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Egypt. J. Chem. **Vol. 67,** No. 7 pp. 289 - 299 (2024)

Egyptian Journal of Chemistry

Synthesis and Evaluation of a New Chelating Agent Using Tannic Acid and Palmitic Acid from Grape Seed Oil

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Abstract

The objective of this research is to create an affordable chelating agent for eliminating heavy metals from polluted water. Water-soluble tannic acid was combined with a fatty residue to produce a new chelating agent that is soluble in hot water but insoluble in cold water due to the presence of fatty residues that solidify at low temperatures. When this new chelating agent binds to heavy metals (HMs) in warm water, a soluble coordinating complex is formed. Lowering the water temperature causes the complex to become semi-solid and consequently insoluble, allowing for easy removal by scraping. Grape seeds (GS) were utilized as an affordable source of fatty residue. The grape seed was ground and dehydrated. The Grape seeds oil (GSO) was extracted, and hydrolyzed into a mixture of fatty acids and glycerol, followed by individual separation of palmitic acid (PA) using a supercritical carbon dioxide extractor. The produced PA was confirmed based on its chemical analysis, spectral data, and GC mass of its methyl ester. PA was then converted into the corresponding acid chloride, which was esterified with tannic acid to produce various derivatives of tannyl palmitate (TP). Tannyl tripalmitate (TTP) is the desired product, and it was separated according to its molecular weight using column and thin-layer chromatography, with a viscometer. The separated compound was established based on its chemical analyses and spectral data. Finally, TTP was evaluated as a new adsorbent for the removal of heavy metals from synthetic polluted water at different pH, doses, and time intervals. Results showed that TTP is a highly effective adsorbent for the removal of HMs from synthetic polluted water. Furthermore, a total organic carbon test (TOC) was conducted to verify the complete removal of the chelating agent and coordinating complex. The results showed complete removal of organic material from the synthetic polluted water after the scraping of the floating fatty layer.

Keywords: Supercritical extractor, heavy metals, tannyl tripalmitate, grape seed oil, palmitic acid, column chromatography, chelating agent.
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1. Introduction

Grape seeds (GSs) are often considered a by-product, but they actually contain beneficial compounds that offer various health benefits. They are rich in phytochemicals like proanthocyanins, which support heart health, skin rejuvenation, and cognitive function. GS extract is also known for its antioxidant properties and has been studied for its potential to enhance immune function and reduce inflammation[1]. Additionally, GSO extracted from GSs is suitable for cooking and has nutritional benefits for the skin. In industry, grape seeds are now being used to produce eco-friendly products such as packaging materials. GSs contain 8-12% palmitic acid, which is a key ingredient in soaps, detergents, cosmetics, and lubricants, as well as a precursor for the synthesis of various chemicals and pharmaceutical compounds[2]. This study explores the use of GSs as a cost-effective source of palmitic acid for the production of tannyl tripalmitate, which serves as a lipophilic component in the newly developed compound.

On the other hand, heavy metals (HMs) are defined as elements with a high mass number, typically in the range of 63.5–200.6 g/mol, and with densities higher than 5 g/cm^3. These metals may pose serious health risks when accumulated in the environment including the food chain, affecting human health[3]. Therefore, the removal of (HMs) is crucial. Various methods can be used to remove HMs from wastewater, including chemical precipitation4[2], ion exchange, adsorption, membrane filtration, and biological processes[5]. Factors such as cost and removal efficiency are important considerations when choosing a removal technique. High concentrations of HMs in water are typically the result of industrial processes, mining activities, and urban runoff, leading to pollution [6]. The technique of chemical and physical adsorption is widely used for the removal of several types of HMs with different concentrations from

wastewater due to its effectiveness, simplicity, and cost-efficiency. In this technique, the metal cation interacts with the adsorbent through electrostatic attraction, ion exchange, and complexation[7]. However, the saturation of adsorption sites over time, results in decreased efficiency, and the disposal of spent adsorbents requires further treatment to prevent secondary pollution[8]. Tannins are natural polyphenolic promising adsorbents for removing HMs as they are readily available, inexpensive, and have a high adsorption capacity[9]. Because tannin molecules contain multiple galloyl and catechol functional groups, they have many active sites for binding with metal cations resulting in the formation of strong coordination complexes, allowing cations to be removed from wastewater. Additionally, tannins are biodegradable, and environmentally friendly compound[10]. Tannin molecules are highly effective for removing heavy metals from aqueous solutions. This is because a single tannin molecule can capture multiple metal ions through various mechanisms. Such mechanism is complexation, where stable metal-tannin complexes are formed by the coordination bonds between metal ions and the phenolic groups of tannins[11]. Another mechanism is ion exchange, where metal ions in the solution is replace by hydrogen ions on the surface of tannins, leading to the adsorption of metal ions onto the adsorbent surface[12]. Additionally, electrostatic attraction occurs between positively charged metal ions and negatively charged functional groups on tannins, resulting in the adsorption of the metal ions[13]. Finally, surface precipitation can occur, leading to the formation of insoluble metal hydroxides or oxides on the surface of tannins and the subsequent removal of metal ions from the solution[14]. On the other hand, tannins can be desorbed with acid or base solutions[15], allowing for the recovery of both tannins and adsorbed heavy metals. This means that tannins can be reused multiple times without a significant loss of adsorption capacity. Tannins can be leached out when it is used directly as an adsorbent for metal recovery from water because they are water-soluble substances. In an attempt to address this issue, scientists have tried immobilizing tannins on matrices that are insoluble in water. Thus, in this study, tannic acid, a water-soluble compound, was chemically modified through esterification with palmitic acid extracted from grape seeds to create a new chelating agent. This new agent is soluble in hot water but insoluble in cold water, allowing it to be easily removed upon cooling.

