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Synthesis, Evaluation, and Application of a Novel Antioxidant Emulsifier Utilizing Mango Kernel Oil, Gluconic Acid, and Quercetin

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Abstract

The objective of this study was to develop a new emulsifier that possesses antioxidant properties and is prepared from naturally occurring compounds. The emulsifier was synthesized by combining oleic acid, gluconic acid, and quercetin. Mango kernel was chosen as a source of oleic acid due to its cost-effectiveness and high oleic acid content. The process involved grinding and drying the kernels, followed by extracting the oil using a Soxhlet device. The extracted oil was further examined to determine its physical and chemical properties, as well as its fatty acid composition. After that, the oil was hydrolysed, producing a mixture of free fatty acids and glycerine, which were then separated from each other. Oleic acid was extracted individually in pure form from the fatty acid mixture using a supercritical CO2 extractor at 28.0 MPa, 313 K. And it was confirmed according to its physical, chemical and spectral characteristics. Oleic acid was then converted to 9-hydroxyoctadecanoyl chloride, followed by its reaction with quercetin and gluconic acid through several steps to produce a novel antioxidant emulsifier known as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl9-((2,3,4,5,6-pentahydroxyhexanoyl)oxy)octa-decanoate or as chromenylgluconyloctadecanoate for simplification. The structures of all prepared compounds were elucidated according to their elemental analysis and spectral data (IR, 1H NMR, 13C NMR and MS). The radical scavenging activity of the newly prepared compound was evaluated using DPPH and ABTS methods, as well as lipid oxidation in sunflower oil with a Rancimat apparatus. The results indicated good antioxidant activity. The compound's surface-active properties and emulsion stability were tested, soy lecithin was used as a reference compound, and the results indicated its efficacy as a surfactant. Albino mice were used for an acute oral toxicity test, with subsequent examination of their liver and kidney functions. The results indicated no significant changes, confirming the safety of the prepared compound. The newly prepared antioxidant emulsifier was used to produce a blend fat spread. This is a typical example of how it can be used. The texture and sensory properties of the spread were evaluated, and the results showed that the properties of the blend fat spread were significantly improved with the use of new antioxidant emulsifier.

Keywords: mango kernel oil, gluconic acid, quercetin, chromenylgluconyloctadecanoate, antioxidant, emulsifier**,** acute toxicity, blended fat spread.

1. Introduction

Emulsifiers are an important class in chemistry named amphiphiles. They typically consist of a hydrophilic head attached to a lipophilic tail, allowing them to hold two immiscible liquids. Surfactants are essential for improving the emulsifying, gelling, film-forming, and foaming qualities of food, pharmaceutical products, and cosmetics [1]. In food industries, they can extend shelf life, and enhance texture. Food emulsifiers can be natural, such as gelatin, arabic gum, cellulose, starch, and phospholipids like lecithin. or synthetic, such as fatty acid esters, mono- and diglycerides, carboxymethylcellulose, and polysorbate [2,3]. The food industry has a keen interest in the production of carbohydrate fatty acid esters due to their tasteless, odourless, non-toxic, antimicrobial, and insecticidal properties [4, 5]. These esters can be created through the esterification of mono, di, or polysaccharides with various fatty acids using chemical or enzymatic reactions[6]. The degree of unsaturation, chain length, linearity, branching degree, cyclization, molecular weight and number and polarity strength of polar functions all significantly impact the surface tension characteristics of the resulting surfactant[7]**.** A more advanced carbohydrate fatty acid can be produced using a non-cyclic sugar, which has less restricted hydroxyl groups compared to the cyclic form [8]. On the other hand, the food industry is highly motivated to produce antioxidants to retard any oxidation process that may lead to food

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deterioration. Those antioxidants may be natural, such as vitamin C, vitamin E, beta-carotene, and flavonoids[9] or synthetic, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). However, due to the reported toxicity of commonly used synthetic antioxidants, their use has been restricted [10]. Phenolic compounds are widely recognized for their strong antioxidant properties, as they can interact with various free radicals by transferring hydrogen atoms, single electrons, sequentially losing electrons in proton transfer, and chelating transition metals [11]. The majority of phenolic compounds have very low solubility in both water and fat, which limits their applications. However, their fat solubility can be enhanced by appending them to a fatty moiety**12**. The naturally occurring polyphenolic flavonoid antioxidant quercetin, also known as 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one, is present in berries, apples, tea, cabbage, berries, nuts, and onions. The many uses of this substance include anti-inflammatory properties, prevention of cardiovascular disease, lowering blood pressure, prevention of cancer cells, regulation of blood sugar, preventing prostate infections, preventing upper respiratory tract infections, and delaying Alzheimer's disease [13]. In addition, quercetin solubility in fat was enhanced via its conversion to the corresponding quercetin palmitate [12].
In this study, we have modified quercetin by attaching it to both hydrophilic (gluconic acid) and lipophilic (fatty acid) groups, creating a new amphiphilic molecule. This modification allows the new quercetin derivative to act as an antioxidant surfactant

2. Materials and Methods

2.1 Materials

Mango fruits were obtained from Ismailia city, Rutin (≥ 98 %), Quercetin, BHT (butylhydroxytoluene), TBHQ (tert. butyl hydro quinine), L-ascorbic acid, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH˙), ABTS radical solution were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Sodium phosphate buffer, potassium dichromate, tween 20, absolute ethanol and all other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany). Animals were obtained from the animal house – National Research Centre.

2.2 Methods

2.2.1 Plant material, Seeds collection and preparation

Optimum mature mango fruits were harvested during the season of August 2022 from Ismailia City, Egypt. The plant was approved for collection and its taxonomic identity was verified by Dr. Hanaa Soliman at the National Centre in Giza, Egypt. Additionally, a voucher herbarium specimen was deposited in a publicly available herbarium at the National Research Centre.
The fruits were first washed with running water and then dried with a towel. The pulp and peels were separated manually, and the seeds were carefully cleaned. The seed shells were then opened with strong scissors, and the kernels were collected. Mango kernels weighing 1500 g were then ground and dried overnight at 40°C. The moisture content was determined, and the dried, ground kernels were stored at -18°C until use.
All methods comply with relevant institutional, national, and international guidelines and legislation. The study is reported following ARRIVE guidelines. The experimental protocol was exempted by the Medical Research Ethics Committee – National Research Centre (Exemption 08421023).

2.2.2 Fat extraction

Oil was extracted from 1500 g of dried, ground kernels using 2.5 L of acetone in a Soxhlet apparatus for 6 hours, with a condensing rate of 5 to 6 drops per second. The solvent was then evaporated using a rotary evaporator until dryness, resulting in mango kernel oil, which was weighed and stored for analysis.[14].

2.2.3 Quality parameters & Fatty acids composition of mango kernel oil

2.2.3.1. Refractive index (RI)

The determination of the refractive index (RI) of the extracted mango kernel oil was performed using a refractometer (Rudolph model J157 at 20˚C). The test was performed three times [15].

2.2.3.2. Free fatty acids (FFA%)

A 0.10 N potassium hydroxide solution was used to titrate a solution containing absolute ethanol and diethyl ether in a 1:1 ratio, with the addition of 1% phenolphthalein indicator. Subsequently, a 5 g oil sample was dissolved in 50 ml of the aforementioned solvent and titrated with 0.10 N potassium hydroxide[15].
Free fatty acids were calculated as % of oleic acid.
FFA % as oleic acid = S × 0.0282 × 100 / W
Where: *S* = titration (ml), *W* = weight of the oil (g).

