# Antioxidant Activity of Methanolic Extracts from Sunflower *(Helianthus annuus* L.) Seed Shells

**H. E. Embaby and Kh. M. Youssef**
Faculty of Agriculture, Department of Food Science Technology, Suez Canal Univ., Ismailia, Egypt
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**Abstract:** Antioxidant properties of methanolic extracts from sunflower seed shells of two varieties (Sakha 53 and Giza 102, oilseed type with black shells) were investigated. Various established *in vitro* systems including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, reducing power (RP), total antioxidant capacity (TAC), and inhibition of a linoleic acid peroxidation assay using ferric thiocyanat (FTC) and thiobarbituric acid (TBA) tests were employed. The extracts of the two varieties exhibited high antioxidant activities and that of Sakha 53 was higher than that of Giza 102 being 63.07% and 52.5% (DPPH, at 0.5 mg/assay), 0.861 and 0.493 (RP, at 1.0 mg/assay, expressed as the absorbance at 700 nm), 0.753 and 0.387 (TAC, at 90 min, expressed as the absorbance at 695 nm), 48.13% and 37.5% (FTC) and 0.616 and 0.686 (TBA, expressed as the absorbance at 532 nm), respectively. The results obtained demonstrated considerable antioxidant activity of extracts from sunflower seed shells with the variety having significant influences on the antioxidant activity.

***Keywords:*** sunflower (*Helianthus annuus* L.), Seed shells, Antioxidant activity

## INTRODUCTION

Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules and thus, prevent or repair the damage of the body cells that is caused by oxygen (Tachakittirungrod et al., 2007). The addition of antioxidants to foods is essential to increase the shelf life and improve the stability of lipids and lipid-containing foods by retarding rancidity, discoloration, or deterioration due to autoxidation. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in processed foods. However, the use of these synthetic antioxidants in food has been restricted because of their carcinogenecity and other toxic properties (Namiki, 1990). Therefore, there is a strong need for effective antioxidants from natural sources as alternatives to prevent deterioration of foods. Recently, growing interest in the substitution of synthetic antioxidants by natural one has led to tremendous development in the research on the screening of natural antioxidants from inexpensive and residual sources from agricultural industries. It has been reported that fruit and seed processing by-products such as peel and husks are found to be a rich source of bioactive compounds that can be used as antioxidant agents and nutraceuticals (Moure et al, 2001). Investigations of plant hulls that possess antioxidant activity have been reported, including those from mung bean (Duh et al., 1997), canola (Amarowicz et al., 2000), sesame (Shahidi et al., 2006) and lentil and pea (Oomah et al., 2011).

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops. Besides palm, soy and rapeseed oil, sunflower oil is ranking fourth with a worldwide production of about 10.6 million metric tons (mt) in 2006 (FAO-STAT, 2008). Sunflowers have been known since the 26th century B.C. and have their origin in the lowlands of Mesoamerica (Pope et al., 2001). Nowadays, two main types of sunflowers are grown, the oilseed and non-oilseed or confectionary types. Sunflower seeds are composed of oil (40%), shell (30%) and meal (30%) (Demir et al., 2005). During the production of sunflower oil, meal and shell are obtained as main by-products. The meal is used primarily in preparing fodders for farm animals; however its nutritional, sensory and functional properties also make it a protein and antioxidant compounds source of interest for human food (Weisz et al., 2009). The major chemical components of sunflower seed shells such as: lipids, proteins, carbohydrates along with oil, moisture content and even the average length, width, and thickness of the seeds were studied (Cancalon, 1971; Perez et al., 2007). Moreover, quantitative analyses of total phenolic compounds and individual phenolic acids in sunflower shell extracts were described (De Leonardis et al., 2005; Weisz et al., 2009). Known that sunflower kernel extracts possess a high antioxidant activity (Nadeem et al., 2010; Žilić et al., 2010), but studies on antioxidant assessment of sunflower seed shells is scanty. Thus, the objective of the present paper was to evaluate the antioxidant activity of methanolic extracts from sunflower seed shells and to compare them with the synthetic commercial antioxidant, namely butylated hydroxytoluene (BHT).