2. EXPERIMENT
2.1 Instruments
Melting points were determined using an Electrothermal™ IA9300 Beginning Ending Recording Model for Pharmacopeia Requirements. Qualitative and quantitative analyses of fatty acids in the extracted fat samples were performed using a capillary gas chromatograph (HP 6890) and reported in relative area percentages. The supercritical CO_2 instrument used in this study was the Separations. Inc., Allentown, USA, model no.7071. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer, with 1H spectra running at 300 MHz. Chemical shifts are reported in δ and were referenced to the solvents. Mass spectra were obtained using a Shimadzu GC MS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were conducted at the Micro-analytical Center of Cairo University. The refractive index was measured using a refractometer (Rudolph model J157), pH was measured using a Pinnacle 530 Benchtop pH meter, and a spectrophotometer (V-730 Spectrophotometer - Japan) was used for metal determination.

2.2 Materials and methods
2.2.1 Chemicals
Ethanol, acetone, diethyl ether, thionyl chloride, sodium hydroxide, sulfuric acid, and tannic acid were bought from the British Drug House (BDH). All other chemicals used in the present study were acquired from Sigma Chemical Company (England, Landon, Ltd., Pools).

2.2.2 Plant material and seeds collection and preparation
In June 2023, grape seeds were collected as a by-product from a juice shop in Giza, Egypt during the grape harvesting season. The seeds were washed, dried with a towel, and left to air dry overnight.

2.2.3 Grape seed oil (GSO) extraction
The grape seeds (1000 g) were crushed and dried for 12 hours at 40 °C to determine the moisture content. The dried sample was then separated into four equal parts. GSO was extracted from each part using diethyl ether (600 ml) in a Soxhlet apparatus at a condensation rate of 5 or 6 drops per second for 6 hours. The solvent was recovered using a rotary evaporator, and the obtained grape seeds oil was weighed and stored for analysis[16].

2.2.4 Quality parameters
2.2.4.1. Refractive index (RI)
A refractometer (Rudolph model J157) was used to measure the refractive indexes (RI) of GSO samples[17] at 20°C. Each measurement had been carried out in replicate, adhering to the AOAC (2005) guidelines.

2.2.4.2. Acid value (AV)
Using a 1% phenolphthalein indicator, a mixture of absolute ethanol and diethyl ether in 1:1 by volume was carefully neutralized with 0.10 N potassium hydroxide solution. After dissolving about 5 g of the GSO samples in 50 ml of the neutralised ethanol-diethyl ether solvent, the mixture was titrated with 0.10 M potassium hydroxide while being shaken constantly for 15 seconds. Next, the percentage of oleic acid was used to calculate the percentage of free fatty acids[17].

FFA % as oleic acid = S × 0.0282 × 100 / W

Where: S = titration (ml), W = weight of the oil (g).

2.2.4.3. Iodine value (IV)

The Hanus method[17] was used to calculate the tested samples' iodine value. In a nutshell, 20 milliliters of chloroform were used to dissolve about 0.25 grams of the GSO sample, which was weighed into a 500-milliliter glass stopper flask. After that, 25 ml of Hanus iodine solution (13.2 g of pure I_2 in 1 L CH_3COOH) was added, and the mixture was left to stand in the dark for 30 minutes. 100 ml of distilled H_2O was then added, and everything was well shaken after adding 10 ml of 15% KI solution. I_2 underwent titration using a 0.10 N sodium thiosulphate solution while being shaken continuously until the yellow solution nearly became colourless. As an indicator, a few drops of 1% starch solution were added, and the titration was carried out until the blue colour was developed.

Iodine value = (B – S) × N × 12.69 / W

Where: B = mL $Na_2S_2O_3$ solution required for blank; S = mL $Na_2S_2O_3$ solution required for test sample; N = Normality of $Na_2S_2O_3$ solution, and W = weight of sample in g

2.2.4..4. Peroxide value (PV)

The tested oil's peroxide value (PV) was determined[17]. 10 millilitres of chloroform were added to a flask fitted with a ground-glass cap containing two grams of the GSO, which was then shaken for 10 minutes. Next, two grams of sodium bicarbonate ($NaHCO_3$) and 15 millilitres of glacial acetic acid were added. Following agitation, 1 mL of a saturated KI solution was added, shaken for 1 minute, and then allowed to sit in the dark for 5 minutes. 75 mL of distilled water and 0.5 mL of starch solution were added right away after the allotted amount of time had passed. The blue colour was eliminated by titrating the resultant solution with a $Na_2S_2O_3$ (0.01 N) solution. The identical methods were used to create a blank, but without the sample. The peroxide value of the tested oil was calculated using the given equation.

*PV= [(V1-V2) * N * 1000] / W   [meq.O₂/kg]*

Where: $V1$ = volume of sodium thiosulfate solution consumed in the titration of the sample (mL), $V2$ = volume of sodium thiosulfate consumed in the titration of the blank (mL), N = the normality of sodium thiosulfate, W = weight of fat taken to denote (g).

2.2.4..5. Determination of unsaponifiable matter content

The GSO samples' saponification value was determined[17]. Where, 50 mL of alcoholic KOH solution (35–40 g KOH was dissolved in 20 mL water and diluted to one litre with 95% alcohol) was added to a 250–300 mL conical flask containing 5 g of the tested sample and refluxed for about 30 minutes. After the flask was cooled and the fat was fully saponified, it was titrated with 0.5 M HCl using phenolphthalein. The following formula was used to determine the saponification value:

Saponification value = 28.05 (B–S)/W

Where: B = mL HCl required for blank; S = mL HCl required for test sample; W = weight of the sample in g.

2.2.4..6. Total polar materials (TPM):

TPM was determined in GSO samples and measured by column chromatography[18].

2.2.4..7. Polymer content (PC):

The PC was determined[18] by adding one gram of GSO to methanol (125 ml) containing 1% H_2SO_4. The mixture was then boiled under a reflux condenser for 2 hours and cooled to room temperature. The methanol insoluble was filtered and washed with methanol until no sulphuric acid remained. The washed insoluble polymers were then dissolved in petroleum ether (25 ml) and transferred to a pre-weighed flask. The solvent was evaporated under a stream of nitrogen and the flask was weighed again.

2.2.5 Fatty acids composition

The fat extracted was analyzed for its fatty acid composition using GC-MS after esterification[17]. The fat was converted into methyl esters by shaking a solution of fat (0.1 g) in heptane (2 mL) with methanolic potassium hydroxide solution (0.2 mL, 2 N). The resulting fatty acid methyl esters were then analyzed using a gas chromatograph. The nitrogen flow rate was 0.6 mL/min, and the hydrogen and air-flow rates were 45 and 450 ml/min, respectively. The oven temperature was maintained at 195˚C. The injector and detector temperatures were 230˚C and 250˚C, respectively. Fatty acid methyl esters were identified by comparing their retention times with a known fatty acid standard mixture. Peak areas were automatically computed by an integrator.