2.2.3.3. Iodine value (IV)

According to the AOAC method[15], the iodine value of the extracted mango kernel oil was determined. 25 ml of Hanus iodine solution (13.2 g pure I2 in 1 L CH3COOH) was added to a solution of the oil sample (0.25 g) in chloroform (20 mL), and left in the dark for 30 minutes. Then, KI solution (10 mL, 15%) was added and shaken thoroughly, followed by dilution with distilled water (100 ml). Finally, it was titrated with Na_2S2O_3 solution (0.10 N) in the presence of a few drops of 1% starch solution as an indicator. A blank was performed omitting the oil, and the iodine value was calculated as grams of iodine per 100 g of oil.
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

2.2.3.4. Peroxide value (PV)

Glacial acetic acid (15 mL) and sodium bicarbonate (2 g) were added to a stirred solution of the oil sample (2 g) in chloroform

(10 mL). This was followed by the addition of saturated KI solution (1 mL) with continuous stirring. The solution was then kept in the dark for 5 minutes. After that, dilution with distilled water (75 mL) and the addition of starch solution (0.5 mL) indicator were performed. The solution was titrated with a $Na_2S_2O_3$ solution (0.01 N). The blank was carried out using the same procedures but without the sample[15]. The peroxide value of the tested oil is given by the equation:

*PV= [(V1-V2) * N * 1000] / W [meq.O_2/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).

2.2.3.5. Determination of saponification value

An oil sample (5 g) was dissolved in 50 mL alcoholic KOH solution (35–40 g KOH were dissolved in 20 mL water and diluted to one liter with alcohol, 95%) and refluxed for 30 minutes, then it was left to cool. Finally, it was titrated with HCl (0.5 M) in the presence of phenolphthalein indicator[15].The saponification value was calculated by the following formula:

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.

2.2.3.6. Total polar materials (TPM):

Column chromatography was used to determined TPM in the extracted mango kernel oil sample[16].

2.2.3.7. Polymer content (PC):

An oil sample was dissolved in a solution of methyl alcohol (125 ml) with 1% H2SO4 and refluxed for two hours. The formed precipitate was filtered off, washed with methanol. Finally, the washed precipitate was dissolved in petroleum ether and transferred to a pre-weighed flask. The solvent was then evaporated, and the flask was weighed again to determine the weight of the precipitate[17].

2.2.3.8 viscosity

A computerized Brookfield viscometer model RV-DV (Laboratories., Inc., USA) was used to determine the viscosity of mango kernel oil[18].

2.2.3.9 Fatty Acids Composition

Gas chromatography[19] was used to determine the fatty acid composition of the extracted mango kernel oil. A methanolic potassium hydroxide solution (0.2 ml, 2 N) was added to a solution of fatty acids (0.1 g) in heptane (2 ml) and vigorously shaken to produce a mixture of methyl esters. These esters were then identified using a gas chromatograph with nitrogen, hydrogen, and air flow rates of 0.6, 45, and 450 ml/min, respectively. The oven, injector, and detector temperatures were 195, 230, and 250 ˚C, respectively. The identification of the fatty acid methyl esters was done by comparing their retention times with a known fatty acid standard mixture. Their concentrations were calculated by the computer from the integration of the area under the peak.

2.2.4 Oil hydrolysis.

The triacylglycerol molecule of an oil is hydrolysed under restricted conditions to afford glycerol and a mixture of free fatty acids. Therefore, using a high-pressure reactor at 250 °C and 2 MPa with 500 ml of distilled wate, mango kernel oil (174.15 g) was hydrolysed. The reaction was then left to cool, resulting in the formation of two layers of the reaction mixture, which were separated using a separating funnel. The upper layer, which contained the fatty acids, was washed with distilled water several times and was dried over anhydrous sodium sulphate. Finally, it was filtered off and weighed.[20,21]

2.2.5 Confirmation of oil hydrolysis.

***2.2.5.1* By thin layer chromatographic analysis (TLC)**

Thin-layer chromatographic analysis was used to confirm oil hydrolysis. A silica gel plate (20x20) was activated at 110 °C for 1.0 h. At the baseline (2 cm from the bottom), spots of a standard known fatty acid, oil, and the hydrolyzed fat were spotted individually. A solvent consisting of n-hexane, diethyl ether, and acetic acid at a volumetric ratio of 80:20:1, respectively, was used as the developing solvent. The plates were developed until the solvent reached the front line (15 cm from the start line). Then, iodine vapor was used to visualize the samples on the TLC. When the withdrawn sample showed only one spot with no tail and with a rate of flow similar to that of the known fatty acid spot but not the oil spot, the fatty acids were considered formed[8].

2.2.5.2 By ^{1}H NMR spectrum

The ^{1}H NMR spectrum reviled a signal at δ10.23 which is characteristic for carboxylic hydrogen of the free fatty acids[8].

2.2.5.3 By IR spectrum

The IR spectrum of the hydrolyzed oil shows no ester band[8].

2.2.6 Individual extraction of Oleicacid

A Gallenkamp electrothermal melting point apparatus was used for determination of all melting points. On a Pye UnicamSp 3-300 and Shimadzu FT IR 8101 PC infrared spectrophotometer the infrared spectra were recorded for potassium bromide. On a Varian Mercury VX-300 NMR spectrometer the NMR spectra were recorded. At 300 MHz ^{1}H spectra were run. Chemical shifts are quoted in δ and were related to that of the solvents. On a Shimadzu GCMS-QP-1000EX mass spectrometers at 70 e.V. the mass spectra were recorded. At the Micro-analytical Center of Cairo University elemental analyses were carried out.

Using a supercritical CO_2 extractor[22,23] at28.0 MPa, and 313 K oleic acid was extracted individually in a pure form from the fatty acid mixture (158.44 g, 90.98% of the extracted mango kernel oil). The extracted oleic acid (70.88 g, 98.7% of the actual oleic acid content in the mango kernels oil) was confirmed according to its elemental analysis, melting points, GC-MS and mass spectra (MS) of its methyl ester which detect the corresponding molecular ion peak.

Yield (70.88 g, 98.7 %), (pale yellow liquid),m.p.(13 °C),**IR (KBr)** ν_{max}/cm^{-1}: 3022-2961 (=CH), 2921-2850 (CH- aliphatic), 2788 (OH), 1729 (C=O), 1621(C=C).**^{1}H NMR ($CDCl_3$):** δ 0.91 (t, 3H, J=6.8Hz, 18-CH_3), 1.27-1.34 (m, 20H, 4-7 and 12-17-CH_2), 1.55(m, 2H, 3-CH_2), 2.07 (m, 4H, 8,11-CH_2), 2.21 (t, 2H, J=6.9 Hz, 2-CH_2), 5.34-5.41(m,2H, 9,10-CH),11.4 (s, D_2O-exchangeable, 1H).**^{13}C NMR (DMSO – d_6):** δ14.0, 22.8, 24.6, 27.5, 29.1, 29.3, 29.6, 29.8, 29.9, 31.8, 34.6, 130.4,

179.3. **MS *(m/z)*:** (295M^+), 156, 126, 111, 45, 71, 55. **For** $C_{18}H_{34}O_2$ (282.47): **Calcd:** C, 76.54; H, 12.13 %. **Found:** C, 76.5; H, 11.2 %.

2.2.7 Synthesis of 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 9-((2,3,4,5,6-pentahydroxyhexanoyl)oxy)octadecanoate or {chromenylgluconyloctadecanoate} (11).