## MATERIALS AND METHODS

### Materials

Seeds of two available varieties of sunflower (*Helianthus annuus*), Sakha 53 and Giza 102 (oilseed type with black shells) were obtained from the agronomy department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, linoleic acid, butylated hydroxytoluene (BHT), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents and all other chemicals used were of analytical grade.

### Preparation of Extracts

Whole sunflower seeds were shelled and the separated shells were ground into a fine powder using a household flour-mill (Braun, Germany). Fifteen grams of the ground shells were extracted with 100 ml of

methanol in a shaker at room temperature for 24 hr. Subsequently, the extracts were filtered through a Whatman No. 2 filter paper and the residue was re-extracted under the same conditions. The combined filtrates were evaporated to dryness under vacuum at 40 °C using a rotary evaporator (Strike 300, Steroglass, Perugia, Italy). The dried extract was stored at –20 °C until analysis and the yields were calculated. The percentage of dry extracts of Sakha 53 and Giza 102 were 8.1% and 8.9%, respectively. The extraction process was carried out in triplicate.

**DPPH Radical-Scavenging Assay**

The capacity of the prepared extracts to scavenge the 'stable' free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Hatano et al (1988). Extracts at different concentrations (0.1–0.5 mg) were dissolved in 4 mL of methanol and then added to a methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed for 15 s and then left to stand at room temperature for 30 min. The decrease in the solution absorbance, due to proton-donating activity, was measured at 517 nm using a spectrophotometer (6505 UV/Vis, Jenway LTD., Felsted, Dunmow, UK). The control contained all reagents except the extract and the DPPH radical scavenging activity of BHT was assayed for comparison. The DPPH radical-scavenging activity was calculated using the following formula:

DPPH radical – scavenging activity (%) = $[(1- A_1 / A_0) \times 100]$, Where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the samples (extracts and BHT).

**Determination of the Total Antioxidant Capacity**

The total antioxidant capacity of the extracts was determined according to the method of Prieto et al (1999). The methanolic extract (1 mg/mL, 0.1 mL) was combined with 0.3 mL of a reagent solution consisting of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was incubated at 95 °C for 90 min. After the mixture had been cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. Readings were taken each 30 min. The antioxidant activity was expressed as the absorbance of the sample. The antioxidant activity of BHT (1 mg/mL) was also assayed for comparison.

**Measurement of the Reducing Power**

The determination of reducing power was performed as described by Oyaizu (1986). Different concentrations of the extracts (0.2–1.0 mg/mL in methanol) were mixed with the sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50 °C for 20 min, then 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged for 10 min at 3000g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power. The reducing power of BHT was also determined for comparison.

**Ferric Thiocyanate (FTC) Method**

The efficacy of inhibiting lipid peroxidation of the extracts was determined according to the method described by Zin et al (2002). Four mg of the extract were individually dissolved in 4 mL of methanol. Then, the extract solution was successively mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 mL), 0.05 M phosphate buffer pH 7.0 (8 mL) and distilled water (3.9 mL). The mixture was then kept in a screw-cap container at 40 °C in the dark. Subsequently, the degree of oxidation was measured using ferric thiocyanate method (Kikuzak and Nakatani, 1993) as follows. Every 24 hr, 0.1 mL of this reaction mixture was drawn and mixed with 75% ethanol (9.7 mL), 30% ammonium thiocyanate (0.1 mL) and 0.02 M ferrous chloride in 3.5% hydrochloric acid (0.1 mL). After 3 min, the intensity of formed red color was measured at 500 nm. Absorbance was measured until the control, where there was no addition of sample extract, reached maximum absorbance. BHT was used as a positive control. The inhibition percent of linoleic acid peroxidation was calculated as (%) inhibition =[1–(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)]×100.

**Thiobarbituric Acid (TBA) Test**

TBA test was conducted (Mackeen et al., 2000) instantly after the control sample from FTC test had reached its maximum absorbance value. In brief, 1.0 mL of 20% aqueous trichloroacetic acid and 2.0 ml of 0.67% aqueous thiobarbituric acid were added to 2 ml of sample solutions acquired from FTC test. The mixture was then placed in a boiling water bath for 10 min. After cooling under tap water, the mixture was centrifuged at 3000g for 30 min. Finally, the absorbance of the supernatant at 532 nm was measured by using the spectrophotometer.

