

## Synergistic Antioxidant Scavenging Activities of Grape Seed and Green Tea Extracts against Oxidative Stress

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### Abstract

Grape seed and green tea extracts are reported to produce antioxidant scavenging activities against free radical toxicity. This study aimed to investigate the hypothesis that a specific combination of these extracts presents a synergistic antioxidant scavenging activities. The extracts of grape seed, green tea and their mixtures were characterized by phytochemical studies and tested for phenolics and flavonoids. *In vitro* antioxidant activity for individual extract and its mixtures was determined by DPPH, hydroxyl and superoxide free radical scavenging methods. The amount of total phenolics varied among the different extracts and ranged from 43.74 to 67.68 mg of gallic acid equivalents (GAE) / g dry weight, whereas total flavonoids content ranged from 4.25 to 11.66 mg of quercetin equivalents (QU) / g dry weight. The present results suggest that both extracts reported a highly contents of total phenolic and flavonoids compounds, also the mixtures of these extracts can synergistically enhance antioxidant activity. Antioxidant potential from mixture 2 (grape seed extract 200 mg; green tea extract 100 mg) was comparable to that of standard. HPLC results showed that the most abundant components in the mixture 2 extract were epigallocatechingallate while the lowest was the procyanidine.

**Keywords:** antioxidant activity, DPPH, grape seed extract, phenolics, synergism, tea extract

### Introduction

Oxidative stress, induced by oxygen radicals, proved to be a major factor in various degenerative diseases, such as cancer, malignant diseases, diabetes and atherosclerosis (Muramatsu *et al.*, 1995; Bandyopadhyay *et al.*, 1997; Apel and Hirt, 2004). Antioxidant has been described as any substance that interferes with the response of any substance with oxygen (Poljsak *et al.*, 2013). Moreover an antioxidant is any substance that hinders a free radical reaction (Rahman 2007). The presence of antioxidant systems protects the cell membrane against damage caused by free radicals. Enzymatic antioxidants include: superoxide dismutase, glutathione peroxidase and catalase and non-enzymatic antioxidants include: glutathione and vitamins systems. In general supplementation by antioxidants, like vitamins A, C, E, cysteine and glutathione is known to decrease free-radical damage caused by radiation treatment (Deger *et al.*, 2003; El-Beltagi *et al.*, 2011). There are numerous interests in natural antioxidants extracted from medicinal plants, vegetables and fruits, which might help to prevent oxidative damage (Yilmaz and Toledo, 2004). Of the naturally polyphenolic compounds, catechins and their polymers, procyanidins have obtained a large interest due to their

antioxidant properties that can perform scavenging action on free radicals superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl (DPPH), metal chelating properties, reduction of hydroperoxide formation and their effects on cell signaling pathways and gene expression (Jacob *et al.*, 2008). Catechins and procyanidins are found mostly in green tea and red wine, respectively (Perumalla and Hettiarachchy, 2011). Important health advantages were related with these compounds including the prevention and the treatment of several cardiovascular diseases and cancers (Jeong and Kong, 2004). Grape seed extract (GSE) is a by-product derived from the grape seeds (*Vitis vinifera*) that is extracted, dried and purified to produce a polyphenolic compound rich extract (Lau and King, 2003). Grape seeds composition and properties have been extensively investigated, and reported to have helpful effects on human health such as the decreasing of low-density lipoprotein (Teissedre *et al.*, 1996; Vigna *et al.*, 2003), reduction of cardiovascular diseases and cancer (Waterhouse, 1994). Several groups have studied GSE for antimicrobial, anticancerogenic, antihypertensive, antihyperglycemic, cardiovascular and hypercholesterolemic properties (Clifton, 2004; Peng *et al.*, 2005; Jung *et al.*, 2006; Broker *et al.*, 2006). The seed extracts of *Vitis vinifera* have been showed to have antimicrobial and antioxidant properties (Jayaprakasha *et*

al., 2001), decrease the hazard of chronic disease by protecting against ROS mediated damage (Caillet *et al.*, 2006) and inhibit the incidence of stroke due to its radical scavenging action and inhibition of lipid peroxidation (Uchida *et al.*, 1995).