This preparation takes place via the acylation of 9-hydroxyoctadecanoyl chloride (5) with 7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3,5-dihydroxy-4H-chromen-4-one (8). Each of them was produced through several steps as follows.

***2.2.7.1 Synthesis of 9-hydroxyoctadecanoyl chloride* (5)**

***2.2.7.1.1 Synthesis of 9-Hydroxyoctadecanoic acid*(2)**

Oleic acid was sulfated, followed by hydrolysis to afford 9-Hydroxy octadecanoic acid[24]. To achieve this, sulfuric acid (10 mL, 96%) was added dropwise to a stirred, cooled oleic acid (1) (70.5 g, 250 mmol) in an ice bath over 30 minutes. Then, water (500 mL) was quickly poured over the mixture. The resulting mixture was stirred under reflux for one hour, and then left to cool, resulting in the formation of two layers. The aqueous layer was removed using a separating funnel, and the fatty layer was washed several times with water. Next, an alcoholic KOH solution (140 mL) was added, and the mixture was stirred under reflux for 8 hours. The solvent was then evaporated using a rotary evaporator, and the crude product was collected and neutralized with dilute sulfuric acid. Finally, the formed hydroxy stearic acid **2** was washed with hot water until neutralization. Recrystallization was performed with ethanol, and the mixture was left to stand at room temperature overnight.

Yield (63.06g, 84%), (mp. 74-75 °C), (white wax-like), **IR (KBr)** ν_{max}/cm^{-1}: 3622 (OH alcoholic), 2921-2847(CH-aliphatic),2797 (OH acidic), 1729 (C=O). **^{1}H NMR ($CDCl_3$):** δ 0.91 (t, 3H, J=7.0 Hz, 18-CH_3), 1.25-1.32(m, 22H, 4-8 and 12-17-CH_2), 1.56(m, 2H, 3-CH_2), 2.05 (m, 4H, 9,11-CH_2), 2.2 (t, 2H, J=7.1 Hz, 2-CH_2), 3.3(m, 1H, 10-CH), 4.6 (s, D_2O-exchangble, 1H), 10.85 (s, D_2O-exchangble, 1H).**^{13}C NMR (DMSO – d_6):** δ14.0, 23.7, 24.7, 25.8, 28.0, 29.2, 29.6, 29.8, 31.4, 343.7, 38.9, 74.83, 175.5. **MS *(m/z)*:** (299 M^+), 157, 143, 84, 59, 57. **For** $C_{18}H_{36}O_3$ (300.48): **Calcd:** C, 71.95; H, 12.08 %. **Found:** C, 71.68; H, 12.24 %.

2.2.7.1.2 Synthesis of9-(benzyloxy)octadecanoic acid (3) {Blocking of the alcoholic OH group}

9-Hydroxyoctadecanoic acid**(2)** (63 g, 209.6 mmol) was dissolved in acetone (150 mL). K_2CO_3 (28.9 g, 209.8 mmol) was added. The reaction was stirred under nitrogen for 2 h at room temperature, followed by a slow addition of benzyl bromide (24.90 mL, 209.6 mmol). The mixture was stirred at 40˚C under nitrogen for 3 h. Then it was cooled in an ice bath and acidified to pH 6.0 using acetic acid. Deionized water (300 mL) was added, and the formed suspension of 9-(benzyloxy)octadecanoic acid **(3)** was filtered off[12],then it was heated with deionized water (where any benzyl bromide residue will react with hot water to form water-soluble HBr and alcohol soluble benzyl alcohol) and filtered off, followed by other washing with ethanol, then it was dried under vacuum.

***2.2.7.1.3 Synthesis of 9-(benzyloxy)octadecanoyl chloride*(4)**

Oxalyl chloride (17.15 ml, 200 mmol) was added dropwise to a stirred 9-(benzyloxy)octadecanoic acid **(3)** (24.54 g, 200 mmol) and refluxed[25] for 4 h**.,** andthe upper condenser opening should be closed with a piece of cotton and a few grams of anhydrous sodium sulphate. The mixture was then heated under a vacuum in the presence of water to get rid of the unreacted oxalyl chloride and the byproducts. A quantitative recovery of 9-(benzyloxy)octadecanoyl chloride**(4)** was obtained.

2.2.7.1.4 Synthesis of 9-hydroxyoctadecanoyl chloride* (5) *{Hydrogenation to get rid of protection}

9-(benzyloxy)octadecanoyl chloride **(4)** (77.7g, 190 mmol) was stirred in acetone (200 mL). Pd/C (4 g) was added to the solution. The reaction was continued for 2 h. under hydrogen at room temperature. Pd/C was filtered off, and the filtrate was evaporated under vacuum, washed, and recrystallized from ethanol to produce 9-hydroxyoctadecanoyl chloride **(5).**

Yield (16.48 g, 94%), (mp. 74-75 °C), **IR (KBr)** ν_{max}/cm^{-1}: 3483-3194 (OH alcoholic), 2933-2844 (CH- aliphatic),1815 (C=O). **^{1}H NMR ($CDCl_3$):** δ 0.89 (t, 3H, J=7.1 Hz, 18-CH_3), 1.25-1.33 (m, 22H, 17-11 &7-4-CH_2), 1.4 (m, 4H,10-8-CH_2), 1.53(m, 2H, 3-CH_2), 2.15 (t, 2H, J=7.0 Hz, 2-CH_2), 3.5(m, 1H, 9-C), 4.8 (s, D_2O-exchangble, 1H).**^{13}C NMR (DMSO – d_6):** δ14.0, 22.7, 25.0, 25.5, 28.3, 29.3, 29.6, 29.8, 31.7, 37.2, 46.7, 71.8, 170.9. **For** $C_{18}H_{35}ClO_2$ (318.93): **Calcd:** C, 73.41; H, 10.10; Cl, 8.67; O, 7.82 %. **Found:** C, 73.38; H, 10.12; Cl, 8.65%

***2.2.7.2Acylation of 9-hydroxyoctadecanoyl chloride (5) with Quercetin derivative* (9)**

***2.2.7.2.1 Blocking of the active hydroxyl groups of rutin* (7)**

K_2CO_3 (33.95 g, 245.7 mmol) was added to rutin **(6)** (50 g, 81.9 mmol) in acetone (150 ml) and stirred under nitrogen for 2 h. at room temperature. Then, benzyl bromide (29.18 ml, 245.69 mmol) was slow added and the mixture was stirred at 40˚C under nitrogen for 3 h. After that, the reaction was acidified to pH 6.0 using acetic acid and cooled in an ice bath, followed by addition of deionized water (300 ml), the formed suspension was filtered and heated in deionized water (where any benzyl bromide residue will react with hot water to form water-soluble HBr and alcohol-soluble benzyl alcohol) and filtered off, followed by other washing with ethanol and then it was dried under vacuum[12].

2.2.7.2.2 Hydrolysis of glycosidic bond of rutin

The filtered residue (66.00 g, 75 mmol) was added to a solution of ethanol (150 ml) and hydrochloric acid (36%, 150 ml). The mixture was heated under reflux for 1 h. Then it was left to cool to room temperature, and the formed hydrolysate was filtered and washed with distilled water until neutralization[12].