2.2.6 Oil hydrolysis

Using distilled water (250 ml) and a high-pressure reactor, grape seed oil (GSO) (170 g, 18.42% of the dried seed) was hydrolyzed for three hours at 250 °C and 2 MPa. Let the reaction to cool to room temperature to separate the fatty and non-fatty layers. After being separated, the fatty layer was filtered and dried over anhydrous sodium sulfate. Using the 1H NMR spectrum and TLC, the formation of free fatty acids was verified[19].

A signal was detected at δ10.88 in the 1H NMR spectrum, suggesting the presence of carboxylic hydrogen in the free fatty acids.

After coating 20x20 plates with a slurry of 60g silica gel in (15g/kg) water, they were dried and activated for one hour at 110°C for the TLC analysis. The hydrolyzed fat's fatty acid mixture and standard spots of known fatty acids and oil were added to the activated thin layer plates. Diethyl ether, acetic acid, and n-hexane were combined in an 80:20:1 volumetric ratio to create the developing solvent. Iodine vapour was used to visualize the spots of various components on the plates as they were developed until the solvent reached the front line, which was 15 cm from the start line. When the withdrawn sample had a tail-free spot and a flow rate that resembled the known fatty acid spot but not the oil spot, it was considered that the fatty acids had formed.

2.2.7 Extraction of free individual palmitic acid (PA) using supercritical CO_2 extractor.

The mixture of fatty acids (147.86 g, 86.6% of the extracted oil) was separated and cooled to 7°C, causing the saturated fatty acids to solidify while the unsaturated fatty acids remained in liquid form. They were then easily separated from each other by filtration. PA was subsequently extracted[20] from the solid fraction using a supercritical CO_2 extractor at 35.0 MPa and 328°K. The resulting PA (14.18 g, 98.2% of the existing PA) was confirmed through elemental analysis, melting points, GC-MS, and mass spectra (MS) of its methyl ester which detects the corresponding molecular ion peak.

Yield (14.18 g, 98.2%), **m.p.** (62.5-63°C), **IR (KBr)** v_{max}/cm^{-1}: 2778 (OH), 2993-2858 (CH- aliphatic), 1770 (C=O). **^{1}H NMR (CDCl3):** δ 10.89 (s, 1H), 2.20 (t, 2H), 1.57(m, 2H), 1.28-1.33 (m, 24H), 0.94(t, 3H) .**13C NMR (DMSO – d$_6$):** δ14.0, 22.3, 24.5, 29.1, 29.4, 29.8, 32.0, 35.9, 179.7. **MS *(m/z)*:** (255 M$^+$), 197, 141, 59, 56. **For** $C_{18}H_{36}O_3$ (300.48): **For** $C_{16}H_{32}O_2$ (256.43): **Calcd.:** C, 74.94; H, 12.58 %. **Found:** C, 75.12; H, 12.44 %.

2.2.8 Synthesis 3,4-bis((3-((3,5-dihydroxy-4-(palmitoyloxy)benzoyl)oxy)-4,5-dihydroxybenzoyl)oxy)-6-(((3-((3,5-dihydroxy-4-(palmitoyloxy)benzoyl)oxy)-4,5-dihydroxybenzoyl)oxy)methyl)tetrahydro-2H-pyran-2,5-diyl bis(3,4-dihydroxy-5-((3,4,5-trihydroxybenzoyl)oxy)benzoate) or Tannyl tripalmitate (TTP)

This compound was prepared through an esterification reaction between tannic acid and palmitoyl chloride as in scheme 1.

2.2.8.1 *Synthesis of palmitoyl chloride*

The extracted PA (13.8 g, 53.82 mmol) was stirred in diethyl ether (70 ml), and then thionyl chloride (6.4 g,-53.82 mmol) was added and refluxed for 3 hours. The upper condenser opening was closed with a piece of cotton and a few grams of anhydrous sodium sulfate. The mixture was then heated under a vacuum to remove the unreacted thionyl chloride and by-products. A quantitative recovery of the product was obtained.

Yield (14.61 g, 98.8 %), (mp.12-13°C), **IR (KBr)** v_{max}/cm^{-1}: 2920-2836 (CH- aliphatic), 1807 (C=O). **^{1}H NMR (CDCl$_3$):** δ 0.90 (t, 3H, J=7.0 Hz, 16-CH$_3$), 1.23-1.32 (m, 24H, 15-4CH$_2$), 1.55 (m, 2H, 3-CH$_2$), 2.65 (t, 2H, J=7.1 Hz, 2-CH$_2$). **^{13}C NMR (DMSO – d$_6$):** δ14.1, 22.6, 24.9, 28.3, 29.3, 29.6, 46.7, 172.0. **For** $C_{18}H_{31}ClO_2$ (274.87): **Calculated::** C, 69.91; H, 11.37; Cl, 12.90; O, 5.82 %. **Found:** C, 69.77; H, 11.61; Cl, 12.60 %

2.2.8.2 *Acylation of palmitoyl chloride with tannic acid*

A solution of palmitoyl chloride (14 g, 50.93 mmol) in diethyl ether (50 ml) with triethyl amine (0.5 ml) as a catalyst, was slowly added to a stirred solution of tannic acid (34 g, 20 mmol) in diethyl ether (100 ml). The mixture was then stirred under reflux for 1 hour. Afterward, it was poured over acidified ice-cold water to produce water-soluble triethylamine hydrochloride. The mixture was filtered at a low temperature, as fatty derivatives became semisolid at low temperatures. The residue was then dried under a vacuum.

This reaction produced numerous isolable products with varying levels of esterification (mono-, di-, tri-, tetra-, penta,…….., palmitates). The most effective derivative acting as a chelating agent is believed to be tannyl tripalmitate.