It is believed that humans have consumed tea (*Camellia sinensis*, family *Theaceae*) for more than 5000 years and currently it is still one of the most usually used beverages worldwide, second only to water (Graham, 1992; Demeule *et al.*, 2002). Green tea is made from leaves of *Camellia sinensis* that have undergone minimal oxidation during processing. Recently green tea become the main topic of medical studies suggesting that regular tea drinkers may have a reduce risk of promoting heart disease and certain types of cancer (Zaveri, 2006; Yamada and Watanabe, 2007). Green tea has been considered a drug since old times and has been recommended for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and, in general, to prolong life (Cabrera *et al.*, 2006; Neturi *et al.*, 2014). The chemical composition of green tea extract is complex, consisting of polyphenols (catechins and flavanoids), alkaloids, volatile oils, polysaccharides, amino acids, lipids, vitamin C, minerals and other compounds (Karori *et al.*, 2007; Afify *et al.*, 2011). Among these, the largest component present in green tea leaves is carbohydrates (including cellulosic fiber) and the simplest compounds are catechins, a group of flavanoids called flavan-3-ols (Yilmaz, 2006). Catechins and other polyphenols act as antioxidants *in vitro* by isolating metal ions and by scavenging ROS and nitrogen species (Dufresne and Farnworth, 2002; Frei and Higdon 2003; Wiseman *et al.*, 1997). The aim of the present study was to investigate the synergistic antioxidant scavenging activities of grape seed and green tea extracts and their mixtures using different systems and can be used for decreasing the harmful effects of free radicals and may be valuable as natural nutritional health supplement.

## Materials and Methods

### Chemicals

Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, aluminum chloride and chemical HPLC-grade standards (purity  $\geq 95\%$ ) of gallic acid, (-) catechin gallate, (-)-gallo catechins, (-)-catechin, (-)-epigallocatechin, (-)-epicatechin, procyanidin B1, procyanidin B2, procyanidine C1, (-)-epigallocatechingallate, (-)-gallocatechin gallate, procyanidine C2 and (-)-epicatechingallate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were obtained from Merck (Darmstadt, Germany). Acetonitrile and phosphoric acid were of HPLC grade, while the other reagents used in the experiments were of analytical grade.

### Grape seed extract (GSE)

Ripened grapes (*Vitis vinifera* L) of the most popular grape cultivars grown in Egypt, 'Roumy Ahmer' (red grape cultivar), were bought from the local market. Following manual separation of the seeds from whole berries, grape seeds (GS) were dried in oven at 50 °C for 3 days and later were grounded to fine powder by grinder. One hundred g of GS powder was extracted with 500 ml of ethanol 50% and left for one day at

room temperature. The extract was filtered through Büchner funnel followed by ethanol removal using vacuum rotary evaporator at 40 °C. The residue gained was dissolved in water and kept at 4 °C until further use (Ahn *et al.*, 2002). The selection of ethanol 50% as the organic solvent was based on the fact that ethanol when mixed with water facilitates the solubility of the bioactive compounds compared to pure water (Nawaz *et al.*, 2006).

### Green Tea Extract (GTE)

Green tea leaves were bought from the local market. Ethanol extract was prepared according to Gramza *et al.* (2006). Extracts were prepared by triplicate 24 hours maceration of grinded 100 g tea leaves with 250 ml ethanol 95% at ambient conditions. Collected extracts were filtered and centrifuged (7000 rpm, 15 min), ethanol was evaporated on rotary evaporator. The powdered ethanol extract was kept frozen (-18 °C) until further use.

### Mixture preparation

After extraction of both GSE and GTE they were mixed together to obtain two different mixtures with ratio of 1:1 (100 mg GSE, 100 mg GTE) and 2:1 (200 mg GSE, 100 mg GTE), respectively. The mixtures were kept frozen at -20 °C until further use.

### Determination of total soluble phenolic contents

The total soluble phenolic contents of GSE, GTE and each mixture solution were determined using Folin-Ciocalteu reagent (Erkan *et al.*, 2008). One ml of GSE, GTE and each mixture solution (0.1 mg/ml) was mixed with 7.5 ml of Folin-Ciocalteu reagent which was diluted with distilled water. After keeping it at room temperature for 5 min, 7.5 ml of 60 mg/ml of Na<sub>2</sub>CO<sub>3</sub> solution were added. After 2 hrs at room temperature the absorbance was measured at 725 nm against blank using Genway spectrophotometer. Gallic acid was used as a standard and expressed as mg gallic acid equivalents per gram of grape seed dry matter (mg GAE/g DW). The samples were analyzed in triplicates.