2.2.7.2.3 Acylation with 9-hydroxyoctadecanoyl chloride

To a cold stirred acetone (100 ml) in ice medium, H_2SO_4 (8 ml) was dropped wisely, Then, the hydrolysate **(8)** (40.08 g, 70 mmol) was added, followed by addition of 9-Hydroxyoctadecanoic acid chloride **(5)** (22.33 g, 70 mmol). The mixture was stirred under reflux for 3 h. During definite time intervals, thin layer chromatographic analysis of withdrawn samples was carried out in order following the esterification reaction progress. At the completeness of the reaction, the organic layer was

washed with deionized water several times. Dried over Na_2SO_4 and then taken to dryness under vacuum and afforded the corresponding acylated compound 7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4-oxo-4H-chromen-3-yl 9-hydroxyoctadecanoate**(9).**

Yield (16.48 g, 94%), (mp. 74-75 °C), **IR (KBr)** ν_{max}/cm^{-1}: 3483-3194 (OH alcoholic), 2933-2844 (CH- aliphatic),1815 (C=O). **^{1}H NMR ($CDCl_3$):** δ 0.89 (t, 3H, J=7.1 Hz, 18-CH_3), 1.25-1.33 (m, 22H, 17-11 &7-4-CH_2), 1.4 (m, 4H,10-8-CH_2), 1.53(m, 2H, 3-CH_2), 2.15 (t, 2H, J=7.0 Hz, 2-CH_2), 3.5(m, 1H, 9-C), 4.8 (s, D_2O-exchangble, 1H).**^{13}C NMR (DMSO – d_6):** δ14.0, 22.7, 25.0, 25.5, 28.3, 29.3, 29.6, 29.8, 31.7, 37.2, 46.7, 71.8, 170.9. **For** $C_{18}H_{35}ClO_2$ (318.23): **Calcd:** C, 73.41; H, 10.10; Cl, 8.67; O, 7.82 %. **Found:** C, 73.38; H, 10.12; Cl, 8.65%

***2.2.7.*3 <u>Acylation with gluconic acid</u>**

To a stirred refluxed acetone (150 ml), potassium hydroxide was gradually added until the PH reached 10.5, the mixture was left to cool to room temperature, and filtered off. The filtrate was stirred, and gluconic acid (12.16 g, 62 mmol) was gradually added, followed by the addition of 7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4-oxo-4H-chromen-3-yl 9-hydroxyoctadecanoate **(9)** (53.01 g, 62 mmol). The reaction mixture was stirred under reflux for three hours and then, the reaction was left to cool. Acetone was evaporated using a rotatory evaporator. The remainder was washed with ice/cooled water several times till neutralization, to get rid of potassium hydroxide and the unreacted gluconic acid, (where the very cooled water could not dissolve the fatty derivatives as they were found to be semi-solid at low temperatures). Then, the residue was washed with warm water (where gluconate derivative **10** will be dissolved, while compound 9 does not), the filtrate was evaporated under a vacuum, and compound **10** wasthen afforded.

***2.2.7.*4 Hydrogenation (getting rid of protection)**

The formed ester **10** was stirred in acetone (250 ml) in the presence of Pd/C (2 g) and stirred at room temperature for 2 hours under hydrogen. Then, filtration of Pd/C was performed followed by evaporation of the filtrate. Thus, the remainders were benzene and the final product 11, which were separated from each other using hot water. Compound **11** dissolved in the hot water while benzene did not. The water was then evaporated from the aqueous solution, and the remainder was washed and recrystallized from ethanol to produce 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 9-((2,3,4,5,6 pentahydroxyhexanoyl)oxy)octa decenoate **(11).**

Yield (40.41 g, 88.28%), mp. 63-64°C (pale yellow flakes). **IR (KBr) ν_{max}/cm^{-1}**= 3322-3257 (9 OH), 1739, 1723, 1716 (3 C=O), 1522-1486 (C=C aromatic), 1291, 1278 (2 C-O). **^{1}H NMR (300 MHz, DMSO-d6):**δ 9.41-8.64 (4s, D_2O-exchangble, 1H), 7.06 (d, 1H, J = 2.2 Hz), 6.57(dd, 1H, J = 2.1 Hz, 8.7 Hz), 6.50 (d, 1H, J = 8.51 Hz), 6.09 (d, 1H, J = 2.2 Hz), 5.94 (dd, 1H, J = 2.3 Hz), 5.55-4.23 (5s, D_2O-exchangble, 1H), 4.00 (m, 1H), 3.10-4.20 (3s, 2H), 2.26 (t, 2H, J = 7.5 Hz), 1.49-1.23 (m, 28H), 0.91 (t, 3H). **^{13}C NMR (75 MHz, DMSO-d6):** δ 173.4, 171.9, 167.1, 162.2, 158.9, 153.5, 147.7, 146.3, 133.4, 122.9, 120.5, 118.2, 115.0, 104.5, 98.3, 94.2, 75.1,73.2, 72.1, 70.0, 68.9, 63.7, 34.5, 31.9, 29.6, 29.3, 28.9, 26.5, 25.6, 25.1, 22.2,14.1. For $C_{39}H_{54}O_{15}$ (762.38): Calculated: C, 61.14; H, 7.14%. Found: C, 61.31; H, 7.04 %

2.2.8 Evaluation of the newly prepared compound as an antioxidant

2.2.8.1 DPPH• radical scavenging assay

chromenylgluconyloctadecanoate **(11)** radical scavenging activity was determined[12] based on its efficiency in trapping the free radical of the violet DPPH• (1,1-Diphenyl-2-picryl hydrazyl) and causing its reduction to the stable yellow DPPH molecule (1,1-Diphenyl-2-picryl hydrazine). The activity was compared to those of Quercetin and BHT (butyl hydroxyl toluene) individually. Variable concentrations (0.1 mL) (0.2, 0.4, 0.6, 0.8, and 1 mmol/L EtOH) of each series (chromenylgluconyloctadecanoate (11), quercetin, and BHT) were mixed with a DPPH• solution (3.9 mL, 0.063 mmol) individually and kept in the dark for 30 minutes at room temperature, causing the reduction of the deep violet DPPH• (1,1-Diphenyl picryl hydrazyl) to the yellow DPPH (1,1-Diphenyl picryl hydrazine). Finally, a portable hyperspectrometer (Spectronic 21D, Milton Roy Boulder, Colorado, USA) was used to determine the absorbance of the reaction mixture at 517 nm. The blank used was L-ascorbic acid. The percentage of DPPH radical-scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0- A_1)/A_0] \times 100]$

Where A_0 was the absorbance of the blank and A_1 was the absorbance of the sample or standard. All analyses were performed in triplicate. The data were recorded as mean values.

2.2.8.2 ABTS$^{•+}$radical cation scavenging assay

An antioxidant reduces the dark blue 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{•+}$) into a colorless ABTS molecule. The preparation of the $ABTS^{•+}$ solution was achieved by treating an ABTS radical solution (7 mmol) with $K_2S_2O_8$ (140 mmol) as an oxidizing solution at room temperature and kept in the dark for 12 hours. This was followed by its dilution with absolute ethanol until the absorbance at 734 nm was 0.700 ± 0.02. Then, samples (0.2 mL) of variable concentrations of each series (chromenylgluconyloctadecanoate (11), quercetin, and BHT) (0.2, 0.4, 0.6, 0.8, and 1 mmol/L EtOH) were mixed individually with the previously prepared blue $ABTS^{•+}$ solution (5 mL) for 6 minutes at room temperature. The absorbance of the final reacting solution was measured at 734 nm**[18].**

Radical scavenging activity (%) = $[(A_0- A_1)/A_0] \times 100]$

Where A_0 was the absorbance of the blank and A_1 was the absorbance of the sample or standard. All analyses were performed in triplicate. The data were recorded as mean values.

