

Preparation and Characterization of Nanohybrid Antioxidant from Propyl Gallate and Evaluation of its Antioxidant Activity

Zahraa Raheem Murshidy¹, Abeer Muhammedali Alnasrawi^{2*}, Ali Abed Alkadhmi Al-Ghanimi¹

¹. Department of Biology, College of Science, University of Kerbala, Kerbala, Iraq.

². Department of Clinical Laboratories, College of Applied Medical Science, University of Kerbala, Kerbala, Iraq.

*Corresponding Author Email: abeeralnasrawi@gmail.com

Abstract

In this study a nanohybrid antioxidant Mg/Al-PG-LDH was prepared by intercalating propyl gallate (PG) between magnesium/ aluminum layered double hydroxides. Identification of the prepared compound by the FT-IR revealed that some functional groups had been shifted either to higher or lower frequencies indicating the preparation of new compound. X-ray diffraction analysis confirmed the crystalline nature of the prepared compound. The antioxidant activity of the nanohybrid compound was evaluated using three methods. In the ABTS cation radical scavenging assay the IC₅₀ of PG and Mg/Al-PG-LDH were (50 and 100) µg/ml, respectively. By using ferric reducing antioxidant power (FRAP), analysis of the results allowed to rank the antioxidants used in this study as follows: Mg/Al-PG-LDH < Trolox < PG. The standard antioxidant butylated hydroxyl anisole exhibited the highest chelating effect in compare with the nanohybrid antioxidant and PG.

Keywords: *Propyl gallate, Nanohybrid antioxidant, FT-IR, XRD, ABTS, FRAP, and Chelating effect*

Introduction

Since ancient times, food additives have been used to achieve many goals including preserving food, improving its texture, enhancing or improving its appearance and prolonging its shelf life [1]. Antioxidants are considered as the important class of food additives due to their function in scavenging free radicals. Two methods can be used to categorize this type of additives. Depending on their function, there are primary and secondary antioxidants. On the other hand, there are natural and synthetic antioxidants depending on their nature [2].

In the last few decades, antioxidants have attracted worldwide attention, especially from the scientific community, consumers, and food manufactures [3].

One of the most primary synthetic antioxidants is propyl gallate (PG, propyl 3, 4, 5- trihydroxybenzoate). Its synthesis is carried out by condensation of propanol and gallic acid [4]. PG is a phenolic compound

extensively used in food, cosmetics and pharmaceutical industries [5].

Its antioxidant activity is due to its ability to suppress chain initiation or break chain propagation of peroxidation of unsaturated fatty acids [6]. Recently, nanotechnology encompasses many fields, especially the food sector. It offers the possibility of synthesis of novel materials with new properties quite different from their bulk counterparts [7].

Many advantages in food sector can be achieved by using nanotechnology such as food sensory improvement, increasing absorption of nutrient and bioactive compounds, extending food shelf life, improving food safety and production of antimicrobials to face pathogenic bacteria in food [8]. The purpose of this study is to prepare and characterize a nanohybrid antioxidant compound from PG and evaluate its antioxidant activity using different methods.

Materials and Methods

Preparation of Nanaohybrid Antioxidant from PG

The method described by [9] with some modifications was followed in preparing the nanohybrid antioxidant under study. Briefly, 50 ml of PG solution (2.4%) was dropped under stirring within 2 hrs on 50 ml of magnesium aluminum layered double hydroxides (2%).

The mixture was placed in a shaker incubator at 37 °C for 18 hrs and then incubated at 40°C for 24 hrs in a static incubator. The mixture was subjected to centrifugation at 5000 rpm/20 min., washing several times with deionized water, drying at 40°C, and finally storage for further uses. The prepared nanohybrid PG, the bulk PG and magnesium aluminum layered double hydroxides were given the symbols Mg/Al-PG-LDH, PG and LDH, respectively.

Characterization of nanohybrid PG

Characterization by FT-IR

PG, LDH and Mg/Al-PG-LGH samples were ground finely with potassium bromide and pressed into thin pellets. FT-IR scans were performed within (400-4000) cm^{-1} .

Characterization by X-ray Diffraction (XRD)

X-ray Diffractometer was used to record the XRD spectra of the nanohybrid antioxidant Mg/Al-PG-LDH and LDH within the range (2-50) ° 2 Θ .

In Vitro Antioxidant Activity of the nanohybrid PG

Three methods were used to evaluate the antioxidant activity of Mg/Al-PG-LDH and PG. These methods were:

ABTS Radical Cation Scavenging Assay

The 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity was measured according to the method of [10] with minor modifications.