### Determination of total flavonoids contents

Ethanol extracts of GSE, GTE and each mixture solution (250 ml) were mixed with 1.25 ml of distilled H<sub>2</sub>O and 75 ml of a 5% NaNO<sub>2</sub> solution. After 5 min, 150 ml of a 10% AlCl<sub>3</sub>.H<sub>2</sub>O solution was added and filtered for 6 min. About 500 ml of 1M NaOH and 275 ml of distilled H<sub>2</sub>O were added to the mixture, mixed well and the intensity of color was measured at 510 nm. The content of total flavonoid concentration was calculated using quercetin (QU) as a standard (Jia *et al.*, 1999). The results were expressed as mg of quercetin equivalents per gram of grape seed dry matter (mg QU/g DW). The samples were analyzed in triplicates.

### HPLC analysis of GTE and GSE mixture extracts

Sample solution of GTE and GSE mixture 200 µg/ml was analyzed by LC/DAD/MS HPLC, Agilent (serious 1100 quartary pump). LC/DAD/MS HPLC conditions was as follows, column type; Reversed phase Zorbax Eclipse Plus C<sub>18</sub> (Agilent, 3.5 µm); column dimensions, 4.6 × 100 mm; column oven temperature, 35 °C; mobile phases, (A) water / phosphoric acid 1000, 1 (v/v), (B) acetonitrile / phosphoric

acid 1000, 1 (v/v); a gradient program, (B) 0% (0 min) = 15%, (20 min) = 30%, (25 min) = 100%, (45-61 min) = 0 (62-77 min); detector type, UV and Mass detector; monitor wavelengths, 210 and 280 nm; injection volume, 10  $\mu$ L. Phosphoric acid has an absorbance at 210 nm, and that was the reason for correcting the HPLC chromatograms by subtracting the base line. All extracts were prepared in triplicate and each extract was analyzed in triplicate.

#### DPPH scavenging activity

The antioxidant activity of plant ethanol extracts was measured based on the radical scavenging ability in reaction with a stable DPPH free radical according to Blois (2002). Briefly, 0.1 mM of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml ethanolic extracts (10, 50, 100, 150  $\mu$ g/ml). The mixture was shaken and allowed to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm. The radical scavenging activities BHT and BHA were determined also as positive controls. Minimum absorbance of the reaction mixture indicated maximum free radical scavenging activity. Purple colored stable free radicals were reduced to yellow colored diphenylpicrylhydrazine when antioxidant was taken. The corresponding blank readings were taken and the capability to scavenge DPPH radical was calculated using the following equation,

$$\text{DPPH}^{\cdot} \text{ scavenging activity (\%)} = [(A_0 - A_1/A_0)] \times 100,$$

where,

$A_0$  = The absorbance of control reaction (containing reagents except the test compounds).

$A_1$  = The absorbance in presence of the tested extracts.

#### Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured according to the methods of Singh *et al.* (2002). About 0.1 ml of ethanolic extracts (10, 50, 100, 150  $\mu$ g/ml) were added in different test tubes. About 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of dimethylsulfoxid (DMSO) (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were taken to these tubes, and the reaction was started by adding 0.5 ml ascorbic acid 0.22%. Test tubes were capped tightly and heated on a water bath at 80-90 °C for 15 min. The reaction was inhibited by addition 1 ml of ice cold TCA (17.5 %w/v). About 3 ml of Nash reagent (75 g ammonium acetate, 3 ml glacial acetic acid, and 2 ml acetyl acetone were mixed and increased to 1 L with distilled water was added to all tubes and left at room temperature for 15 min for the color development. The intensity of yellow color formed was determined spectrophotometrically at 412 nm against the reagent control. The radical scavenging activities of BHT and BHA were also measured as positive controls. The percentage of hydroxyl radical scavenging activity was calculated by using the formula,

$$\text{Hydroxyl radical activity (\%)} = 1 - [A_1/A_0] \times 100,$$

where,

$A_1$  = absorbance of tested extracts.