2.2.8.3 Lipid oxidation in sunflower oil

The Rancimat 673 (Metrohm Co., Herisou, Switzerland) was used to determine the oxidative stability of refined sunflower oil[12]. Samples of the refined sunflower oil were added to (0.4 mmol/L) TBHQ (tert-Butylhydroquinone) and chromenylgluconyloctadecanoate**(11)**individually, and compared to a control sample. An air stream was bubbled through the oil at 110 ± 0.2°C and a flow rate of 10 ± 0.2 L/h. The volatile degradation products passed through a second vessel

containing distilled water, resulting in a rise in its conductivity. Using the software provided with the instrument, the intersection of the tangent lines was used to determine the induction period (IP).

2.2.9 Evaluation of the newly prepared compound as a surfactant

A surface tensiometer (POWEREACH JK99B, Shanghai Zhongshan Digital Technical Apparatus Co., Ltd., China) was used to determine the surface-active properties of the prepared surfactant.

2.2.9.1 Oil–water interfacial tension γo/w

The purified sunflower oil was first prepared by adding 50 mL of deionized water to 500 g of sunflower oil with stirring at room temperature for 1 hour. This was followed by centrifugation, which resulted in the formation of two layers. The aqueous layer, which contained free fatty acid, was removed, while the oily layer was treated with 25% (w/w) activated charcoal and left overnight. Subsequent centrifugation and filtration were performed to obtain pure sunflower oil. This oil is considered acceptable[26] for use in oil-water interfacial tension measurements only when its interfacial tension against deionized water is still higher than 25 mN/m for 1 hour.

According to the Du Noüy method[27], the sunflower oil–water interfacial tensions were measured as follow: individual solutions of chromenylgluconyloctadecanoate (**11**) and soy lecithin (0.01% w/v) were dissolved in deionized water at 20°C. Then, a horizontally suspended clean and heated-to-redness platinum ring of known geometry was dipped in a sample of the prepared solutions and pulled out afterwards. The interfacial tension is measured as the maximum force needed to pull the ring through the interface.

2.2.9.2 Determination of critical micelle concentration (CMC) by air-water surface tension method

A surface tensiometer equipped with a Wilhelm plate was used to measure the surface tensions[28] of the newly prepared chromenylgluconyloctadecanoate (**11**) solutions at 25°C and at different concentrations, and the results were compared to those of soy lecithin. The lower edge of a horizontally suspended clean and heated-to-redness platinum ring of known geometry was brought into contact with the surface of a homogeneous aqueous surfactant solution (50 ml) which was placed individually in Petri dishes. The sample vessel was lifted until the plate dipped into the tested liquid. The surface tension was measured as the maximum force needed to pull the plate through the surface. The CMC was determined as the break point formed by plotting surfactant concentration versus surface tension.

2.2.9.3 Emulsion stability

Using an Ultra-Turrax homogenizer T25, an individual solution of chromenylgluconyloctadecanoate (**11**) and soy lecithin was mixed with distilled water (40 mL, 0.5% w/v) at 9500 rpm for 1 min. Then, the oil phase (60 mL) was slowly added, and the mixture was homogenized for 2 minutes at 13500 rpm to form an emulsion[29,30], which was then placed in a 100-mL graduated cylinder at 25 °C. At different time intervals (0, 4, 8, 12, 16, 20, and 24 hr), the volume of the separated oil phase was measured. The percentage of the separated oil over a given period was calculated according to the following formula:

Separation % = (Volume of separated oil phase / Total Volume of oil) × 100

2.2.10 Acute toxicity test and Liver and kidney function tests:

In individual housing, 22–25 g albino mice were kept under conventional settings (12 hour light/dark cycle, 25±3°C temperature, 35–60% relative humidity). The study report adheres to the ARRIVE criteria. The National Research Center's Medical Research Ethics Committee approved the experimental procedure (Exemption - 08411023). Six groups of animals were used, each consisting of five animals. One group was used as a control and fed only standard mice feed, while the other groups were fed different oral dosages of the prepared chromenylgluconyl octadecenoate mixed with standard mice feed[12].

To demonstrate the safety of chromenylgluconyloctadecanoate as a food additive, tests were conducted on the liver and kidney function of those albino mice. Thus, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphates (ALP), Creatinine, and Urea were determined spectrophotometrically[31,32].

A statistically significant difference between the experimental and control data was determined using a one-way analysis of variance (ANOVA) and a Tukey post hoc test in Microsoft Excel 2016 MSO (16.0.10361.20002) 32-bit.

2.2.11 Uses of Chromenylgluconyloctadecanoate(11) as an antioxidant emulsifier for blended fat spreadproduction

The Hydrophilic-lipophilic balance (HLB) values of non-ionic surfactants can be calculated using Griffin equation[33,] which is expressed by the following formula:

HLB=20(MH/ M)

where MH is the molecular mass of the hydrophilic moiety, and M is the molecular mass of the whole surfactant molecule.

Based on the (Griffin, 1949) equation, the calculated HLB value of the newly prepared chromenylgluconyloctadecanoate (11) was determined to be 8.4. This indicates that it could be used as a water-dispersible, wetting, and spreading agent, and also be appropriate for use in water-in-oil emulsions. As an example of its application, it has been used to prepare blended fat according to the percentages outlined in Table (1).

Table (1): Blended fat spread components

Ingredient	Amount (%)
Lipid phase (65 %)	
Anhydrous milk fat	9.75
sunflower oil	45.4945
Palm kernel oil	9.75
β-carotene	0.0065
Water phase (35 %)	
Water	34.4
Emulsifier	0.6

All components of the lipid phase were mixed and melted at 80°C, on the other hand, 0.6 % soy lecithin was mixed with water (34.4 %) at 60°C for the control sample, and it was replaced with chromenylgluconyloctadecanoate**(11)** for the tested sample. Anhydrous milk fat was used to ensure that the only emulsifier present was chromenylgluconyloctadecanoate or soy lecithin. Lipid and water phases were mixed at 30°C for 10 min using a Black & Decker MX300 homogenizer (New Britain, CT, USA) for emulsification. The sample temperature was then fast decreased to 5°C with stirring at 37 rpm using an electrical ice cream machine (MoulinexKrups GVS141 Ice Cream Maker with 1.6 L bowl) for crystallization. Finally, the sample was stored at 5°C for 24 h. before analysis.

2.2.11.1Fatty acid profiles, quality parameters, and oxidative stability of anhydrous milk fat, sunflower oil, palm kernel oil, and blended fat spread.

The fatty acids composition of anhydrous milk fat, sunflower oil, palm kernel oil and the prepared blended fat spread was analyzed using GC after esterification[19]**.**The refractive index, acid value, peroxide value, iodine value, and saponification number were determined according to A.O.C.C. (2005). The polar content was also determined[16].Additionally, the oil oxidative stability of anhydrous milk fat, refined sunflower oil, refined palm kernel oil, blended fat spread with soy lecithin (0.6 %) and blended fat spread with chromenylgluconyloctadecanoate (0.6 %) was measured[18] individually using Rancimat 673 (Metrohm Co., Herisou, Switzerland).

2.2.11.2 Colour Analysis

A Color Flex colorimeter that had been calibrated with black and white standards was used to measure the color values of each sample. The L* (lightness; 100 = white, 0 = black), a* (redness, red, green), and b* (yellowness, yellow, blue) values of the samples were measured[34].