The ABTS reagent was generated by mixing equal volumes of 2 mM ABTS and 4.9 mM Potassium persulfate and the mixture was kept in the dark at room temperature for 16 hrs.

The reagent absorbance was then adjusted to 0.700 ± 0.02 at 734 nm. Various dilutions ranged from 1 mg/ml to 5 $\mu\text{g/ml}$ were prepared from Mg/Al-PG-LDH, PG, and the standard antioxidant trolox. The reaction was initiated by adding 3 ml of the reagent to 0.3 ml of test sample, mixed well and incubated at room temperature for 10 min.

The absorbance was measured at 734 nm and the radical inhibition percentage was calculated according to the following equation:

$$\text{PI}\% = [1 - (\text{At}/\text{Ar})] \times 100$$

Where At and Ar represent the absorbance of the sample and ABTS, respectively.

Ferric Reducing Antioxidant Power (FRAP) Assay

The method described by [11] was used to evaluate FRAP of Mg/Al-PG-LDH and PG. For comparison, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was chosen as a standard. Three solutions were used to prepare FRAP reagent include 10 mM 2,4,6-tripyridyltriazine (TPTZ), 20 mM ferric chloride, and 0.3 M sodium acetate buffer (pH= 3.6). The three solutions were mixed together in the ratio (1:1:10).

Various concentrations ranged (5-100) $\mu\text{g/ml}$ were prepared from the two compounds under study. 200 μl of the test sample were added to 3 ml of FRAP reagent, mixed well, left at room temperature for 4 min. and the absorbance was measured at 593 nm.

Ferrous ion Chelating Activity

Ferrous ion chelation by Mg/Al-PG-LDH, PG and the standard antioxidant butylated hydroxy anizole (BHA) was determined according to the method described by [12] with some modifications. Concentrations in the range (10-1000) $\mu\text{g/ml}$ were prepared from the above compounds. Briefly, 1 ml of the test sample was added to a mixture constructed of (1ml of 0.375 mM FeSO_4 .

7 H_2O , and 1 ml of 1 mM Ferrozine). The mixture was vortexed well and incubated in the dark at room temperature for 10 min. Absorbance was recorded at 562 nm against blank. Control solution was prepared by mixing 1.5 ml of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.5 ml of Ferrozine.

The percentage of chelating effect was calculated according to the following equation:

$$\text{Chelating effect \%} = \frac{(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100}{100}$$

Results and Discussion

Characterization of Mg/Al-PG-LDH and PG by FT-IR

The FT-IR spectrum of PG is illustrated in figure 1. The three bands observed at (3504, 3468, and 3223) cm^{-1} is assigned to O–H stretching. The symmetric and a symmetric stretching vibration of CH_3 were observed at (2879 and 2968) cm^{-1} , respectively. The appearance of the band at 1693 cm^{-1} belongs to the ester carbonyl (C=O) stretching. Skeletal stretching of benzene ring was observed at (1616, 1539, and 1469) cm^{-1} .

The two bands appeared at (868 and 764) cm^{-1} is due to the bending of benzene CH [13]. Figure 2 (a) showed the FT-IR spectrum of the LDH. The characteristic band in this spectrum is observed at 1383 cm^{-1} which belongs to the nitrate group between LDHs. A broad band can be observed at 3527 cm^{-1} is due to the stretching of different types of OH groups, while the bands observed at (400-600) cm^{-1} are assigned to the vibration of Mg–O and Al–O bonds [13,14].

The results of the FT-IR spectrum presented in figure 2(b) confirmed the preparation of nanohybrid antioxidant Mg/Al-PG-LDH by intercalation of PG into LDHs. The band at 3506 cm^{-1} belongs to the stretching vibration of phenolic OH which seems to be interfered with aromatic CH stretching. The ester

carbonyl C=O stretching can be observed at 1662 cm^{-1} which shifted to lower frequency in comparison with FT-IR spectrum of PG (Figure 1). The two bands at (1570 and 1496) cm^{-1} are attributed to the skeletal stretching of benzene ring. In comparison with the FT-IR spectrum of LDH in figure 2 (a), the nitrate group (NO_3) shifted to a lower frequency since it appeared at 1373 cm^{-1} . Finally, out of plane bending of aromatic CH observed at 833 cm^{-1} which shifted to a lower frequency [13, 14].