**Statistical Analyses**

All tests were conducted in triplicate. The data are reported as means ± SD. Analysis of variance (ANOVA) accompanied with Duncan test using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago) was conducted to identify the significant difference among samples ($p < 0.05$).

## RESULTS AND DISCUSSION

**DPPH Radical Scavenging Activity**

The DPPH radical assay is commonly used for the determination of antioxidant activity of pure antioxidant compounds as well as different plant extracts (Yu and Zhou, 2004). DPPH is a stable organic free radical with deep violet color, which gives absorption maxima within the 515–528 nm range. Upon receiving a proton from any hydrogen donor, mainly from phenolics, it loses its chromophore and becomes yellow. The decrease in the absorbance depends on the concentrations of the antioxidant and the radical, the molecular structure of the antioxidant, and its kinetic behavior (Amarowicz et al., 2000). The scavenging activity (SCA%) of the sunflower shells extracts compared to BHT for DPPH radical is shown in Fig 1. In this experiment, time was constant—30 min—for all extracts and the synthetic antioxidant (BHT), while the

concentration of hydrogen donors/radical scavengers added was different. The results indicate that, at different concentrations (0.1mg to 0.5 mg/assay), the methanolic extracts of sunflower shells from both varieties (Sakha 53 and Giza 102) exhibited a strong DPPH scavenging potency. Also, the scavenging activity of the extracts increased when the amounts added increased. These results were similar to those reported in hulls extracts from other sources such as, sesame hulls (Shahidi et al., 2006) and mung bean hulls (Duh et al., 1997). Moreover, scavenging activity of the extract of Sakha 53 (63.07% at 0.5 mg/assay) was significantly ($p$ <0.05) higher than that of Giza 102 (52.5% at 0.5 mg/assay) and this result suggests that variety might have significant influences on the antioxidant activity of sunflower shells. This finding was supported by the observation that many barley varieties differed significantly in their antioxidant properties (Zhao et al., 2008).

It has been proven that the antioxidant activity of plant extracts is mainly ascribed to the concentration of the phenolic compounds; by increasing the concentration of phenolic compounds or the degree of hydroxylation of them, their DPPH radical scavenging activity increases (Silva et al., 2006; Mohsen and Ammar, 2009). Thus, the observed differences between antioxidant activities of Sakha 53 and Giza 102 varieties could be attributed to the level of total phenols. Generally, the results indicate that the extracts from both varieties had high hydrogen donation ability; however, when compared to BHT, they showed significantly ($p < 0.05$) lower DPPH radical scavenging activity.

**Reducing Power**

Reducing power is associated with the antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes (Yen and Chen, 1995). In this assay the yellow color of the test solution changes to various shades of green and blue, depending upon the reducing power of each extract. The presence of reductants (i.e. antioxidants) causes the reduction of the $Fe^{3+}$/ferricyanide complex to the ferrous form. Therefore, the $Fe^{2+}$ can be monitored by measuring the formation of the blue color at 700 nm (Chung et al., 2002).

Fig. 2 depicts the reducing powers of the methanolic extracts of sunflower shells as well as BHT at different concentrations. The results indicate that sunflower shells extracts exhibited a great reducing power. Thus, phenolics present in the sunflower shells extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products. Furthermore, the reducing power of Sakha 53 was significantly higher ($p < 0.05$) than that of Giza102 at different concentrations. For example, At 1.0 mg, the reducing power of Sakha 53 and Giza 102 were 0.861 and 0.493, respectively, while that of BHT was 1.773 (expressed as the absorbance at 700 nm). Therefore the reducing power was in this order: BHT > Sakha 53 > Giza 102. These results are in good agreement with those reported by Duh et al (1997) for mung bean hulls. Also, the reducing power of the sunflower shells extracts were similar to those reported by Amarowicz et al (2000) for crude tannins of canola and rapeseed hulls. Thus, the extracts may contain reductones and react with freeradicals to stabilize and terminate radical chain reactions. An interesting observation was that the trend for reducing power of both varieties was similar to their DPPH radical scavenging activities, when a comparison between Figs. 1 and 2 is made.