$A_0$  = absorbance of control (containing all reagents except the test compounds).

#### Superoxide anion radical scavenging activity

Superoxide radicals were produced by the method of

Giannopolites and Ries (1977) modified by Siddhurajua *et al.* (2000). All the solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo induced reactions were completed in aluminium foil-lined box with two 30W fluorescent lamps. The space between the reaction solution and the lamp was regulated until the intensity of illumination reached about 4000 lux. A 30  $\mu$ L aliquot of various concentrations (10, 50, 100, 150  $\mu$ g/ml) of extracts was mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63  $\mu$ M nitro blue tetrazolium and 100  $\mu$ M EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25 °C. The reaction mixture, without sample extract, was used as a control. The radical scavenging activities of BHT and BHA were also determined as positive controls. The scavenging activity was calculated as follows:

$$\text{Superoxide anion radical scavenging activity (\%)} = 1 - [A_1/A_0] \times 100,$$

where,

$A_1$  = absorbance of tested extracts,

$A_0$  = absorbance of control (containing all reagents except the test compounds).

#### Statistical analysis

Each statistical analysis was carried out with SPSS10 version software. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples and p-values <0.05 were considered significant. All the measurements were done in triplicate.

## Results and Discussion

#### Total phenolic and total flavonoids content of extracts

Total phenolic and total flavonoids content of grape seed and green tea extracts individually and the prepared mixtures were examined (Table 1). The amount of total phenolics varied among the different extracts and ranged from 43.74 to 67.68 mg GAE/ g dry weight. Total flavonoids content ranged from 4.25 to 11.66 mg QU /g dry weight. The highest phenolic and total flavonoids contents were significantly recorded in mixture 2 (200 mg GSE: 100 mg GTE) extract. In contrast, the lowest contents of total phenolic and total flavonoids were noticed in GTE extract. This result indicated that mixture 2 extract presented a large amount of total phenolic and flavonoids than grape seed and green tea extracts individually and the prepared mixture 1. It can be establish the order of all the extracts with highest value of total phenolic and total flavonoids content as follows: mixture 2 (200 mg GSE: 100 mg GTE) > mixture 1 (100 mg GSE: 100 mg GTE) > GSE > GTE.

#### HPLC phenolic composition of the mixture 2 (200 mg GSE: 100 mg GTE)

Polyphenol composition of mixture 2 was determined by LC/DAD/MS HPLC analysis and the results are shown in Fig. 1. The separation of the phenolic compounds in mixture 2 was achieved within 60 min. Identified Compounds (arranged according to their appearance in the attached chromatogram) are as follows in Table 2. The obtained results reported the presence of highly quantities of natural antioxidant compounds in mixture 2 (200 mg GSE: 100 mg GTE) with a highly relatively percentages when the Epigallocatechingallate