Characterization of Mg/Al-PG-LDH by XRD

XRD was conducted to analyze the crystalline nature of the nanohybrid antioxidant prepared in this study. Figure 3 (a) shows XRD spectrum of LDH. It was well known from previous studies that there are three diffraction planes in the LDH spectrum included (003), (006) and (009) which appeared at (10.47°, 22.03°, and 34.44°), respectively.

The three planes have a crystalline distances of (0.84, 0.41, and 0.26) nm, respectively, too. After intercalation of PG between LDHs, results of crystallographic analysis are given in Figure 3 (b). The new diffraction planes were appeared at (8.69, 15.06, and 24.1)° 2 θ with crystalline distance of (1.01, 0.58, and 0.36) nm, respectively.

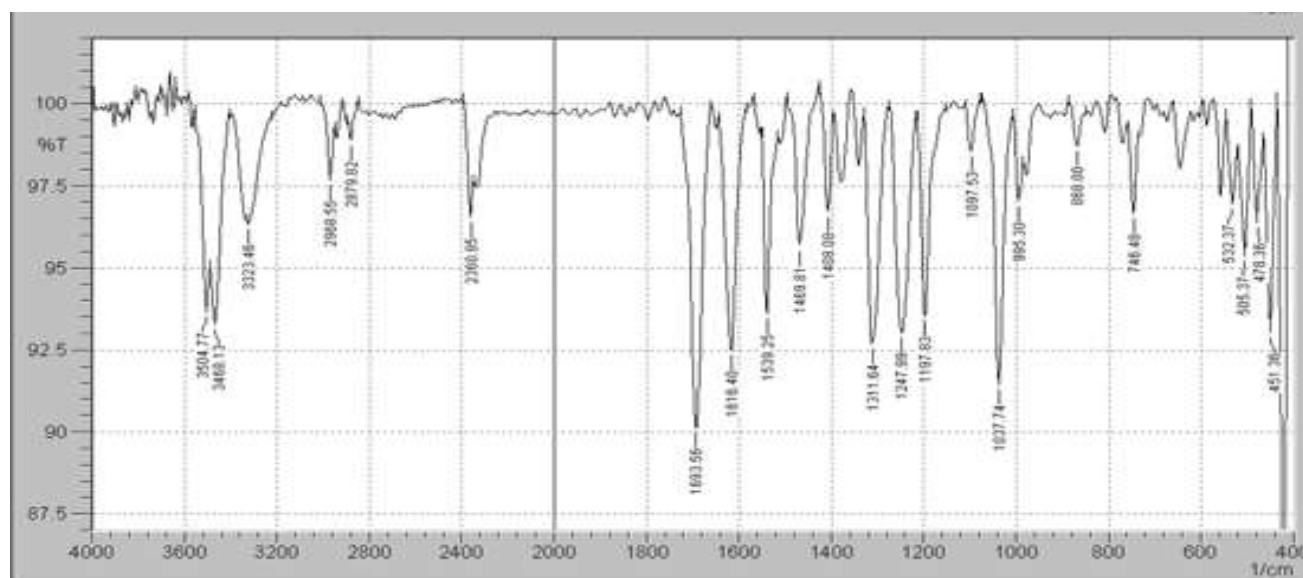


Figure 1: the FT-IR spectrum of PG

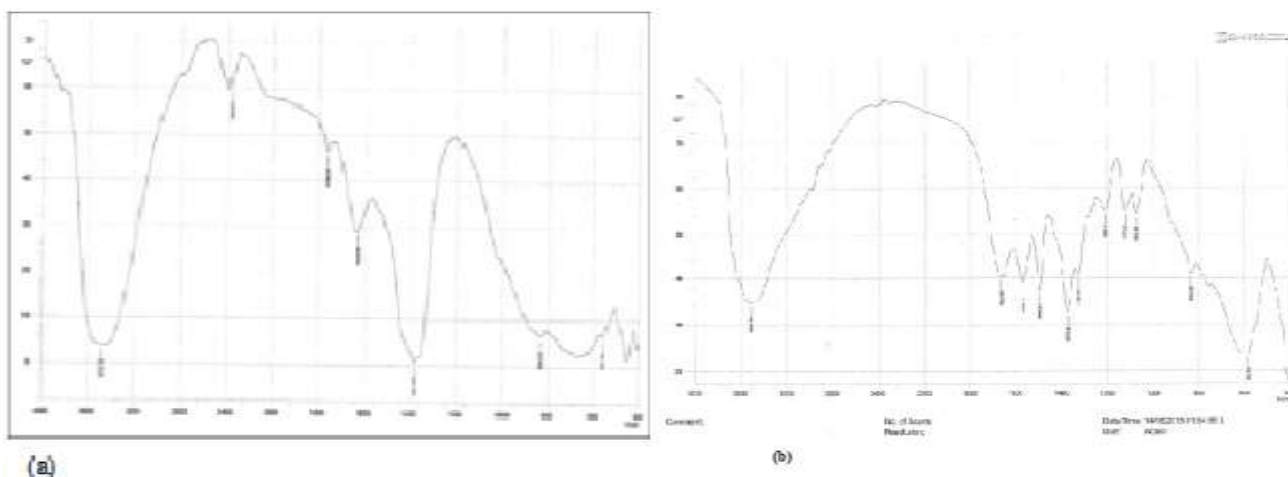


Figure 2: FTIR spectrum of Magnesium /Aluminum layered double hydroxide (a) and Mg/Al-PG-LDH (b)

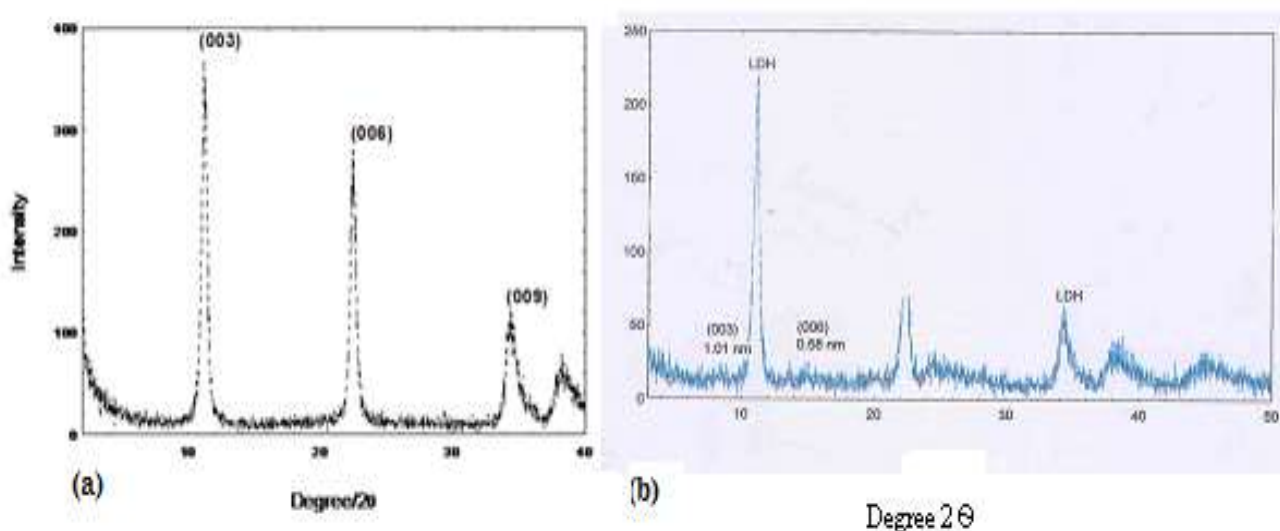


Figure 3: XRD analysis of Magnesium/Aluminum layered double hydroxide (a) and Mg/Al-PG-LDH (b)