2.2.8.3 Separation of tannyl tripalmitate (TTP)
2.2.8.3.1 Low-pressure chromatography

Low-pressure chromatography using silica gel was utilized to separate the various products. Thus, all products were dissolved in petroleum ether at a ratio of 3:10 w/v. The resulting solution was then applied to a (60 cm x 4.0 cm) silica gel column and eluted with petroleum ether. The eluent was collected in 20 ml fractions and tentative identification was performed using TLC.

2.2.8.3.2 TLC detection

The silica gel plates were activated at 120 °C for 1 hour before use. A few microliters of each fraction collected from column chromatography were loaded onto the marked points approximately 10 mm from the bottom of the silica plate. The plates were then developed in a mixture of petroleum ether, diethyl ether, and acetic acid (80:90:1, v/v/v) at room temperature, and the separated spots were visualized using iodine fumes. Samples with the same rate of flow (Rf value) were collected together in one category.

2.2.8.3.3 Viscometric measurements

Each category was individually dried under vacuum, and then the intrinsic viscosities (η) were measured for each category[21] in the same volume of distilled water at 25 °C using the Brookfield viscometer Model T.C.500 (Brookfield Engineering Laboratories Stoughton, MA, USA). The molecular weights (M) were calculated using the equation[22] described by **R.X. Yan** $\eta = 3.38 \times 10^{-3} M^{0.43}$

This step was carried out till getting molecular weight so close to that of TTP (2416.45 gmol^{-1}). It is known that there are many different structures for tannyl tripalmitate, depending on the position of the hydroxyl function at which the esterification reaction takes place. However, all of these structures have nearly the same chelation activity.

2.2.8.4 Elemental analysis and spectral data of tannyl tri palmitate (TTP)

IR (KBr) v_{max}/cm^{-1}: 3435 (OH), 3071 (C-H aromatic), 2924-2830 (CH- aliphatic), 1730 (C=O), 1565 (C-C), 1210 (C-O) cm^{-1}. **^{1}H NMR** (CDCl3, 300MHz): δ 0.90(t-9H), 1.25-1.33 (m, 72), 1.65 (m, 6), 2.55 (t, 6H), 3.80 (m, 2H), 4.42 (dd, 2H), 4.61(m,1H), 4.75(m,2H), 4.92 (m,2H), 6.90-7.19 (20, aromatic protons), 8.75-9.48(s, 22 phenolic protons). **^{13}C NMR** (CDCl3, 75MHz): δ=14.0 22.6, 25.0, 29.3, 29.6, 31.9, 33.5, 63.9, 68.8,72.4, 74.5, 74.8, 94.4, 109.3, 113.9, 115.5, 120.4,129.6, 134.5, 141.8, 145.2, 152.1, 154.4,

165.0, 172.3 ppm. **For** $C_{124}H_{142}O_{49}$ (2416.45 gmol^{-1}), **Calculated:** C, 61.63; H, 5.92; O, 32.44 %. **Found** C, 61.7; H, 5.0 %.

2.2.9 Evaluation of tannyl tri palmitate (TTP) as a low-cost adsorbent for heavy metals

2.2.9.1. Preparation of standard calibration curves for the adsorbates.

Freshly prepared stock solutions of 1000 mg/L for each analytical grade metal salt of $CuSO_4.5H_2O$, $NiSO_4.6H_2O$, $ZnSO_4.7H_2O$, and $CdSO_4.H_2O$ were prepared. All salts were dried and cooled in a desiccator before weighing. Serial dilution in 50 ml volumetric flasks was performed to prepare intermediate standard solutions for each metal (5, 10, 15, 20, 25, and 30 mg/L), and their absorbance's were measured spectrophotometrically to obtain a calibration curve for each type of metal ion used. Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+} were determined spectrophotometrically at 324.8 nm, 232 nm, 213.73 nm and 228.87 nm respectively.

2.2.9.2 Preparation of synthetic polluted water

1000 mg/L solution of each metal salt ($ZnSO_4.7H_2O$, $CdSO_4.H_2O$, $NiSO_4.6H_2O$, and $CuSO_4.5H_2O$) was prepared by dissolving 1g of each solute individually in 1 litre of ultrapure water. Then, the solutions were diluted to a new concentration of 30 mg/L, and samples of 50 ml were placed in 250 ml conical flasks.

2.2.9.3 Preparation of adsorbent solution

A 1 mmol/L solution of TTP was prepared by dissolving 2.416 g of solute in 1 liter of distilled water. This solution was then diluted to a new concentration of 0.05 mmol/L.

2.2.9.4 Investigation of pH effect on the extraction of heavy metals

Aquatic solutions containing TTP (0.05 mmol/L, 50 mL) were individually added to 50 mL samples of Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+} metal ion solutions with initial concentrations of 30 ppm. The pH was adjusted using H2SO4 and NaOH solutions[23,24] with the help of a pH meter (Pinnacle 530 Benchtop pH meter). The mixtures were stirred at 40°C for 90 minutes and then cooled in a fridge for 25 minutes, resulting in the formation of a thin fatty layer of the chelate complex on the solution surface. This layer was carefully removed by scraping. The concentration of the remaining heavy metal in the clear solution was determined using a UV-visible spectrophotometer (V-730, made in Japan). A TOC test was conducted on the clear solution to confirm the absence of any traces of the chelating agent or coordinating complex. The percentage of heavy metal removal was calculated using Eq (1).

Metal ion removal (%) = [(Co − Cf) / Co] × 100

Where Co: initial metal ion concentration of the test solution (ppm); Cf: final concentration of the test solution (ppm).

2.2.9.5 Investigation of the effect of contact time on the extraction of heavy metals

The adsorption behaviour of solutions containing initial metal ions concentrations of 30 ppm in 50 ml for each metal adsorbate (Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}) and (0.05 mmol/L, 50 mL) adsorbent (TTP) was studied at different time intervals[23]. The experiments were conducted with continuous stirring at pH 5 and 40 °C. Samples were exposed to these conditions for 20, 40, 60, 80, 100, 120, and 140 minutes. Afterward, the mixtures were cooled in a fridge for 25 minutes, leading to the formation of a thin fatty layer of chelate complex on the solution surface. This layer was removed by scraping, and the concentration of the heavy metal remaining in the clear solution was determined using a UV-visible spectrophotometer (V-730, manufactured in Japan). Furthermore, a Total Organic Carbon (TOC) test was carried out on the clear solution to confirm the absence of any traces of the chelating agent or coordinating complex. The percentage of heavy metal removal was calculated using Eq (1).

