 $^{a}$ |

Each value represents the mean of ten replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level $p \leq 0.05$.

terpinene (7%), as well as sabinene (5%), alpha-terpineol (5%) and alpha-terpinene (3%) constituting a total of about 80% of essential oil(the other one comprises largely phenols) **Novak *et al*., (2002).** Several compounds including *trans*-anethole, estragole, fenchone and polyphenolics were isolated from fennel plant and some of these interact with potential mechanisms of the body **(Garga, 2009)**. Cinnamon has some interesting medical properties. It contains condensed tanins, oil and coumarins. Cinnamon and their constituents are condelaed and flavonoids have been shown potent anticarcinogenic activities **(Nair, 1998)**; and cinnamaldehyde and related compound have been inhibited *in vitro* growth of 29 kind of human cancer cells **(Lee *et al*., 2007)**. Mint extract (ME) had good total phenolic and flavonoid contents. It exhibited excellent antioxidant activity, as measured by *β*-carotene bleaching and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. It also showed a high superoxide- and hydroxyl-scavenging activity but low iron-chelating ability. A positive correlation was found between the reducing power and the antioxidant activity. The antioxidant activity of ME was found to be comparable to the synthetic antioxidant, butylated hydroxytoluene (BHT) **(Sweetie *et al*., 2007)**. Asian ginseng contains ginsenosides, glycans (panaxans), polysaccharide fraction DPG-3-2, peptides, maltol, B vitamins, flavonoids, and volatile oil **(Ang-Lee *et al*., 2001).** On other hand, the principal active components of ginseng are the ginsenosides or ginseng triterpenoid saponins **(Choi *et al*., 2008; Gillis, 1997 and Attele, 1999).** Furthermore, olive leaves contain high quantities of phenol substances very similar to those present in olives and their derived products **(De Leonardis *et al*., 2008**). There is compelling scientific evidence that olive leaf polyphenols are bioactive compounds (**Khayyal *et al*., 2002 and Somova *et al*., 2003).** Finally, **Sayed-Ahmed, (2016)** found that the TPC was

1104-7129 mg EGA.100 $g^{-1}$ in different food by-products including red onion skin and potato peel powders.

In general, Data of the present study with the others confirmed that such selected formulae could be constituted a good position in food sciences and nutritional applications through their high content of bioactive compounds including phenolics. In this concern, phenolic compounds is found by significant amounts in all tested formulae shows a variety of pharmacological and nutritional effects such as growth-inhibition of tumor and microbial cells, immunostimulatory properties, enhancing reproduction, improving the growth performance (body weight gain, feed consumption, and feed conversion), reduction of cancer risk and protection against cardiovascular diseases, diabetes as well as ageing (**Wang *et al*., 2005; Corzo *et al*., 2007; Durmaz *et al*., 2009 and sayed- Ahmed, 2016)**. Also, the ability of these compounds to acts as antioxidants has been demonstrated in the literature. Several researchers have investigated the antioxidant activity of phenolic compounds such as flavonoid and have attempted to define the structural characteristics of flavonoids that contribute to their activity (**Nieto *et al*., 1993 and Foti *et al*., 1996)**. Also, Phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, *p*-coumaric acids, vanillic, syringic and *p*-hydroxybenzoic appear to be active antioxidants (**Larson *et al*., 2007and El-Sadany, 2001**). Antioxidant activity is fundamental property important for life. Many of the biological functions, such as antimutagenicity, anticarcinogenicity, antiaging and antiobesity, among others, originate from this property (**Cook and Samman, 1996; Elhassaneen *et al*., 2016 and El-Harbi, 2018**).

**Antioxidant activity of tested formula**

The antioxidant activities of two tested formulae are shown in Table (5). From such data it could be noticed that the tested formulae showed slightly differences in antioxidant activity (AA= 92.74-94.56%). All of the selected tested formulae showed strong activity because of their high phytochemicals content (phenolic compounds and carotenoids). Such data are in accordance partially with that observed by **Elhassaneen *et al*., (2016)** and **Sayed-Ahmed, (2016)** who found that some food processing by-products (onion skin, mango peel powder, eggplant peel and potato peel) including in our tested formulae high in their antioxidant activity due to their phenolic compounds high content.