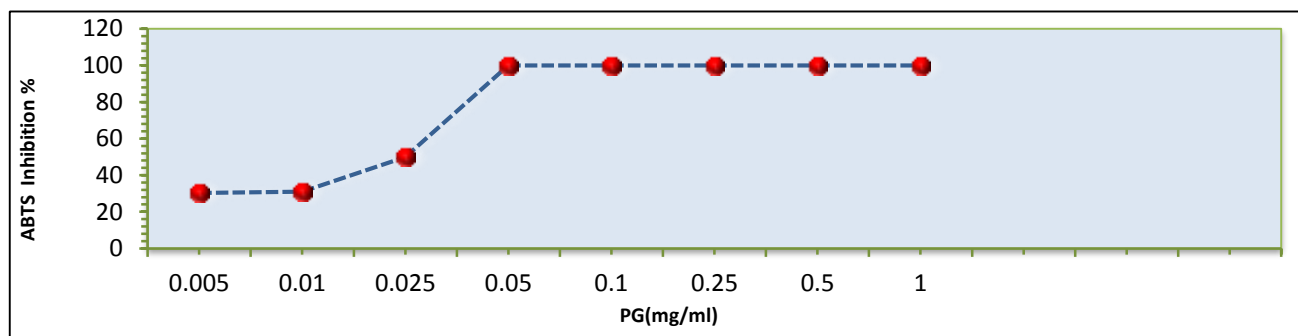


Figure 4: Antioxidant activity of bulk (PG) by ABTS scavenging method

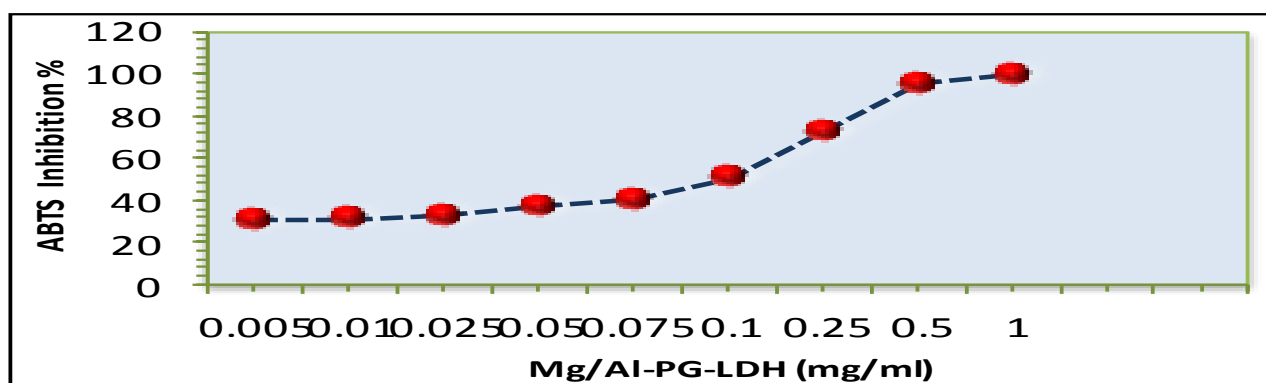


Figure 5: Antioxidant activity of Mg/Al-PG-LDH by ABTS scavenging method

In vitro antioxidant activity of Mg/Al-PG-LDH and PG

ABTS Cation Radical Scavenging Activity

Antioxidant concentration that causes reduction of ABTS (IC_{50} , which was expressed as PI%) was used as an index to compare the antioxidant activity of the compounds used in this study. Antioxidants can react with the radical ABTS solution and donate more electrons to reduce the blue color of the ABTS [15].

Results shown in figures 4 and 5 revealed that IC_{50} values of PG and Mg/Al-PG-LDH were found at (50 and 100) $\mu\text{g/ml}$, respectively. The antioxidant activity of PG is due to its ability to suppress chain initiation or break chain propagation of peroxidation of unsaturated fatty acids [6].

The results of the current study were in agreement with earlier study reported by [16] where the IC_{50} value of the nanohybrid antioxidant octyl gallate was at 75 $\mu\text{g/ml}$.

Another study revealed that intercalation of the flavonoids, quercetin, and catechin to some nanoparticles cause enhancing in their antioxidant activities by inhibiting the oxygen radical ($O\cdot_2$) [17]. Generally, gallic acid and its derivatives possess high scavenging activity due to the presence of many hydroxyl groups in their structures [18].

FRAP Assay

FRAP assay is based on antioxidants abilities to reduce Fe^{3+} to Fe^{2+} forming a blue color in the presence of TPTZ [11]. By observing the results in Figures 6, 7, and 8, it can be noticed that the reducing power of the compounds under study were proportional with the increasing in their concentrations.

Furthermore, analysis of the results allowed to rank the antioxidant used in the present study as follows: Mg/Al-PG-LDH < Trolox < PG. Our results supported by those found by [19] where the bulk tannic acid exhibited high reducing power in compare with the nanohybrid tannic acid (loaded on LDH).