2.2.11.3 Textural Evaluation

Texture measurements were conducted using the TA. XT2i Texture Analyzer (Stable Micro Systems Ltd, Surrey, UK) equipped with a 5 kg load cell[35]The measurements were performed using a back-extrusion cell with a 35 mm diameter compression disc. The samples were carefully placed into acrylic cylindrical containers with dimensions of 50 mm internal diameter and 75 mm height to a depth of 55 mm. A single cycle was applied at a constant crosshead velocity of 1 mm/s reaching a sample depth of 40 mm before returning. The resulting force–time curve was analyzed using the Texture Expert for Windows version 1 equipment software to obtain values for texture attributes such as hardness, spreadability, adhesiveness, and cohesiveness.

The samples apparent viscosity was measured[18]using the RV-DV computerized Brookfield viscometer (Laboratories, Inc., USA). Spindle No. 7 was used to measure the viscosity of samples at 4, 25, and 40 °C while rotating at 100 rpm.

2.2.11.4 Solid fat content (SFC)

Using low-resolution nuclear magnetic resonance (NMR) on an MQ20 Bruker mini spec device from Germany, the solid fat content (SFC) of the prepared blended fat spread supplemented with chromenylgluconyloctadecanoate was determined[36]. Samples were placed in 5 mL NMR vessels after melting at 80°C for ten minutes. The SFC was calculated over a temperature range of 0°C to 40°C. Every sample test observation was made in triplicate, with the temperature change rate set at 6°C/min and the frequency set to measure every 5°C.

2.2.12 Sensory evaluation

The blended fat spread supplemented with Chromenylgluconyloctadecanoate and lecithin, as well as a commercial sample, were assessed by ten independently untrained panelists. The sensory quality attributes were assessed using a hedonic rating system, where a score of 1 meant extremely dislike and 9 meant a strong like. The color, appearance, flavor, texture, odor, spreading ability, and general acceptability of the blended fat spreads were assessed. Panelists were given blended fat spreads on randomly coded white Styrofoam platters and were seated in a room with partitions between the seats and overhead fluorescent lighting. Furthermore, they were asked to rinse their mouths with water before and after evaluating the samples. [37]

3. Results and Discussion

3.1 Fat extraction

Fat is usually extracted from seeds after their grinding and dehydration to increase the surface area and consequently facilitate oil extraction. Thus, when 1500 g of mango kernel oil was ground and dried overnight at 40 °C, the moisture content was found to be (148.8g, 9.92% of kernel weight). Then, oil was extracted from the cake utilizing a Soxhlet apparatus and acetone, and it was found to be (174.15g, 11.61% of kernel weight). These findings were not significantly different from those obtained by [38]Olugbenga Olufemi Awoluetal. (2019).

3.2 Quality parameters & fatty acids composition of mango kernel oil

The extracted mango kernel oil was chemically and physically characterizedas illustrated in Table (2). The results showed that refractive index, Free fatty acid (%), peroxide value, iodine value and saponification number were 1.5821, 0.26 (%), 0.62 (mEq/Kg oil), 48.22(grams of I_2 /100 g oil), 198(mg KOH/g oil) respectively**.** While oxidized fatty acids, polar and polymer contents were less than 1%. This agreed with other previous reports[39].

Table 2: Physicochemical properties of mango kernel oil.

Parameter	mango kernel oil
Refractive index at 25°C	1.5821±0.05
Free fatty acid (%)	0.26±0.04
Peroxide value (mEq/Kg oil)	0.62±0.03
Iodine value (grams of I_2 /100 g oil)	48.22±0.74
Saponification value (mg KOH/g oil)	198±2.70
Oxidized fatty acids (%)	<1%
Polar Content (%)	<1%
Polymer content (%)	<1%

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

The fatty acid composition of mango kernel oil is illustrated in Table (3), it shows that the major unsaturated fatty acid is oleic, while the major saturated one is stearic, and they represent 45.26 and 37.46 % respectively. It also contains myristic acid, palmitic acid, linoleic acid and linolenic acid as 0.69, 9.23, 6.46 and 0.51% respectively. So, mango kernel oil could be categorized as a rich source of oleic acid, as reported by others[38,39].

Table (3): Fatty acid composition of the extracted Mango kernel oil

Fatty acid	Relative Amount (%)
Myristic acid($C_{14:0}$)	0.69±0.14
Palmitic acid ($C_{16:0}$)	9.23±0.41
Stearic acid ($C_{18:0}$)	37.46±1.03
Oleic acid ($C_{18:1}$)	45.26±1.53
Linoleic acid ($C_{18:2}$)	6.46±0.44
Linolenic acid ($C_{18:3}$)	0.51±0.02
Others	0.39
Saturated fatty acid (%)	47.38
unsaturated fatty acid (%)	52.23

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

3.3 Oil hydrolysis.

Oil is mainly composed of triacyl glycerol molecules, at which their hydrolysis afforded glycerol, fatty acid mixture and traces of unsaponified materials. Thus, the extracted oil (174.15 g) was hydrolyzed and afforded a glycerol and fatty acids mixture (158.44g, 90.98% of the extracted oil). Complete oil hydrolysis was confirmed according to TLC analysis, where there was only one spot with no tail and with a rate of flow similar to that of known fatty acid but not oil. Moreover, H1NMR spectra reflect the presence of a carboxylic hydrogen signal at δ 10.27, and the IR spectra revealed the absence of ester absorption, consequently, there was no ester molecule, and this means complete hydrolysis of the triacylglycerol into free fatty acids. This agrees with Hanaa Soliman et al. (2021).

3.4 Individual extraction of oleicacid

Through supercritical CO_2 extractor at 28.0 MPa, 313 K, pure individual oleic acid (70.88 g, 44.44 % of the fatty acids mixture and 98.7 % of the actual oleic acid content in the mango kernels oil) was extracted from the fatty acids mixture[22]. Then, it was confirmed based on its elemental analysis (C, 76.5; H, 11.2 %), melting point (13 °C), gas chromatography (GC) and mass spectra (MS) of its methyl ester which detect the corresponding molecular ion peak ($295M^+$).All data on the significant levels of the extracted oleic acid were generally consistent with those from prior research Hanaa Soliman et al[8, 21].

3.5 Synthesis of 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 9-((2,3,4,5,6-pentahydroxyhexanoyl)oxy)octadecanoate or {chromenylgluconyloctadecanoate} (11).

For the production of this emulsifier molecule, the fatty acid is going to carry both the antioxidant moiety (quercetin) and the hydrophilic moiety (gluconic acid) via esterification reactions. Thus, the suitable fatty acid is

9-hydroxy octadecanoic acid chloride. On the other hand, the Quercetin molecule (a promising natural phenolic antioxidant) possesses five hydroxyl functions, each of which has its role in radical capture. Quercetin esterification may exhaust one, two, three or four of those hydroxyl functions and affords so many isolable products which are associated with the loss of some radical capture centres, and consequently decrease of its antioxidant activity. Thus, to improve its fat solubility and keep its radical scavenging activity, only one hydroxyl function is going to be esterified with a fatty residue. So, rutin, which is a glycosylated quercetin, was used as a starting material. Where rutin active free hydroxyl functions were protected to avoid their esterification, then its glucoside bond was hydrolysed to afford a new active free hydroxyl function at which the esterification process with 9-hydroxy octadecanoic acid chloride will take place. Moreover, water solubility was improved via another esterification with gluconic acid. Then, a hydrogenation reaction is carried out to remove the protecting groups and return the hydroxyl functions (as in Scheme 2). On the other hand, the hydroxyl function of 9-Hydroxyoctadecanoic acid must be protected to avoid its chlorination (as in scheme 1), where this hydroxyl function is going to be esterified with gluconic acid. |Then, a hydrogenation reaction takes place to remove the blocking group and return the hydroxyl function.