**Table 5.** Antioxidant activity of tested formula

| Samples | Antioxidant value[a] AOX (A/h) | Antioxidant activity[b] AA (%) | Oxidation rate ratio[c] (ORR) | Antioxidant activity coefficient[d] (AAC) |
|---|---|---|---|---|
| Formula I | 0.041± 0.021 | 92.74± 4.73[bc] | 0.072± 0.011 | 784.56± 56.78 |
| Formula II | 0.031± 0.021 | 94.56± 5.12[b] | 0.054± 0.021 | 816.20± 60.43 |
| Control | 0.565± 0.031 | 0.00± 0.00 | 0.998± 0.125 | 0.00± 0.00 |
| BHT, 50 mg/L | 0.056± 0.017 | 90.02± 1.34 [bc] | 0.099± 0.012 | 737.27± 21.00 |
| α-tocopherol, 50 mg/L | 0.011± 0.006 | 98.02± 1.43 [a] | 0.019± 0.007 | 876.35± 20.54 |

[a] Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).

[b] Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively

[c] Oxidation rate ratio (ORR ) = R sample / R control

[d] Antioxidant activity coefficient (AAC) = (Abs S 120 - Abs C 120) / Abs C 0 - Abs C 120) x 1000 where: .Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.

[e] Each value represents mean ±SD. Values with the different letters in the same column are significantly different $P \geq 0.05$.

### *β* -Carotene Bleaching (BCB)

BCB assay based on measured the ability of an antioxidant to inhibit lipid peroxidation (LP). In the BCB method, a model system made of *β*-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. The free linoleic acid radical formed upon the abstraction of a hydrogen atom ($H^+$) from one of its methylene groups (-HC=CH-) attacked the β-carotene molecules, which lost the double bonds and therefore, its characteristic orange color. The absorbance of the solution at 470 nm was monitored on a spectrophotometer by taking measurements at 10 min intervals, and the rate of bleaching of *β*-carotene was calculated by fitting linear regression to data over time according to **Marco (1968)**. The decrease in absorbance of *β*-carotene in the presence of different methanolic selected formulae extracts (and well-known antioxidants used as standards) with the

oxidation of $\beta$-carotene and linoleic acid is shown in Figure (1). Such data indicated that the two selected formulae recorded the lowest decreasing. The values of two tested formulae absorbances throught 120 min are coming well i.e. closing the line of 50 mg α-tocopherol and up to the line of 50 mg /L of butyhydroxy toluene (BHT). These data proved the high stability of the all tested two formulae when comparing with that more common standards α-tocopherol and BHT. The present data are in accordance with the obtained by **Ghaly, (2004), Elhassaneen and Abd Elhady, (2014)** and **Sayed-Ahmed, (2016)** who studied the AA stability of many plant parts extracts commonly distributed in the Egyptian local markets and used in the preparation of the present formulae.

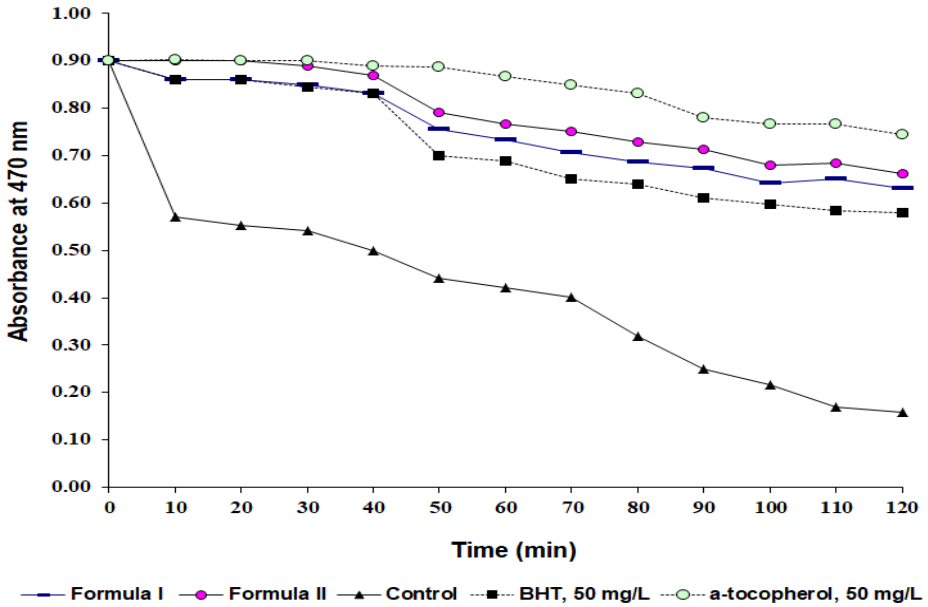



**Figure 1.** Activity of tested formulae assayed by the β-carotene bleaching method (BHT α–tocopherol at 50 mg/L concentration was used as a reference)