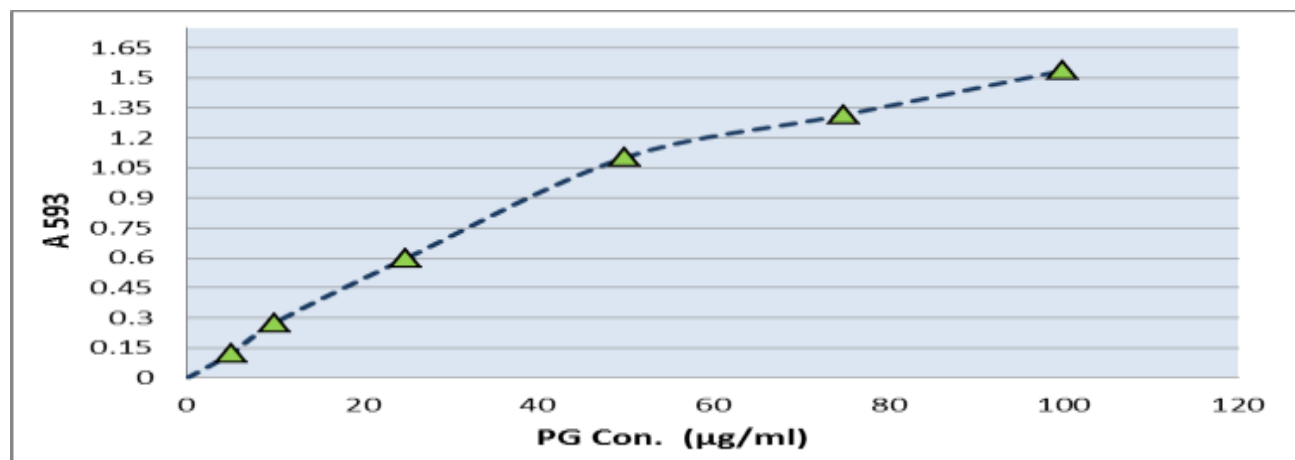


Figure 6: Antioxidant activity of PG by FRAP method

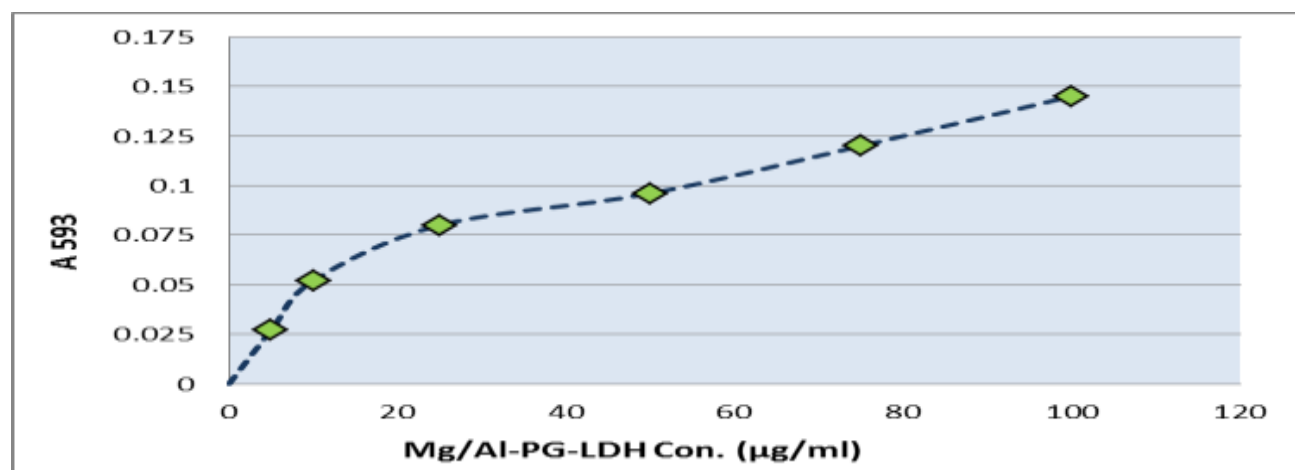


Figure 7: Antioxidant activity of Mg/AL-PG-LDH by FRAP method

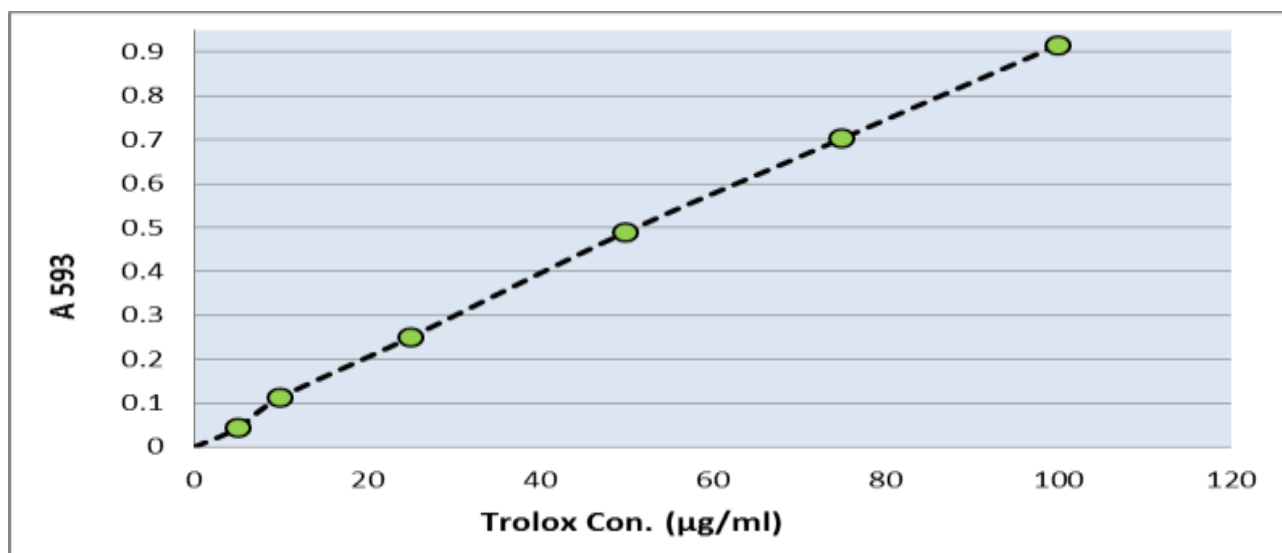


Figure 8: Antioxidant activity of Trolox by FRAP method

Metal chelating effect

From Figures 9,10, and 11, it has been found that the standard antioxidant BHA exhibited the highest metal cheating effect which reached to 30.43 % in compare with Mg/Al-PG-LDH and PG. Results also showed equal chelating effects for the nanohybrid antioxidant and the bulk PG which reached

to (15.14 and 15.24)%, respectively. The results found in this study was not in agreement with those reported by [16] where the metal chelating effect of the nanohybrid octylgallate was higher than its bulk compound and similar to that of BHA.