**Total antioxidant capacity assay**

The assay is based on the reduction of Mo (VI) to Mo (V) by extracts and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The high absorbance values indicated that the sample possessed a significant antioxidant activity. In this assay, the total antioxidant activities of the extracts were measured and compared with those of BHT and the control. According to the results, the extracts of both varieties had significant total antioxidant activities and the effects increased as the reaction time increased. Fig. 3 also declares that the total antioxidant capacity of Sakha 53 was significantly higher ($p < 0.05$) than that of Giza 102 and the total antioxidant capacity of BHT was superior to Sakha 53 and Giza 102. Variations in the antioxidant capacity between the two varieties may be attributed to the difference in the level of phenolic compounds. Also, the values obtained for the total antioxidant capacity coincided well with those of DPPH radical scavenging activity and reducing power in both varieties. Similarly, Pan et al (2008) reported that the ethanolic extracts of longan peel had a high antioxidant capacity and the effect increased with increasing reaction time and increasing concentration.

**Inhibition of Linoleic Acid Peroxidation (FTC and TBA Tests)**

The FTC method was used to measure the peroxide level during the initial stage of lipid oxidation. Peroxides are formed during the linoleic acid oxidation, which react with $Fe^{2}$+ to form $Fe^{3}$+. The latter ions form a complex with the thiocyanate ion and this complex has a maximum absorbance at 500 nm. Low absorbance value in the FTC method indicates a high level of antioxidant activity. The effects of the extracts from sunflower seed shells of both varieties and BHT (as a positive control) in preventing the peroxidation of linoleic acid are shown in Fig 4. The results showed that the extracts significantly retarded the formation of hydroperoxides in the linoleic acid system throughout the incubation period. In the final reaction mixture, the extracts of Sakha 53 and Giza 102 inhibited 48.13% and 37.5% peroxidation of linoleic acid after incubation for 96 h (4 days), respectively. However, these values were significantly ($p < 0.05$) lower than that of the positive

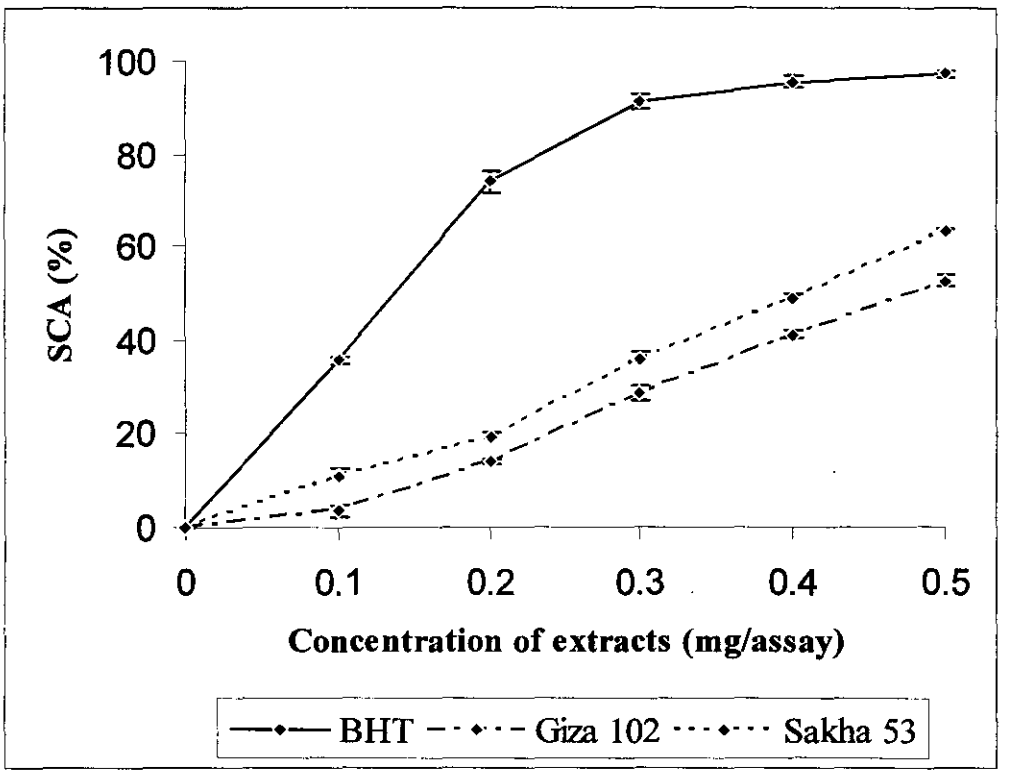


**Figure 1.** DPPH free radical scavenging activity of methanolic extracts from sunflower shells in comparison with BHT. *Results are mean ± SD of three parallel measurements. SCA (%): percentage of scavenging activity on DPPH radical.