**Relationship between total phenolic contents and antioxidant activity of tested formulae**

The total phenolic content of the selected tested formulae investigated in this study varied from 2275 to 38484 mg.100g$^{-1}$ dry weight and the antioxidant activity was AA= 92.74 to 94.56%. The relationship between total phenolic content and antioxidant activity of selected formulae is shown in Table (6). The results indicated that when all selected formulae were

included in the statistical analysis, there was a positive and highly significant ($r^2$= 0.9179 - 0.9462, p< 0.05) relationship between total phenolics and antioxidant activity. This indicates that phenolics can play a major role in the antioxidant activity of tested plant by- products. In similar study, **Velioglu *et al.*, (1998)** reported that the correlation coefficient between total phenolics and antioxidative activities of 28 plant products, including plant parts in our tested formulae was statistically significant. Also, many studies indicated that there was a positive and significant (p< 0.01) relationship between total phenolics and antioxidant activity in different plant parts (**El-Mokadem, 2010; El-Safty *et al.* 2012; Hegazy, 2009; Ahmed *et al.*, 2014; Elhassaneen *et al.*, (2016)** and **Sayed-Ahmed, (2016**).

Onion extracts have been recently reported to be effective in cardiovascular disease because of their hypocholesterolemic, hypolipidemic , anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects, and to possess many other biological activities including antimicrobial , antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities **(Corzo *et al.*, 2007).** In order to determine AA, the five extracts/fractions of red onion peel were studied for their total content of phenolics (TPC), flavonoids(TFC), antioxidant activity (AOA), free radical scavenging activity (FRSA), assayed by DPPH radical in the terms of anti-radical power (ARP) and reducing power (RP), expressed as ascorbic acid equivalents (ASE)/ml. High TPC (384.7 mg GAE/g), TFC (165.2 mg QE/g), AOA (97.4 %), ARP (75.3 ± 4.5) and RP (1.6 ASE/ml) were found for the ethyl acetate (EA) fraction. EA fraction had markedly higher antioxidant capacity than butylated hydroxytoluene (BHT) in preventive or scavenging capacities against $FeCl_3$-induced lipid peroxidation, protein fragmentation, hydroxyl (site-specific and

**Table 6.** Relationship between antioxidant activities (AA) and total phenolic contents of tested formulae (n=16)

| | **Relationship between antioxidant activities and total phenolic contents** | **$R^2$** |
|---|---|---|
| Formula I | Total phenolics ($mg.100g^{-1}$, d.b.) = 135.03 (Antioxidant activity, %) – 09151.5 | 0.9179 |
| Formula II | Total phenolics ($mg.100g^{-1}$, d.b.) = 206.86 (Antioxidant activity, %) - 15447 | 0.9462 |

*P ≥ 0.05 and ** P ≥ 0.01

non-site-specific), superoxide anion and nitric oxide radicals. HPLC and MS/MS analysis showed the presence of ferulic, gallic, protocatechuic acids, quercetin and kaempferol. The large amount of polyphenols contained in EA fraction may cause its strong antioxidant and antimutagenic properties. This information shows that EA fraction of red onion peel can be used as natural antioxidant in nutraceutical preparations **(Singh *et al*., 2004)**. The antioxidant properties of peeled, defatted and roasted apricot kernel flours were evaluated by determining radical scavenging power (RSP), anti-lipid peroxidative activity (ALPA), reducing power (RP), total phenolic content (TPC), assessed by DPPH test, $\beta$-carotene bleaching method, iron (III to II) reducing test and Folin method, respectively. Browning degree of the samples was also measured and found to increase almost linearly with the roasting time. Contrary to browning degree, RSP, RP and TPC did not increase linearly but showed a maximum for 10 min of roasting. Roasting reduced the ALPA values, thus unroasted sample showed the highest ALPA value. RSP, RP and TPC measurements of all samples, were in high correlation (at least, r = 0.92) **(Durmaz *et al*., 2009)**. Ethanol extracts of two varieties of Egyptian oranges (Baladi and Novel) were prepared, and their total phenolic and flavonoid contents, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were determined via standard colorimetric assays. Oil containing extract and butylated hydroxytoluene (BHT) or butylated hydroxylanisole (BHA) was stored at 65°C for 7 days. Total phenolic and flavonoid contents ranged from 559.3 to 591.8 mg tannic acid/100g peel and from 80.94 to 87.71 mg rutin/100g peel, respectively. DPPH scavenging activity was 72.33% and 65.05% for Baladi and Novel, respectively **(Abd El-aal and Halaweish, 2010).** Potato peel powder (PPE) also showed a considerable antioxidant activity in the DPPH radical assay system. The multiple antioxidant activity of PPE was evident as it showed strong reducing power (superoxide scavenging ability) and also ferrous ion chelating potency **(Singh *et al*., 2004).** Extracts of cinnamon have also been shown to have antioxidant effects in part through activating antioxidant enzymes in various tissues that prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, or prevent mutations are important mechanisms in cancer prevention **(Anilakumar *et al*., 2001)**. For GA, the total AA of a compound or substance is associated with several processes that include the scavenging of free radical species (eg. HO., ROO.), ability to quench reactive excited states (triplet excited states and/or oxygen singlet molecular 1O2), and/or sequester of metal ions (Fe2+, Cu2+) to avoid the formation of $HO^{\cdot}$ by Fenton type reactions. In the following sections, we