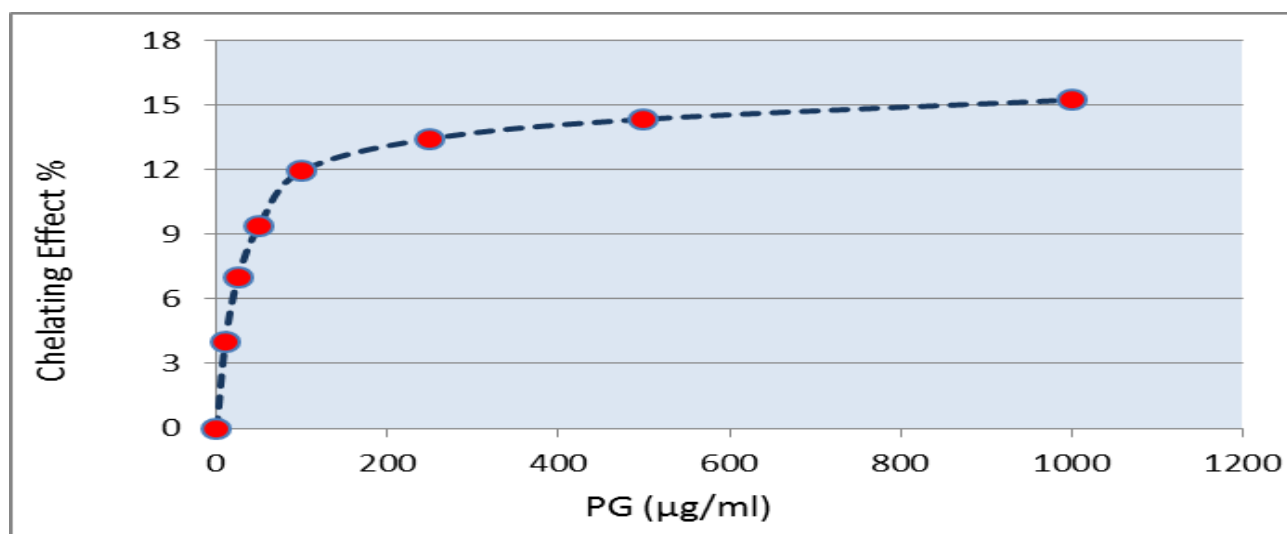


Figure 9: Antioxidant activity of PG by ferrous ion chelating activity

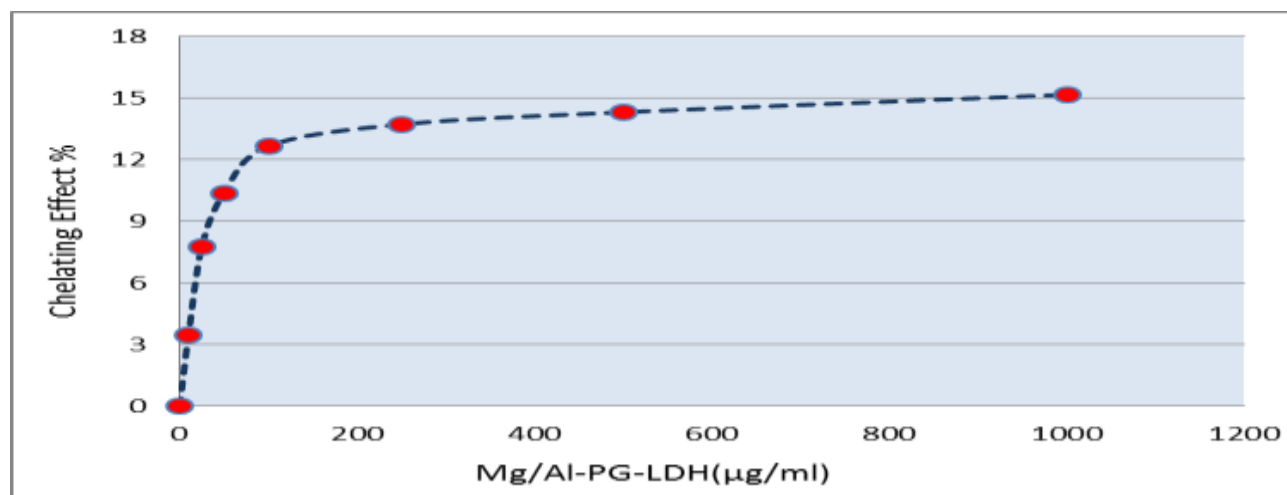


Figure 10: Antioxidant activity of Mg/Al-PG-LDH by ferrous ion chelating activity

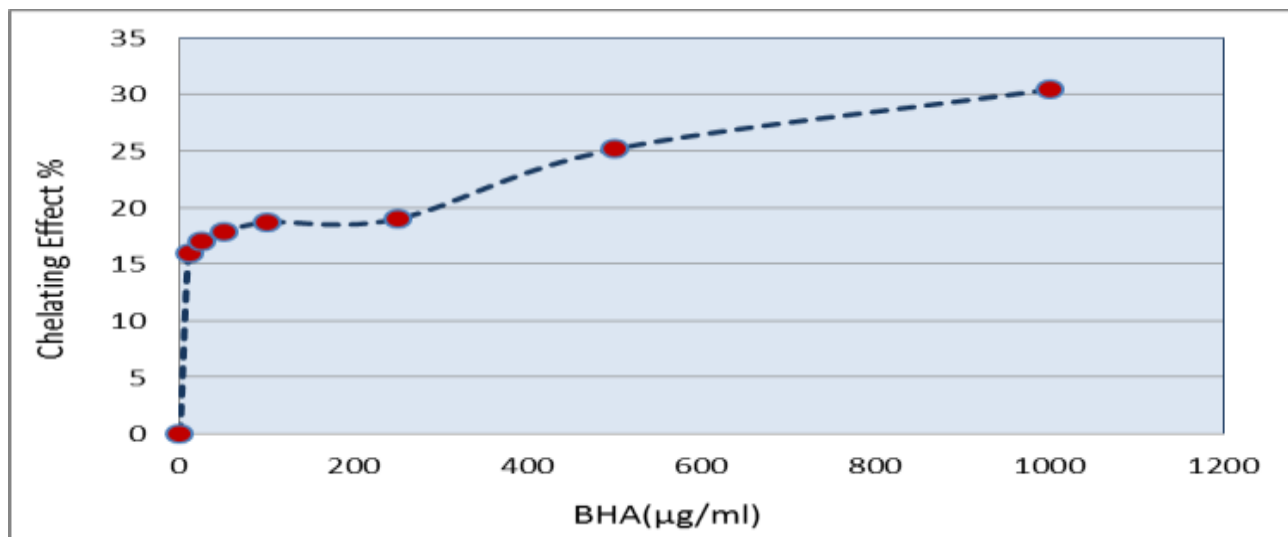


Figure 11: Antioxidant activity of BHA by ferrous ion chelating activity

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