3.5.1 Synthesis of 9-hydroxyoctadecanoyl chloride (5)

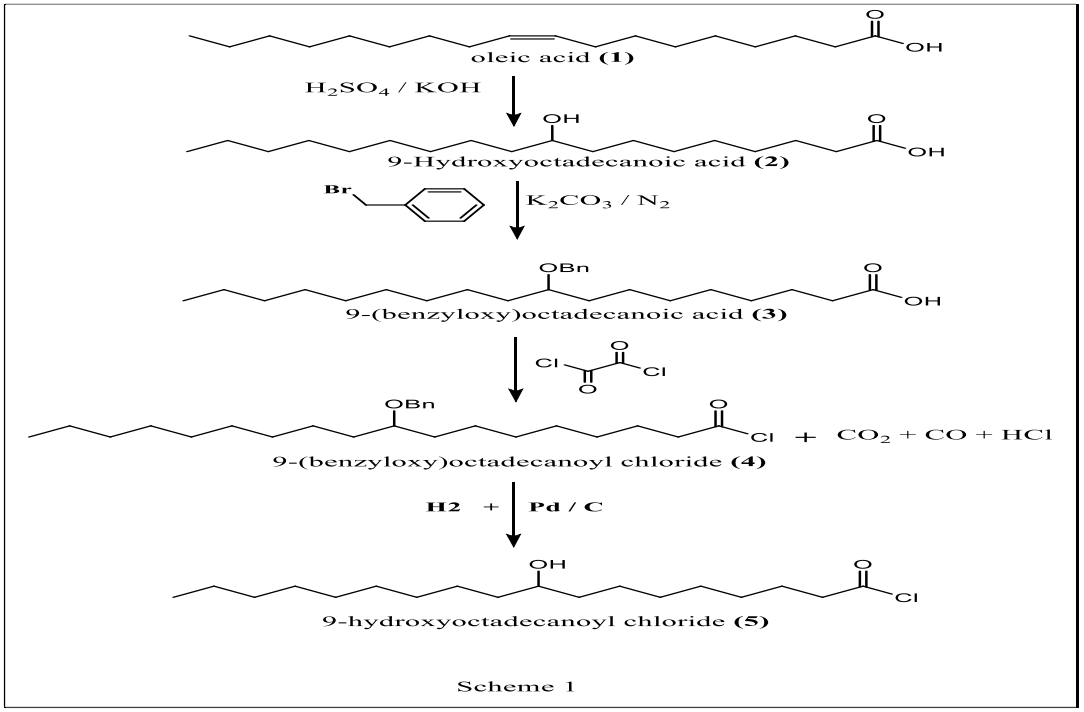
Thus, 9-Hydroxy octadecanoic acid **(2)**was prepared via oleic acid **(1)** sulphating, followed by its hydrolysis. Its IR spectrum reflects absorptions at ν 3622, 2921-2847, 2797 and 1729 cm^{-1} due to OH alcoholic, CH- CH-aliphatic,OH acidic and (C=O) functions respectively. Its H^1NMR shows two D2O-exchangeable signals at δ 4,6 and 10.85 corresponding to alcoholic and acidic OH functions respectively. Moreover, its MS spectrum illustrates a molecular ion peak at m/z 299 (M^+).

Then, the hydroxyl function of 9-Hydroxy octadecanoic acid **(2)** was blocked using K_2CO_3 and benzyl bromide to afford the corresponding 9-(benzyloxy) octadecanoic acid **(3).** The latter **(3)** was refluxed with excess oxalyl chloride and afforded the corresponding 9-(benzyloxy) octadecanoyl chloride (**4)**, after completeness of the reaction, hot water was added to get rid of all the unreacted oxalyl chloride and bi-products, where, oxalyl chloride decomposed in hot water to CO and CO_2gases and water-soluble HCl, this chlorination step is going to facilitate its esterification with the phenolic OH group of rutin. Finally, the deblocking of 9-(benzyloxy) octadecanoyl chloride **(4)** takes place by a hydrogenating process in the presence of Pd/C and afforded the target compound 9-hydroxyoctadecanoyl chloride **(5)** as in scheme 1. The IR spectrum of the latter compound **5** declares bands at 3483-3194 and 1815 cm^{-1} due to alcoholic OH and C=O absorptions respectively. While the H1NMR illustrates a D_2O-exchangble signal at δ 4.8 which is related to the alcoholic OH function, and there was no signal for a carboxylic hydrogen. Moreover, the elemental chemical analysis represents the presence of Cl with a ratio of 8.65%.

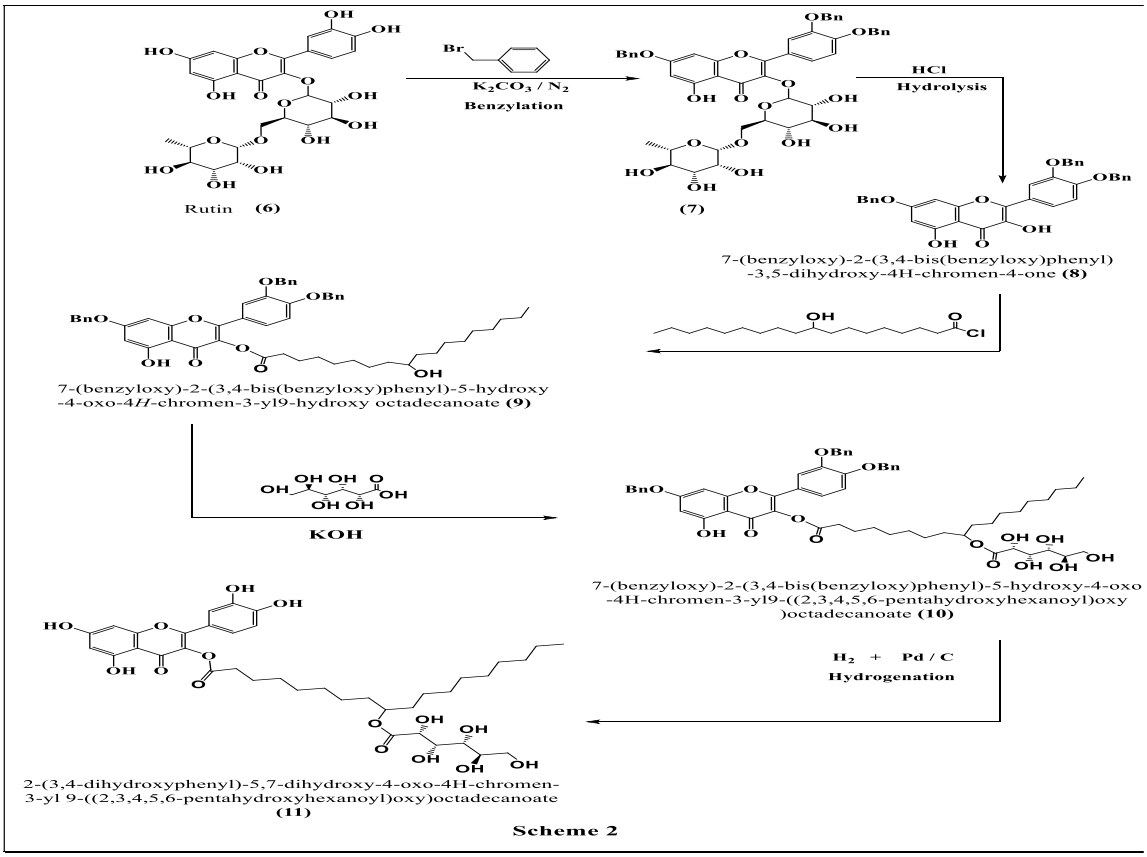
3.5.2 Synthesis of 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 9-((2,3,4,5,6-pentahydroxyhexanoyl)oxy)octadecanoate (11)