2.2.9.6. Investigation of the effect of adsorbent dosage on the extraction of heavy metals.

Various dosages of the adsorbent (TTP) (0.025, 0.05, 0.075, 0.10, 0.125 mmol in 50 mL) were individually added[23,25] to solutions with an initial metal ions concentration of 30 ppm in 50 mL for each metal ($Zn2+$, $Cd2+$, $Ni2+$, and $Cu2+$). The solutions were stirred continuously at pH 5 and 40°C for 90 minutes. Afterward, the mixtures were cooled in a refrigerator for 25 minutes, resulting in the formation of a thin fatty layer of chelate complex on the solution surface. This layer was removed by scraping, and the concentration of the remaining heavy metal in the clear solution was measured using a UV-visible spectrophotometer (V-730, manufactured in Japan). Furthermore, a Total Organic Carbon (TOC) test was conducted on the clear solution to confirm the absence of any traces of the chelating agent or coordinating complex. The percentage of heavy metals (HMs) removal was calculated using Eq (1).

2.2.9.7 Total organic carbon (TOC) analyses (to verify the complete removal of both the chelating agent and coordination complexes).

After removing HMs from synthetic polluted water, it is crucial to ensure complete removal of any remaining chelating agents or coordination complexes. This is achieved by analyzing the total organic carbon[26-28] in the water using an Infitek Total Organic Carbon Analyzer (TOC-M2000 with autosampler). The process involves heating a sample in a furnace at 680 °C to decompose organic compounds into H_2O and CO_2. The evaporated H_2O is removed, and the generated CO_2 is detected by a non-dispersive infrared detector (NDIR) to measure carbon content in parts per million (ppm). Thus, calibration curve of TOC concentration (ppm) versus IR response.

To prepare the calibration curve, a 1 mmol/100 ml solution of TTP is created by dissolving 0.2416 g of the solute in 100 ml of ultrapure deionized water. Standard series solutions with carbon concentrations of 0.0, 4.0, 10.0, 20.0, 40.0, 80.0, and 200.0 ppm are prepared by diluting the initial solution. Before analysis, all samples are heated to 40 °C for complete solubility. The response values of these

solutions are determined to construct a TOC calibration curve for correlating instrument response values with carbon content.

In the analyses process, the carrier gas (O_2) flows through a combustion tube with a platinum-aluminium catalyst at 680°C and a rate of 180mL/min. The sample is injected into the combustion tube, where total organic carbon (TC) is combusted and converted into carbon dioxide. The carbon dioxide is then measured in the gas chamber of an NDIR detector to determine the carbon dioxide concentration. A calibration curve is established using an external standard method to calculate the TC concentration in the sample based on the peak area value obtained from the NDIR detection signal.

Statistical analysis

statistical significance between the control and experimental data was assessed using a one-way analyses of variance (ANOVA) followed by a Tukey post hoc test. Microsoft Excel 2016 MSO (16.0.10361.20002) 32-bit was used for the analysis.

3. Results and Discussions

3.1 grape seed oil (GSO) extraction

To extract GSO efficiently, 1000g of seeds were ground to increase the surface area. A dehydration process was carried out, and the moisture content was measured (73g, 7.3%). The oil was extracted using diethyl ether, resulting in a yield of 170.75g (18.42% of the dry seed weight). This yield aligns well with previous studies[29] by Lei Zhang et al. (2023).

3.2 Quality parameters of the extracted oil

The freshly extracted GSO met the standards for edible oil according to the Egyptian Standard Organization[30] (2005). It had a refractive index of 1.476, an acid value of 0.72 mg KOH/g oil, an iodine value of 134 grams of I2/100 g oil, a saponification number of 189 mg KOH/g oil, a peroxide value of 1.9 mEq/kg oil, and polymer and polar contents of 0.61% and 0.92%, respectively, as shown in Table 1. These findings were consistent with previous research[31].

3.3 Fatty acids composition

Analysis of the esterified fatty acid mixture of grape seed oil using GC-MS revealed that it is predominantly made up of 67.1% linoleic acid (C18:2), known for its beneficial effects on heart health, insulin sensitivity, and blood pressure, as well as its ability to lower total and low-density cholesterol[32]. It also contains 17.3% oleic acid (C18:1), which plays a crucial role in reducing cholesterol and inflammation[32]. Additionally, the oil includes 9.77% palmitic acid (C16:0) and 4.2% stearic acid (C18:0), commonly used in pharmaceutical and industrial products[33]. Traces of myristic acid (C14:0), palmitoleic acid (C16:1), linolenic acid (C18:3), arachidic acid (C20:0), and paulinic acid (C20:1) were also detected. The fatty acid composition obtained in this study aligns with previous findings[34].

Table (1): Physical and chemical properties of the extracted grape seed oil

arameters	The extracted seed oil
Refractive index at 25°C	1.476±0.03
Acid value (mg KOH /g oil)	0.72±0.01
Iodine value (gI₂/100g oil)	134±1.20
Saponification number (mg KOH/g oil)	189±1.42
Peroxide value (meq. /kg oil)	2.91±0.04
Polymer contents (%)	0.61±0.02
Polar contents (%)	0.92±0.02

Data are expressed as mean ± SD values given to represent the means of three determinations

Table (2): Fatty acid composition of the extracted grape seed oil

fatty acid	%
Lauric acid (C12:0)	0.01±0.01
Myristic acid (C14:0)	0.07±0.05
Palmitic acid (C16:0)	9.77±0.32
Palmitoleic acid (C16:1)	0.31±0.002
Stearic acid (C18:0)	4.2±0.16
Oleic acid (C18:1)	17.3±0.13
Linoleic acid (C18:2)	67.1±0.93
Leinolenic acid (C18:3)	0.32±0.01
Arachidic acid (C20:0)	0.17±0.01
Paulinic acid (C20:1)	0.38±.00
others	0.37
saturated fatty acids	14.22
unsaturated fatty acids	85.41

Data are expressed as mean ± SD values given to represent the means of three determinations

3.4 Oil hydrolysis.

The triacylglycerol molecule in any oil can be easily broken down into free fatty acids and glycerol under high temperatures and pressure in the presence of water. When 170.75 g of extracted GSO (18.42% of the dry seed) was hydrolyzed, it yielded 147.87 g of a fatty acid mixture (86.6% of the hydrolyzed oil) and glycerol. This hydrolysis was confirmed through thin-layer chromatographic analyses (TLC), which showed a single spot with no tail, flowing at a rate similar to known fatty acids but not oil. Additionally, the 1H NMR spectrum displayed a carboxylic hydrogen signal at δ10.3.