will discuss the *in vitro* antioxidant capacity of GA for some of these processes **(Ali, 2008).**

In general, the data of this study with the others proved the importance of using selected formulae as natural antioxidants in nutritional therapy. For examples, **Majid *et al.*, (1991)** found feeding of phenolic acid (ellagic, found in PPP) significantly increased the levels of reduced glutathione and glutathione reductase in liver and lungs of male and female mice as well as increase in inhibition of NADPH-dependent lipid peroxidation. The antioxidant activity of four phenolic acids, representative of three chemical groups, present in the all tested plant by-products, upon low density lipoprotein peroxidation was studied *in vitro* in a low density lipoprotein (LDL) oxidation model by **Laranjinha *et al.*, (1994)**. Antioxidants help protect cells from the potentially damaging physiological process known as "oxidative stress" (damage to healthy cells or DNA by unpaired electrons known as free radicals). Oxidative stress is thought to be associated with the development of chronic diseases including cancer, heart disease, conditions of aging including neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Serum glucose concentration of alloxaneor Streptozotocin -induced diabetic rats consumed the tested plant by-product powder/extracts were studied by **Shalaby *et al.*, (2016)**, and **Aly *et al.*, (2017)**). They were noticed that treatment of animals with aloxane or Streptozotocin caused a significant increased ($p \leq 0.05$) in serum glucose concentration compared to normal controls. Supplementation of the rat diets with 0.25% w/w/ by pomegranate peel powder, red onion skin powder, potato peel powder, tomato pomace extract, eggplant peel extract and their mixture decreased the rise of mean serum glucose by the ratio ranged 23- 74.94%. The mixture treatment gave maximum hypoglycemic yield when compared with the tested food processing by-products separated. It could be mean that a combination of different food processing by-products may be more efficient for reducing the serum glucose level because the interactive effects occurred by different categories of bioactive compounds of food processing by-products used.

Antioxidant compounds found in selected formulae such as used in the present study inhibited or delayed the oxidation of other molecules i.e. lipids, proteins, nucleic acid, and carbohydrates by inhibiting the initiation or propagation of oxidizing chain reactions (**Shalaby *et al.*, 2016**). From first principles, it is easy to see that antioxidants might protect a target a proposed pathways as follow: 1) scavenging oxygen-derived species, either by using protein catalysts (enzymes) or by direct chemical reaction proceeds); 2) minimizing the formation of oxygen-derived species; 3) binding metal ions