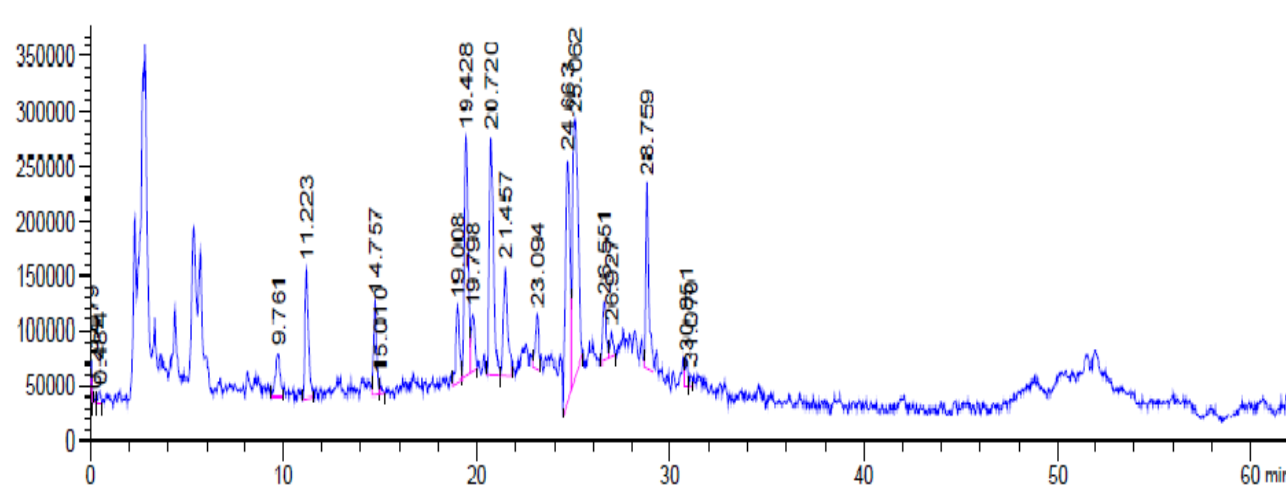


Fig. 1. MS-HPLC chromatogram of mixture 2 (200 mg GSE: 100 mg GTE)

Table 1. Total phenolic and total flavonoids contents of the ethanolic extracts of grape seed, green tea and prepared mixtures

Extracts	Total phenolic*	Total flavonoids**
GSE	46.44±0.35 <sup>c</sup>	4.98±0.08 <sup>c</sup>
GTE	43.74±0.26 <sup>d</sup>	4.25±0.12 <sup>d</sup>
Mixture 1	59.34±0.40 <sup>b</sup>	10.58±0.20 <sup>b</sup>
Mixture 2	67.68±0.52 <sup>a</sup>	11.66±0.23 <sup>a</sup>
LSD 0.05	1.12	0.45

Data with different superscript letters in the same column were differed significantly ( $P \leq 0.05$ ), \* Mean of triplicate determinations  $\pm$  SE expressed as mg GAE acid equivalent /g dry weight, \*\* Mean of triplicate determinations  $\pm$  SE expressed as mg QU equivalent /g dry weight. GSE: grape seed extract, GTE: green tea extract, Mixture 1 (100 mg GSE: 100 mg GTE), Mixture 2 (200 mg GSE: 100 mg GTE)

(EGCG) 19.12%, Epicatechin (EC) 14.22%, Catechin reported 13.38%, Epigallocatechin (EGC) 11.85%, (-) Epicatechingallate (ECG) 7.30%, Procyanidine C1 6.61 %, (-) Catechin Gallate (CG) 6.23%, Procyanidine B1 3.7%, (-) Gallo Catechin (GC) 3.61%, (-) Gallocatechin gallate (GCG) 2.91%, Procyanidine B2 2.81% and Gallic Acid (GA) 2.61%. The major compound in GSE and GTE mixture 2 are catechins such as EGCG followed by EC, Catechin, EGC, ECG, while the lowest compounds was the procyanidine. The obtained results are in accordance with the results of previous studies which revealed that, the main compounds in GSE and GTE are catechins and the content of proanthocyanidins are at least 10 times lowers than catechins (Gramza *et al.*, 2005; Mandić *et al.*, 2009; Wang *et al.*, 2014). Polyphenolic compounds (mainly flavanoids) present in green tea and grape seed extracts have demonstrated potential antioxidant properties due to their redox potential; that enable them to act in various forms such as hydrogen donors, reducing agents, resultant oxygen quenchers, and chelating metal ions in numerous food applications (Gramza *et al.*, 2006). The active hydroxyl groups present in the molecular structure of polyphenols are the active components of the green tea and grape seed extracts that can interact with the free radicals to inhibit lipid oxidation (Mitsumoto *et al.*, 2005). Furthermore, green tea and grape seed polyphenols can exhibit scavenging activity against free radicals (Rice-Evans *et al.*, 1997), peroxy nitrite, superoxide

Table 2. Relative percentage of identified compounds represented in mixtures 2 (200 mg GSE: 100 mg GTE) via MS-HPLC

Retention time	Compound	Relative percentage (%)
9.761	Gallic Acid (GA)	2.61
11.223	(-) Catechin Gallate (CG)	6.24
14.75	(-) Gallo Catechin (GC)	3.61
19.00	Procyanidine B1	3.71
19.42	(-) Epigallocatechin (EGC)	11.85
19.798	Procyanidine B2	2.81
20.72	(-) Catechin	13.38
21.45	Procyanidine C1	6.61
23.09	Procyanidine B2	2.41
24.663	(-) Epicatechin (EC)	14.22
25.06	(-) Epigallocatechingallate (EGCG)	19.12
26.551	(-) Gallocatechin gallate (GCG)	2.91
26.927	Procyanidine C2	1.12
28.759	(-) Epicatechingallate (ECG)	7.30