3.5 Palmitic acid extraction using supercritical CO_2 extractor

Palmitic acid (PA) was extracted with high purity from the solid fraction of the fatty acid mixture using a CO_2 extractor at 35.0 MPa and 55°C. The yield was 14.18 g, representing 98.2% of the actual PA present. The confirmation was based on melting point, GC mass, and spectral analyses as detailed in the experimental section.

3.6 Synthesis 3,4-bis((3-((3,5-dihydroxy-4-(palmitoyloxy)benzoyl)oxy)-4,5-dihydroxybenzoyl)oxy)-6-(((3-((3,5-dihydroxy-4-(palmitoyloxy)benzoyl)oxy)-4,5-dihydroxybenzoyl)oxy)methyl)tetrahydro-2H-pyran-2,5-diyl bis(3,4-dihydroxy-5-((3,4,5-trihydroxybenzoyl)oxy)benzoate) or tannyl teripalmitate

The compound was synthesized via an esterification reaction, as depicted in scheme 1. To facilitate the esterification of the phenolic OH group with a carboxylic acid, an acid chloride was utilized. The extracted PA was chlorinated to form the corresponding acid chloride, as confirmed by the disappearance of the carboxylic OH absorbance in the IR spectrum and chemical analysis. Subsequently, the palmitoyl chloride was esterified with tannic acid, primarily at the para position, which is the most reactive site for esterification. Triethylamine was employed as a catalyst, resulting in the formation of various products with different degrees of esterification (tannyl mono, di, tri, tetra, penta, etc., palmitate). Tannyl tri palmitate was the desired product due to its solidification properties at low temperatures, while lower esterification levels led to poor solidification and higher levels reduced chelation activity. Low-pressure column chromatography using silica gel was used to separate fractions, which were tentatively identified using TLC based on their RF values. Fractions with the same RF value contained the same compound and were collected together. The compound with the highest molecular weight corresponded to the lowest RF value, as indicated in Table 3. The compounds were differentiated by estimating their molecular weight based on viscosity measurements, as shown in Table 4. The isolated tannyl tri palmitate was confirmed through chemical analyses and spectral data. Its IR spectrum exhibited characteristic bands at 3435, 3071, 2924-2830, 1730, 1565, and 1210 cm−1, corresponding to absorptions of (OH), (C-H aromatic), (CH- aliphatic), (C=O), (C-C), and (C-O), respectively. Additionally, its H NMR displayed signals at δ 1.25-1.33 (m, 72) and 8.75-9.48 (s, 22 phenolic protons).

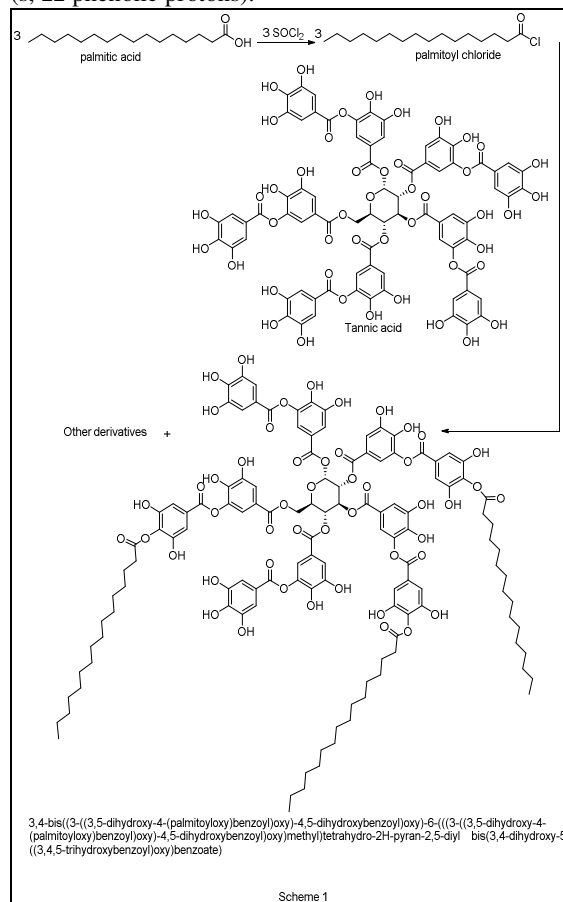3,4-bis((3-((3,5-dihydroxy-4-(palmitoyloxy)benzoyl)oxy)-4,5-dihydroxybenzoyl)oxy)-6-(((3-((3,5-dihydroxy-4-(palmitoyloxy)benzoyl)oxy)-4,5-dihydroxybenzoyl)oxy)methyl)tetrahydro-2H-pyran-2,5-diyl bis(3,4-dihydroxy-5-((3,4,5-trihydroxybenzoyl)oxy)benzoate)

Scheme 1

Table (3): Rate of flow (RF) of tannyl palmitate esters

The compound	RF Value
Tannyl monopalmitate	0.461
Tannyl dipalmitate	0.430
Tannyl tripalmitate	0.405
Tannyl tetrapalmitate	0.374
Tannyl pentapalmitate	0.348
Tannyl hexapalmitate	0.311
Tannyl heptapalmitate	0.281

Data are expressed as mean ± SD values given to represent the means of three determinations.