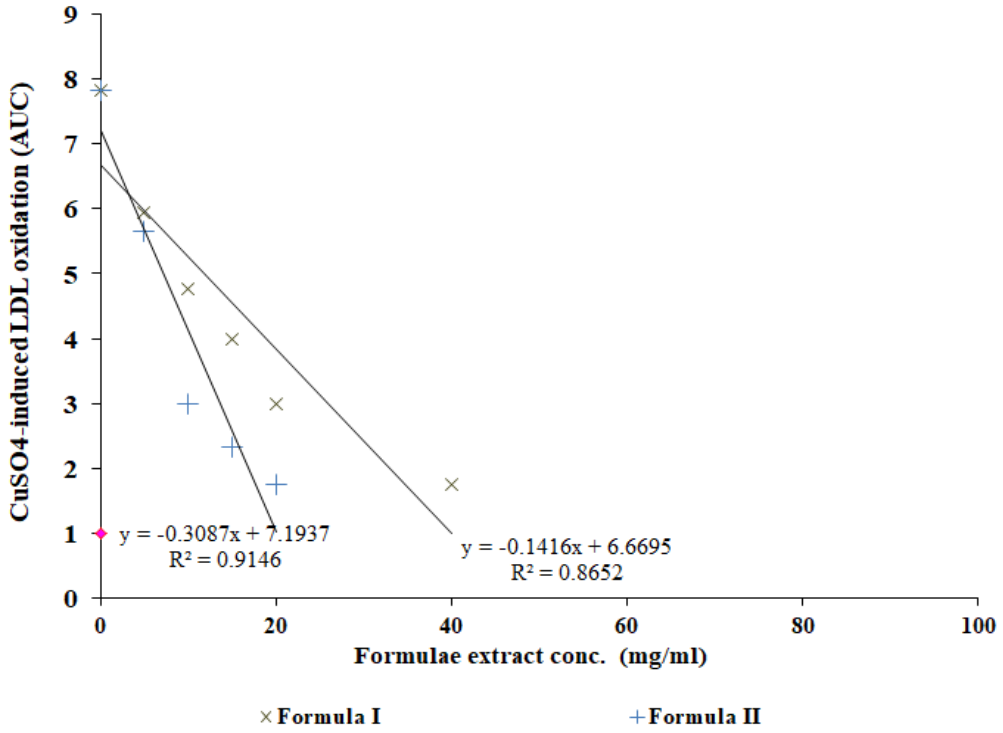
needed to convert poorly reactive species (such as $O_2$ - and $H_2O_2$ ) into nasty ones (such as OH); 4) repairing damage to the target; and 5) destroying badly damaged target molecules and replacing them with new ones (reviewed in **Sayed-Ahmed, 2016 and Elhassaneen *et al.*, 2016**).

Finally, data of the present study with that carried out by the others could be represent the mile stone towards the extension of using parts such OPPE, BPPE, TPPE and their mixture, as natural antioxidants in many different nutritional applications. Synthetic antioxidants such as butylated hydroxyanisole (BAH) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of the last century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity. Thus, the interest in natural antioxidants has increased considerably year after year.

**Inhibition of low density lipoprotein (LDL) oxidation by selected food processing by-products samples**

Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by **tested formulae** samples is shown in Figure (2). From such data it could be noticed that the inhibitive action of the all **tested formulae** samples against CuSO4-induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent fashion. As compared to the **tested formulae samples** acted more dramatically in protecting LDL against oxidation, indicating a possibility those extracts may be more promising in the prevention of atherosclerosis by inhibiting LDL oxidation. The present data are in accordance with that obtained by **Aly *et al.*, ( 2017)** who applied such application on some plant parts/food processing by-products extracts including onion skin, tomato pomace and eggplant peels. Such effect could be attributed to the different bioactive compounds as antioxidants (phenolics, carotenoids, vitamins, volatile components, polysacchrides etc.) contained in such formulae (**Schieber *et al.*, 2001; Somova *et al.*, 2003; Singh *et al*., 2004; Prakash *et al*., 2007; Choi *et al*., 2008).** In similar study, the non-site-specific inhibition of hydroxyl radical induced deoxyribose degradation was also higher in the outer dry layers of red and violet varieties than in their middle and inner layers. The outer layers were also potential inhibitors of nitroblue tetrazolium chloride (NBT) reduction caused by superoxide anions **(Prakash *et al*., 2007).** The non-site-specific inhibition of hydroxyl radical induced deoxyribose degradation was also higher in the outer dry layers of red and violet varieties than in their middle and inner layers. The outer layers were also potential inhibitors of nitroblue tetrazolium chloride (NBT) reduction caused by superoxide anions **(Prakash *et al*., 2007).** Also, Aly *et al*,

(2018) found that tomato pomace extract; onion skin extract; eggplant extract and their mixture extract effectively protect LDL against oxidation *in vitro*, which was attributed to the high levels of polyphenols and vitamins in the extract. Such mechanisms of actions, protecting LDL against oxidation by phenolic compounds, could be included increased the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation **(Majid *et al.*, 1991 and Aly *et al.*, 2017)**. Furthermore, phenolics including phenolic acids exhibited a complex reaction with peroxyl radicals and inhibition of the LDL oxidation **(Laranjinha *et al.*, 1994)**. On the other side, many studies reported that the ‘‘oxidative modification of lipoproteins’’ hypothesis proposes that LDL oxidation plays a key role in early atherosclerosis **(El-Harbi, 2018 and Arief, 2020)**. The oxidized LDL is atherogenic due to its cytotoxic toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which leads to the development of atheromatous plaques **(Hong and Cam, 2015)**. The present data with the others proved that the tested formulae could be used successfully as a promising tool in the prevention of cardiovascular diseases (CVD) including atherosclerosis via inhibiting LDL oxidation process.



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