radicals, chelate copper and iron, preventing metal catalyzed free radical formation (Lin and Liang, 2000). Flavonoids from plant extracts end the radical chain reactions that occur during the oxidation of triglycerides in food systems (fats, emulsions and oils) and thus can act as free radical scavengers (Turkoglu *et al.*, 2007; Mohamed *et al.*, 2010). Presence of O-dihydroxy and O-hydroxyketo groups and C<sub>2</sub>-C<sub>3</sub> double bonds are linked to the scavenging activity of different catechin molecules (Rice-Evans *et al.*, 1997). Thus, EGCG has the most scavenging activity of free radicals followed by ECG, EC, and EGC as the antioxidant functions of the plant catechins depends on the structure, position and number of hydroxyl groups (Hu *et al.*, 2001). Proanthocyanidins in the form of monomeric phenolic compounds, such as catechin,

epicatechin and epicatechin-3-O-gallate, and in dimeric, trimeric and tetrameric procyanidin forms are rich in GSE. These can combine with gallic acid to form gallate esters and ultimately glycosides (Negro *et al.*, 2003; Weber *et al.*, 2007). The red color and astringency taste of the GSE can be attributed to polyphenol rich compounds especially proanthocyanidins which may affect the color and sensory characteristics of the product when used at higher concentrations (Monteleone *et al.*, 2004; Weber *et al.*, 2007).

#### DPPH Radical Scavenging Activity

The free radical scavenging activity of different concentrations (10, 50, 100 and 150  $\mu\text{g/ml}$ ) from extracts of grape seed and green tea extracts individually and the prepared mixtures were determined by the DPPH method compared with BHA and BHT as standard and the results are shown in Fig. 2. Antioxidant molecules can quench DPPH free radicals and convert them to a colourless product, resulting in a decrease in absorbance at 517 nm. All the studied extracts showed significantly high radical scavenging activity ranging from 52.87 to 59.04 at 10  $\mu\text{g/ml}$ , from 63.82 to 77.13 at 50  $\mu\text{g/ml}$ , from 78.04 to 85.83 at 100  $\mu\text{g/ml}$  and from 85.0 to 90.0 at 150  $\mu\text{g/ml}$ . The highest radical scavenging activity in all concentrations was in mixture 2 (200 mg GSE: 100 mg GTE) compared to synthetic food antioxidants BHA, BHT. The results obtained indicated that, higher percentage of DPPH scavenging activity in case of mixture 2 may be attributed to the higher total phenolic and flavonoids contents present in mixture 2. In several studies significant correlation was found between DPPH scavenging activity and the total phenolic content of a number of grape seed and tea extracts from different cultivars (Bakkalbasi *et al.*, 2005). The obtained data from Fig. 2 showed that, all the treatments reported a highly DPPH-Free radical scavenging activity against *in vitro* oxidative stress specially in 150  $\mu\text{g/ml}$ , but the highly quantity of natural antioxidant phenolic compounds and total flavonoids in grape seed extract reflect the highest antioxidant activity in mixture 2 (200 mg GSE: 100 mg GTE) in compare to mixture 1 and GSE, GTE separately. Like other polyphenolic compounds, catechins and procyanidins have an antioxidant property,

which may partially account for their useful role in heart diseases. Catechins and procyanidins have been shown to scavenge several forms of free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, singlet oxygen ( $^1\text{O}_2$ ), superoxide anion ( $\text{O}_2^{\cdot-}$ ), nitricoxide ( $\text{NO}^{\cdot}$ ), hydroxy ( $\text{OH}^{\cdot}$ ) and alkyl peroxy radicals due to their redox potential; that enable them to act in various forms such as hydrogen donors, reducing agents, nascent oxygen quenchers, and chelating metal ions in numerous food applications (Sichel *et al.*, 1991; Santos-Buelga and Scalbert, 2000; Sang *et al.*, 2003; Gramza *et al.*, 2006).

#### Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical created in biological systems and capable of damaging almost every molecule found in living cells and it is considered to be lipid peroxidation process, removing hydrogen atoms from unsaturated fatty acids (Kappus, 1991). The obtained data from Fig. 3 showed that, all concentrations (10, 50, 100 and 150  $\mu\text{g/ml}$ ) of different extracts reported a highly significantly hydroxyl radical scavenging activity against *in vitro* oxidative stress but the highly quantity of natural antioxidant phenolic compounds and total flavonoids in grape seed extract reflect the highest antioxidant activity in mixture 2 (200 mg GSE: 100 mg GTE). The antioxidant potential of GSE is twenty and fifty fold greater than those of vitamins E and C respectively (Shi *et al.*, 2003). Mixture 2 extract at the higher concentration 150  $\mu\text{g/ml}$  has the hydroxyl radical activity of 90% followed by 89.64, 87.54 and 85% of GSE, mixture 1 and GTE extracts respectively, compared with standards such as BHT with 97.06% and BHA with 94.02% scavenging activity. These results indicated that strong  $\text{OH}^{\cdot}$  scavenging activities of mixture 2 extract were closely related to their high levels of phenolic compounds and due to the scavenging of the radical by hydrogen donation. This is similar to the observation of several others researchers who have reported a dose – dependent activity in other foods such as sesame, sorghum, pomegranate peel and seeds, grapes pomace and seeds (Chang *et al.*, 2002; Singh *et al.*, 2002; Zhang *et al.*, 2011; Afify *et al.*, 2012; Abdel-Rahim *et al.*, 2013).

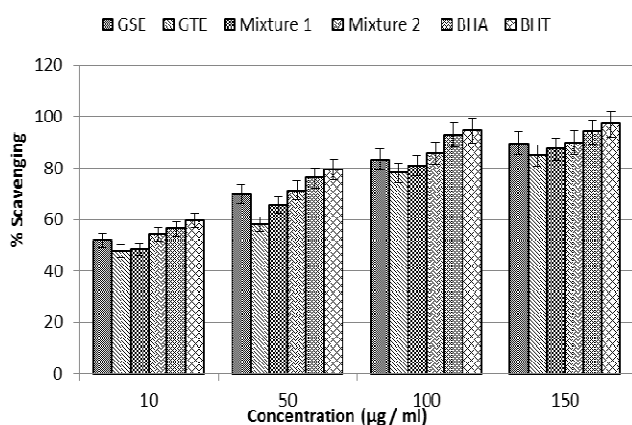


Fig. 2. DPPH radical scavenging activity (%) of different extracts concentrations of grape seed, green tea, prepared mixtures, BHA and BHT. GSE: grape seed extract, GTE: green tea extract, Mixture 1 (100 mg GSE: 100 mg GTE), Mixture 2 (200 mg GSE: 100 mg GTE), BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene

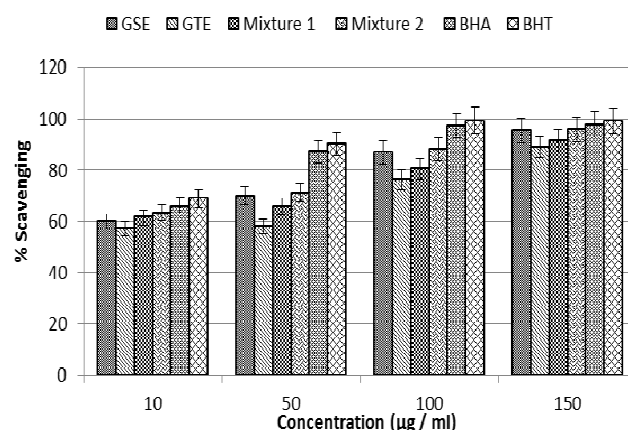


Fig. 3. Hydroxyl radical scavenging activity (%) of different extracts concentrations of grape seed, green tea, prepared mixtures, BHA and BHT. GSE: grape seed extract, GTE: green tea extract, Mixture 1 (100 mg GSE: 100 mg GTE), Mixture 2 (200 mg GSE: 100 mg GTE), BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene

*Superoxide radical scavenging activity*

Superoxide radical scavenging activity data revealed that the scavenging activity tendencies were similar to those obtained for DPPH and hydroxyl radical scavenging activity. Thus, the mixture 2 (200 mg GSE: 100 mg GTE) extract had a significantly higher scavenging activity value at all concentrations than other extracts (Fig. 4). In addition, all the treatments of GSE individually gave a significant increase in superoxide scavenging activity comparing with GTE individually due to the highly quantity of natural antioxidant phenolic compounds and total flavonoids in grape seed extract that reflect the highest antioxidant activity in mixture 2 in comparison with mixture 1. The scavenging activity of mixture 2, GSE, mixture 1, GTE extracts at higher concentration 150 µg/ml were: 95.98, 95.45, 90.51 and 89.16% respectively, compared with standard antioxidants BHA and BHT (97.76%, 99.12% respectively). Superoxide anion plays an important role in plant tissues and is involved in the formation of other cell-damaging free radicals (Bloknina *et al.*, 2003). Superoxide has been observed to directly start lipid peroxidation. It has also been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of superoxide anion radical (Yen and Duh, 1994). Superoxide anions derived from dissolved oxygen by the phenazine methosulphate (PMS) - nicotinamide adenine dinucleotide hydride (NADH) system will decrease nitroblue tetrazolium (NBT) in this system. In this method, superoxide anion reduces the yellow dye (NBT<sup>2+</sup>) to produce the blue formazan, which is measured spectrophotometrically. Antioxidants are able to inhibit the blue NBT formation (Parejo *et al.*, 2002). The decrease of absorbance in presence of antioxidants evidences consumption of superoxide anion in the reaction mixture. The increasing order of the percentage superoxide scavenging activities was mixture 2 > GSE > mixture 1 > GTE. These effects may be attributed to the antioxidant activity of the studied extracts, indicating the phenolic and flavonoids presence in the extracts for governing the O<sub>2</sub><sup>•-</sup> scavenging activities. The antioxidant properties of procyanidins from grape seeds have been shown to suppress superoxide anion and lipid peroxidation and to reduce or delay

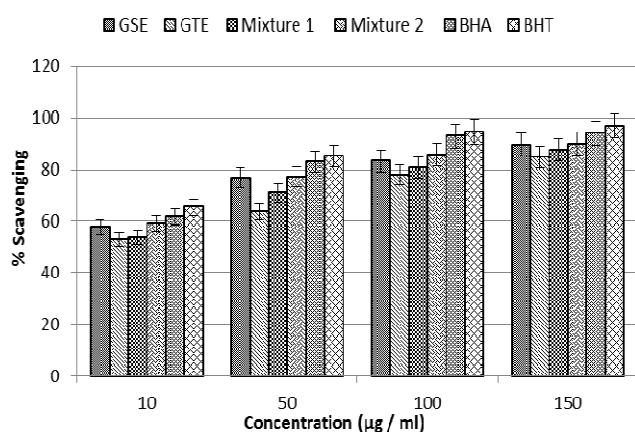


Fig. 4. Superoxide radical scavenging activity (%) of different extracts concentrations of grape seed, green tea, prepared mixtures, BHA and BHT. GSE: grape seed extract, GTE: green tea extract, Mixture 1 (100 mg GSE: 100 mg GTE), Mixture 2 (200 mg GSE: 100 mg GTE), BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene

the formation of conjugated dienes during all stages of lipid peroxidation including induction, propagation, and breakdown (Maffei-Facino *et al.*, 1996). In fact, phenolics are one of the major classes of natural antioxidants found in plants that remove such free radicals. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and to break down peroxides. It is clearly shown that the number of phenolic-OH groups present in the structure of an antioxidant molecule isn't always the only factor determining its antioxidant activity. Positions of phenolic-OH groups, presence of other functional groups in the whole molecule, such as double bonds and their conjugation to (-OH) groups and ketonic groups, also play important roles in antioxidant activities (Rice-Evans *et al.*, 1996; El Gengaihi *et al.*, 2015).

**Conclusions**

The mixture of GSE and GTE (200 mg: 100 mg) was found to be an effective antioxidant more than GSE and GTE separately. Based on the achieved results, it can be concluded that GSE and GTE mixture have the potency to act as a powerful antioxidant and can be used for decreasing the harmful effects of free radicals or reactive oxygen species (ROS) and lipid peroxidation thereby may be valuable as natural nutritional health supplement. In addition, potential use of these natural plant extracts could offer the food processing industries an alternative solution to synthetic chemical antioxidants.

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