Table (4): Expected molecular weight (M Wt E) and average molecular weight obtained from viscosity measurements (M Wt V)

The compound	M Wt E, gmol^{-1}	M Wt V, gmol^{-1}
Tannyl monopalmitate	1969.69	1884.7±34.31
Tannyl dipalmitate	2208.11	2099.75±42.44
Tannyl tripalmitate	2416.45	2322.11±37.98
Tannyl tetrapalmitate	2654.87	2562.36±39.73
Tannyl pentapalmitate	2891.32	2775.85±41.84
Tannyl hexapalmitate	3129.55	2989.56±38.49
Tannyl heptapalmitate	3370.11	3297.73±37.84

Data are expressed as mean ± SD values given to represent the means of three determinations.

3.7 Evaluation of as a low-cost adsorbent for heavy metals

According to Figure 1, it is assumed that a tree molecule of TTP can bind to twenty-one molecules of heavy metal due to the three fatty residues being connected to the para positions. However, if the fatty residue were linked to the ortho or meta position, TTP could potentially capture more heavy metals. Hence, the optimal ratio between TTP and heavy metal should be at least 1:7.
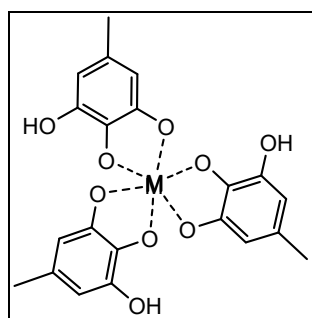
Figure 1: One of the assumed coordinating complexes

3.7.1. Standard calibration curves of the adsorbates.

Figures 2-5 show the standard calibration curves for each adsorbate. The absorbance-concentration equation for each adsorbate was determined separately using Microsoft Excel 2016, enabling the calculation of the concentration of any sample based on its absorbance.

3.7.2 Investigation of pH effect on the extraction of heavy metals

The elimination of HMs (Zn^{2+}, C^{d2+}, Ni^{2+}, and Cu^{2+}) from a 50 ml solution with an initial metal ion concentration of 30 ppm at 40°C for 80 minutes using 0.05 mmol TTP was significantly affected by pH. Figure 6 demonstrates the gradual removal of the HMs at low pH (2-3), followed by rapid increases at pH 3-4. The highest removal rates were achieved at pH 5, with 98% of Zn^{2+}, 96.6% of Cd^{2+}, 95.9% of Ni^{2+}, and 93.8% of Cu^{2+} being eliminated. Removal efficiency slightly decreased at pH 6-7. These findings are in line with previous studies[35]. At low pH, the abundance of H^+ and H_3O^+ ions compete with metal ions for adsorption, causing reduced absorption of heavy metals. At moderate pH levels, the active sites of TTP become deprotonated, creating more negatively charged sites. This boosts the electrostatic attraction between the adsorbent surface and metal cations, resulting in increased metal absorption.
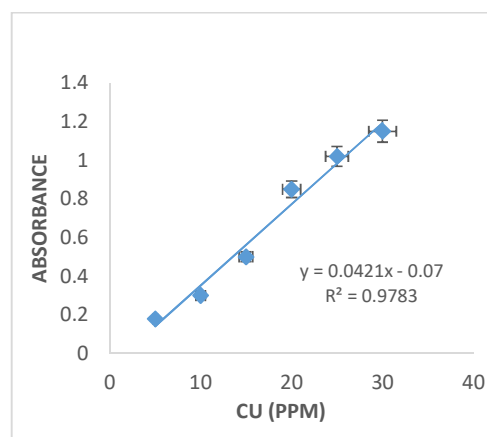

Figure 2: Calibration curve for Cu^{2+} concentration in ppm versos absorbance

Data are expressed as mean ± SD values given to represent the means of three determinations.
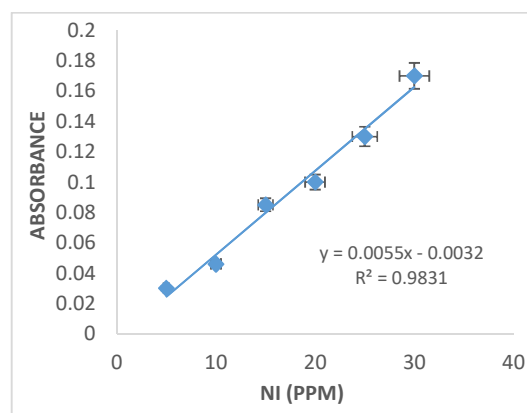

Figure 3: Calibration curve for Ni^{2+} concentration in ppm versos absorbance

Data are expressed as mean ± SD values given to represent the means of three determinations.
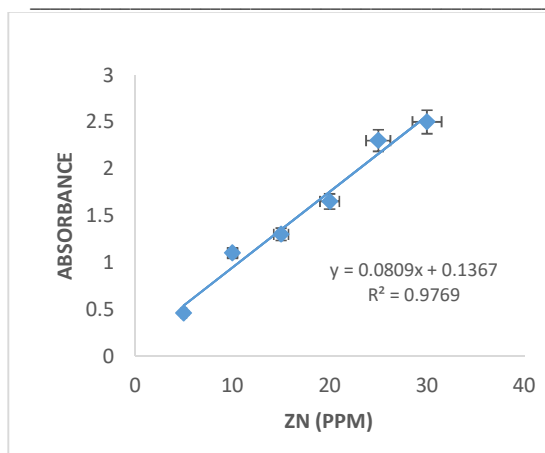
Figure 4: Calibration curve for Zn^{2+} concentration in ppm versos absorbance
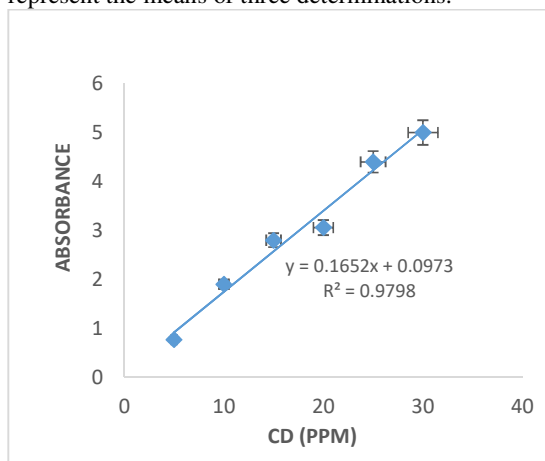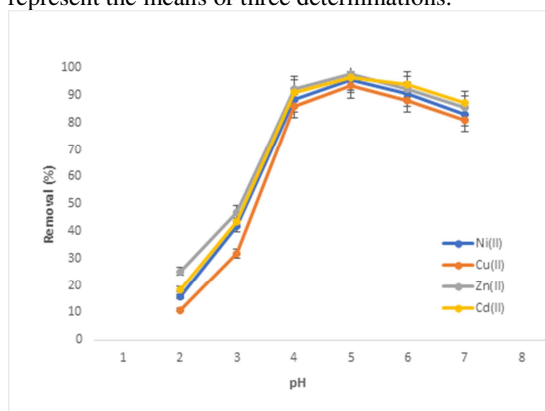Data are expressed as mean ± SD values given to represent the means of three determinations.