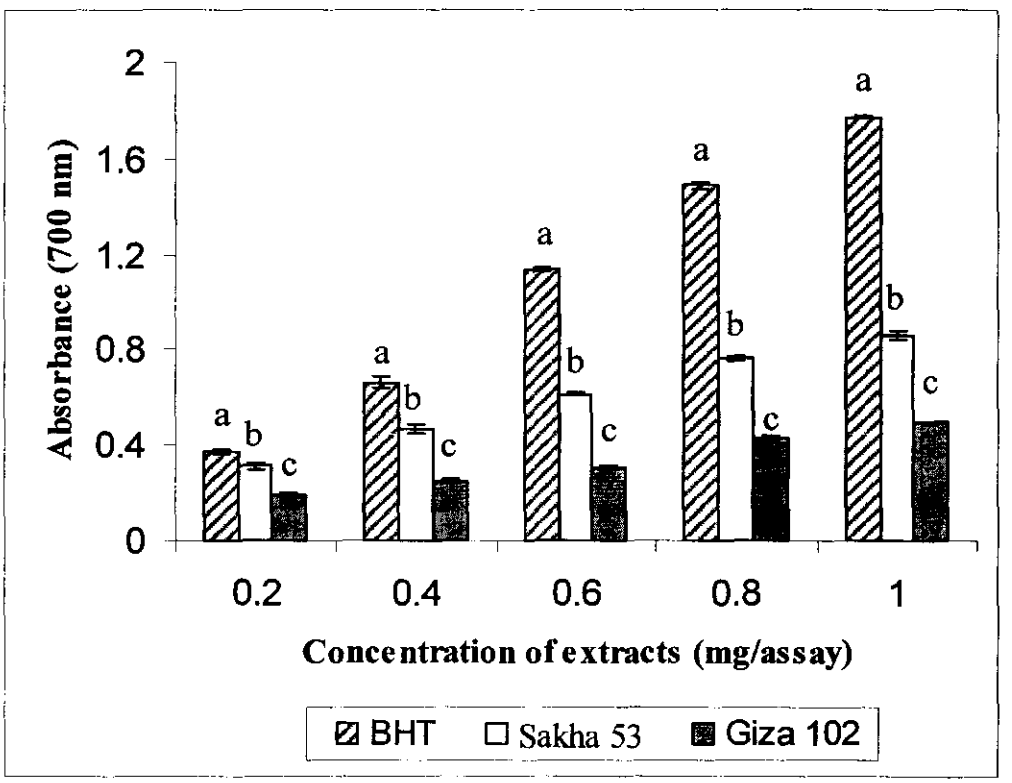


**Figure 2.** Reducing power of methanolic extracts from sunflower shells. Values with different letters (for each concentration) are significantly different ($p < 0.05$). Error bars indicate standard deviations ($n = 3$).

control, BHT (90.57%). Moreover, Sakha 53 exhibited higher inhibition in the linoleic acid peroxidation system than Giza 102. Another interesting observation is that the efficiency of the extracts from both varieties in preventing the peroxidation of linoleic acid was similar to their DPPH radical scavenging activities, reducing powers and total antioxidant capacities as shown in Figs 1, 2, 3 and 4. Similar results were obtained for seed coats from red and black beans (Tsuda et al., 1994) and tamarind (Siddhuraju, 2007).