Figure 5: Calibration curve for Cd^{2+} concentration in ppm versos absorbance
Data are expressed as mean ± SD values given to represent the means of three determinations.

Figure 6: Effect of pH on removal of (Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}) at an initial concentration of 30 ppm, 90 minutes and 0.05mmol of Tannyl tripalmitae at 35 °C Data are expressed as mean ± SD values given to represent the means of three determinations.
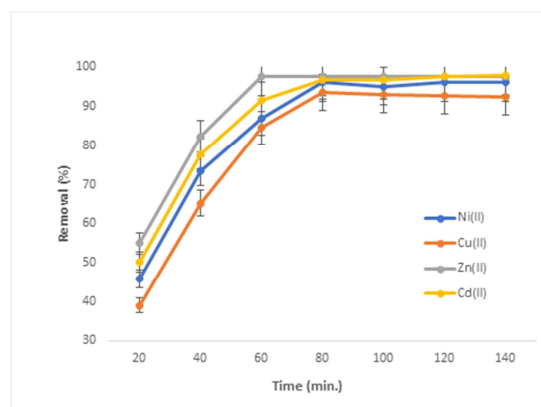

Figure 7: Effect of time on removal of (Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}) at an initial concentration of 30 ppm and 0.05mmol of Tannyl tripalmitae at 35 °C and pH 5 for 90 minutes
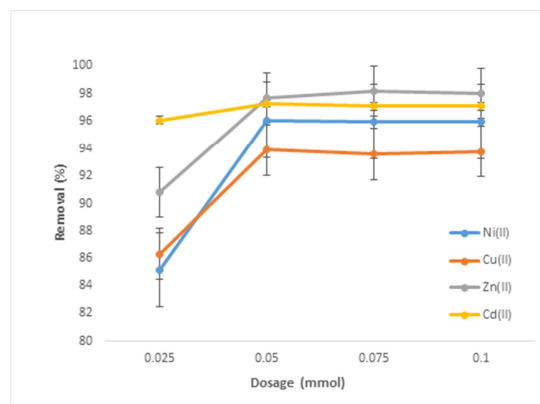Data are expressed as mean ± SD values given to represent the means of three determinations.

Figure 8: Effect of Adsorbent dose of Tannyl tripalmitae (mmol) on removal of (Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}) at an initial concentration of 30 ppm and at 35 °C and pH 5 for 90 minutes
Data are expressed as mean ± SD values given to represent the means of three determinations.

3.7.3 Investigation of contact time on the extraction of heavy metals

The study examined the effectiveness of TTP in removing HMs (Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^2) from a 30 ppm initial metal ion concentration in a 50 mL solution at 40°C and pH 5. The experiment was conducted over different time intervals (20, 40, 60, 80, 100, 120, and 140 minutes). The results showed a steady increase in removal percentages up to around 80 minutes, with removal percentages of 97.8% for Zn^{2+}, 96.7% for Cd^{2+}, 96.2% for Ni^{2+}, and 93.6% for Cu^{2+}. After 80 minutes, there was no significant improvement in removal efficiency, as shown in Figure 7.

3.7.4 Investigation of the effect of adsorbent dosage on the extraction of heavy metals.

The study aimed to eliminate HMs (Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}) from a 30 ppm initial metal ion concentration in a 50 ml solution at 40°C and pH 5 within a 90-minute timeframe. Various concentrations of TTP (0.025, 0.050, 0.075, and 0.1 mmol in 50 mL) were examined. Results revealed that at a 0.025 mmol concentration, removal percentages were 90.8% for Zn^{2+}, 96.1% for Cd^{2+}, 96.1% for Ni^{2+}, and 93.9% for Cu^{2+}. Increasing the concentration to 0.050 mmol resulted in maximum removal percentages of 98.2% for Zn^{2+}, 97.1% for Cd^{2+}, 96.0% for Ni^{2+}, and 93.6% for Cu^{2+}. There were no significant differences observed at higher concentrations. Additionally, consistent removal percentages were noted for cadmium ions across all concentrations as illustrated in Figure 8.

3.7.5 Total organic carbon (TOC) analyses (to verify the complete removal of both the chelating agent and coordination complexes).

A Total Organic Carbon (TOC) test was conducted on each clear sample solution after removing the floating fatty layer to confirm the absence of chelating agents or coordinating complexes. This test was carried out after each treatment (pH variation, time interval variation, and adsorbent dose variation). The results showed that no organic carbon was detected in any of the samples after removing the fatty layer.

4. Conclusion

A new low-cost chelating agent, Tannyl tripalmitate, was created by esterifying tannic acid with PA . This agent effectively removes HMs from synthetic polluted water. TTP is soluble in hot water but solidifies into a semi-solid at lower temperatures. When added to a hot solution, it captures HMs to form a soluble metal complex. As the solution cools, the complex forms a floating thin semi-solid layer that can be easily removed by scraping. TTP showed excellent efficiency in removing HMs from synthetic polluted water. Additionally, TOC testing confirmed that the chelating agent and coordinating complex were completely removed by scraping.

5. Acknowledgments

Authors' contributions:

Hanaa collected materials, designed the study, conducted sequencing alignment, drafted the manuscript, and conducted physicochemical analyses Usama performed statistical analysis, prepared figures, contributed to writing, and reviewed the final manuscript.

Competing interests

The authors declare that there are no conflicts of interest.

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