After the control sample had reached its maximum absorbance value in FTC test, TBA test was conducted on the samples. This test measures the thiobarbituric acid reactive substances content at a later stage of lipid oxidation. In this test, a low absorbance value indicates higher thiobarbituric acid reactive substances inhibitory activity (Ismail et al., 2010). Fig. 5 shows that the extracts of sunflower shells exhibited a high thiobarbituric acid reactive substances inhibitory activity with Sakha 53 showing a stronger activity ($p < 0.05$) than Giza 102. Also, the strength of thiobarbituric acid reactive substances inhibitory activity of BHT was superior to both extracts ($p < 0.05$). The trend of thiobarbituric acid reactive substances inhibitory activity of the sunflower shells extracts is rather similar to the trend of FTC test. This suggests that reduction of thiobarbituric acid reactive substances content in Sakha 53 and Giza 102 samples could be attributed to the lower hydroperoxides accumulation in the respective samples. Besides, secondary antioxidant compounds that might present in these extracts may also contribute to the inhibition of hydroperoxides decomposition (Ismail et al., 2010). In general, seed coats may play an important role in protecting from oxidative damage by possessing endogenous antioxidants such as phenolic compounds. The sunflower seed shell extract was reported to contain protocatechuic, chlorogenic, caffeic, syringic, ferulic and o-cinnamic acids, noting that chlorogenic acid is predominant amounting up to 59.1 mg/100 g in the shells (Weisz et al., 2009). Also, Total phenolic acid content determined by HPLC varied from 40.8 to 86.0 mg/100 g of shells and depended on the variety of sunflower (Weisz et al., 2009).

## CONCLUSIONS

Methanolic extracts from the sunflower seed shells of varieties, Sakha 53 and Giza 102, were found to possess antioxidant activity (determined by DPPH, reducing power, total antioxidant capacity and lipid peroxidation inhibition). The antioxidant activity increased with increasing reaction time and concentration. The extract from Sakha 53 exhibited a higher activity than that of Giza 102. Thus, the variety had some influences on the antioxidant properties which could be attributed to the level of phenolic compounds.

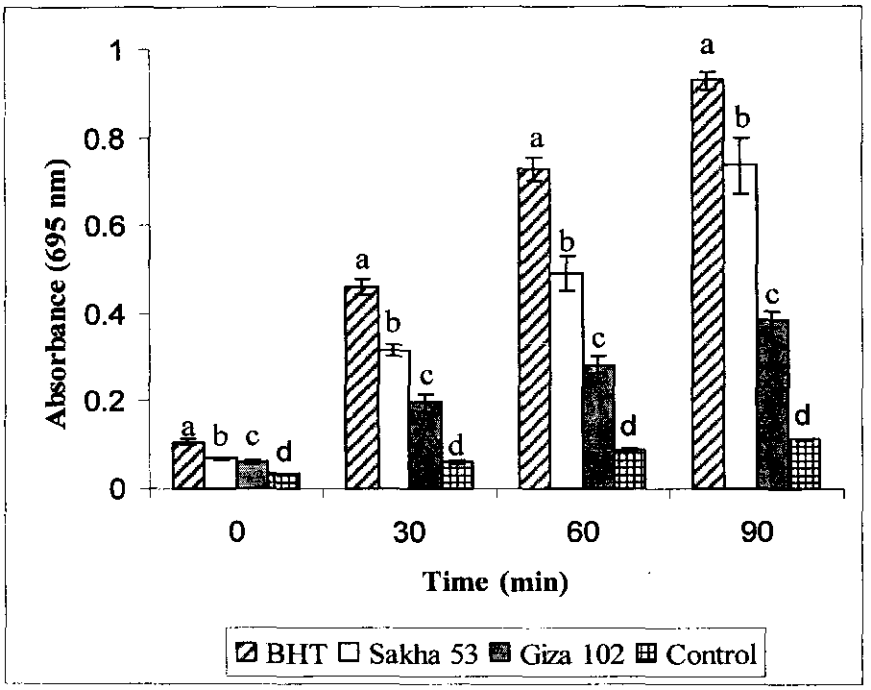


**Figure 3.** Total antioxidant capacity of methanolic extracts from sunflower shells. Values with different letters (for each time) are significantly different ($p < 0.05$). Error bars indicate standard deviations ($n = 3$).

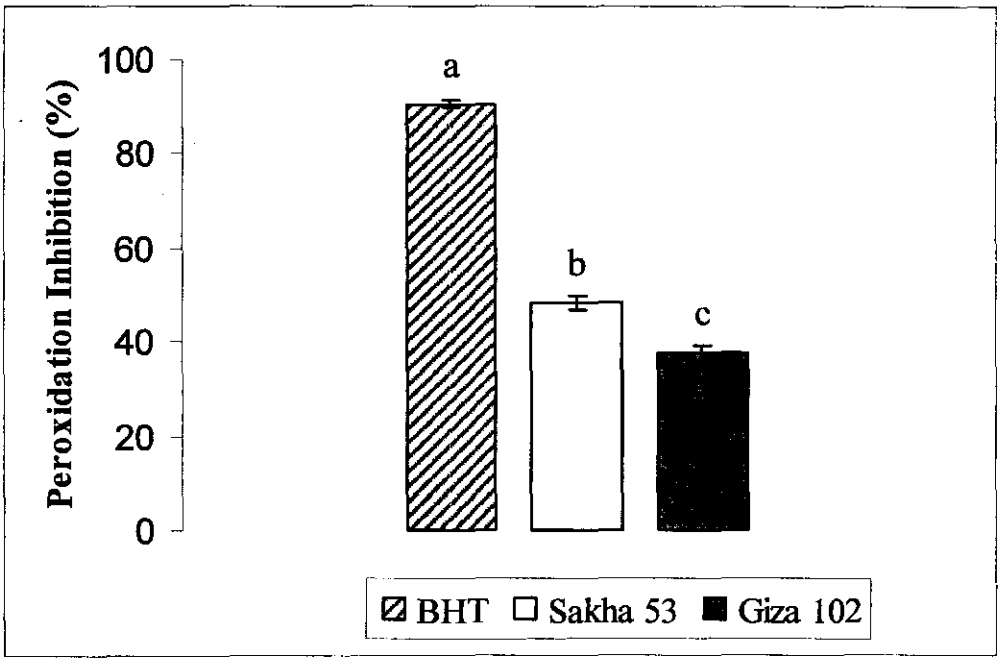


**Figure 4.** Hydroperoxides inhibitory activity of sunflower shells extracts measured by ferric thiocyanate test. Different letters indicate significant differences ($p < 0.05$). Error bars indicate standard deviations ($n = 3$).

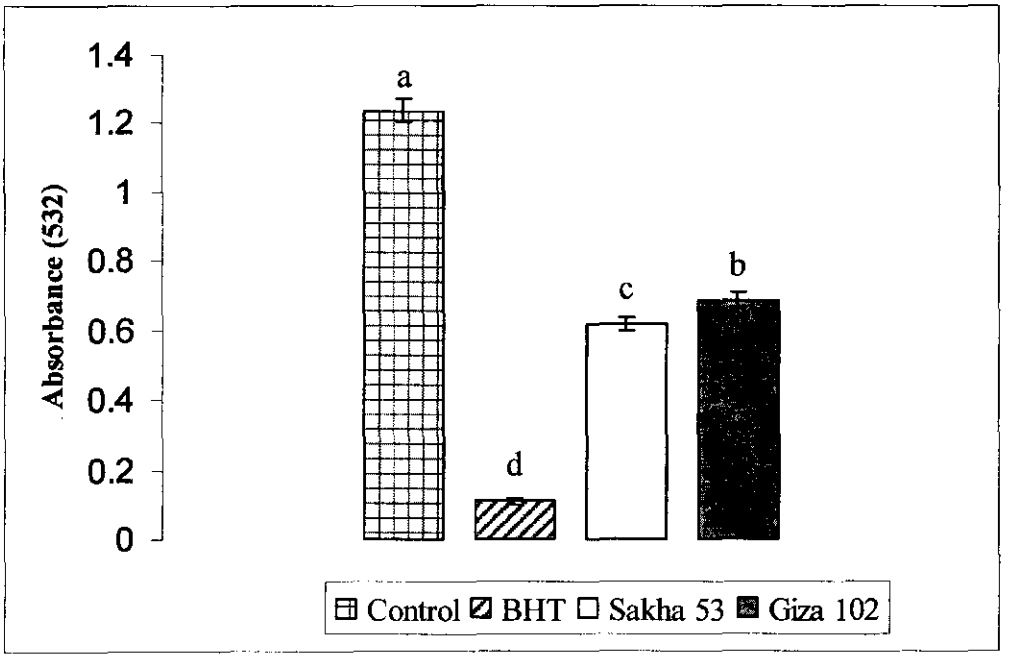


**Figure 5.** Thiobarbituric acid reactive substances inhibitory activity of sunflower shells extracts measured by thiobarbituric acid test. Different letters indicate significant differences ($p < 0.05$). Error bars indicate standard deviations ($n = 3$).

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