The active phenolic OH groups of Rutin **(6)** were blocked using K_2CO_3 and benzyl bromide to avoid the unrequired esterification process. Then, a new active OH function was formed when the glucoside bond was hydrolyzed by HCl. Esterification reaction with the previously prepared 9-hydroxy octadecanoic acid chloride **(5)** took place with this newly formed OH function and produced 7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4-oxo-4H-chromen-3-yl 9-hydroxyoctadecanoate **(9)** which is a fat-soluble compound. To enhance its water solubility to be used as an emulsifier, it underwent further esterification with the hydrophilic gluconic acid in a strong alkali medium to prevent the gluconic acid cyclization and afforded 7-(benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-4-oxo-4H-chromen-3-yl-9-((2,3,4,5,6-pentahydroxyhexanoyl) oxy)octadecanoate **(10).**

Finally, compound **10** washydrogenated to remove the protecting groups and return the OH functions of quercetin, thus maintaining its antioxidant activity, and producing the final desirable antioxidant emulsifier 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 9-((2,3,4,5,6-pentahydroxyhexanoyl)oxy) octadecanoate **(11) or** Chromenylgluconyloctadecanoate**(11)** for simplificationas in scheme 2. The IR spectrum of the final product revealed absorptions of four phenolic OH and five alcoholics OH functions at 3322-3257cm^{-1}in addition to three bands at 1739,1723 and 1716 cm^{-1} corresponding to three carbonyl absorptions. Also, the H1NMR showed four D_2O-exchangeable signals at δ 9.41-8.64 related to the four phenolic OH and other five D_2O-exchangeable signals at δ 5.55-4.23 due to the five alcoholics OH functions in addition to the fatty aliphatic protons at δ 0.91-4.0.



Scheme 1



Scheme 2

3.6 Evaluation of the prepared compound as an antioxidant

3.6.1 DPPH• radical scavenging assay

The radical scavenging activity of chromenylgluconyloctadecanoate **(11)** was measured in terms of its ability to capture and reduce the free radical from violet DPPH• (1.1-Diphenyl-2-picryl hydrazyl) which has absorption at a wavelength of 517 nm. to the stable yellow DPPH molecule (1,1-diphenyl-2-picrylhydrazine). Thus, by monitoring the decrease in absorption, the radical scavenging activity of an antioxidant molecule can be determined.

According to the comparison of the scavenging activity of the newly prepared chromenylgluconyloctadecanoate, its precursor quercetin and the well-known antioxidant BHT: butylated hydroxyl toluene, it was found that there is no significant difference between the activity of chromenylgluconyloctadecanoate and quercetin. However, both of them show higher antioxidant activity than the famous oxidant BHT as shown in Figure (1). This is in agreement with another report. [12].

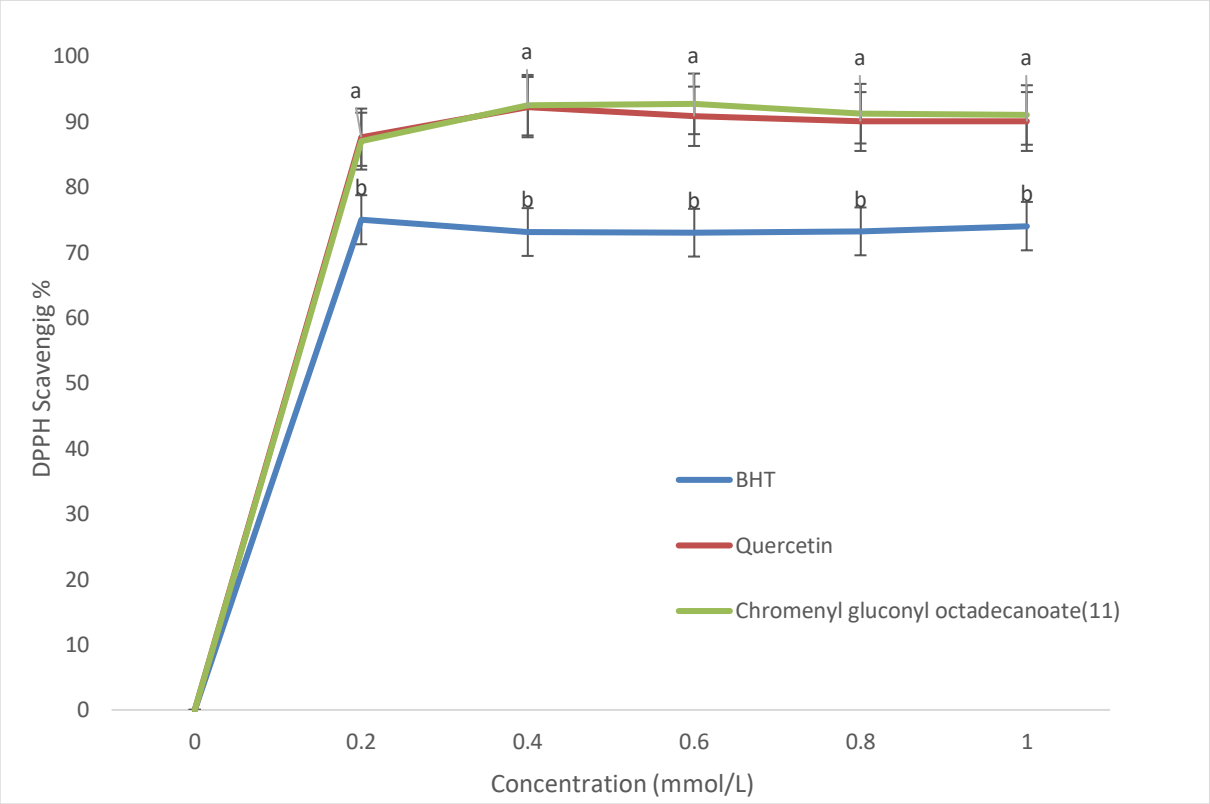


Fig. 1**:** DPPH free radical scavenging activity of different concentrations (0-1 mmol/L) of （Quercetin, Chromenylgluconyloctadecanoate and BHT: butyl hydroxyl toluene).
(Data are expressed as mean ± SD values given to represent the means of three determinations.)

3.6.2 ABTS•+radical scavenging assay

With an absorption at 734 nm, the dark blue radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfinate) (ABTS•+) is reduced by an antioxidant to a colourless ABTS molecule. Therefore, one way to assess an antioxidant molecule's capacity to scavenge radicals is to monitor drops in the absorption of (ABTS•+). The radical scavenging activities of chromenylgluconyloctadecanoate, quercetin, and BHT towards ABTS•+ were compared.

The results indicated that neither chromenylgluconyl octadecenoate nor quercetin activity was significantly different from the other's and that both displayed higher activity than BHT when the antioxidant concentration was higher than 0.4 mmol/L as shown in Figure (2), This finding is consistent with another findings[12].

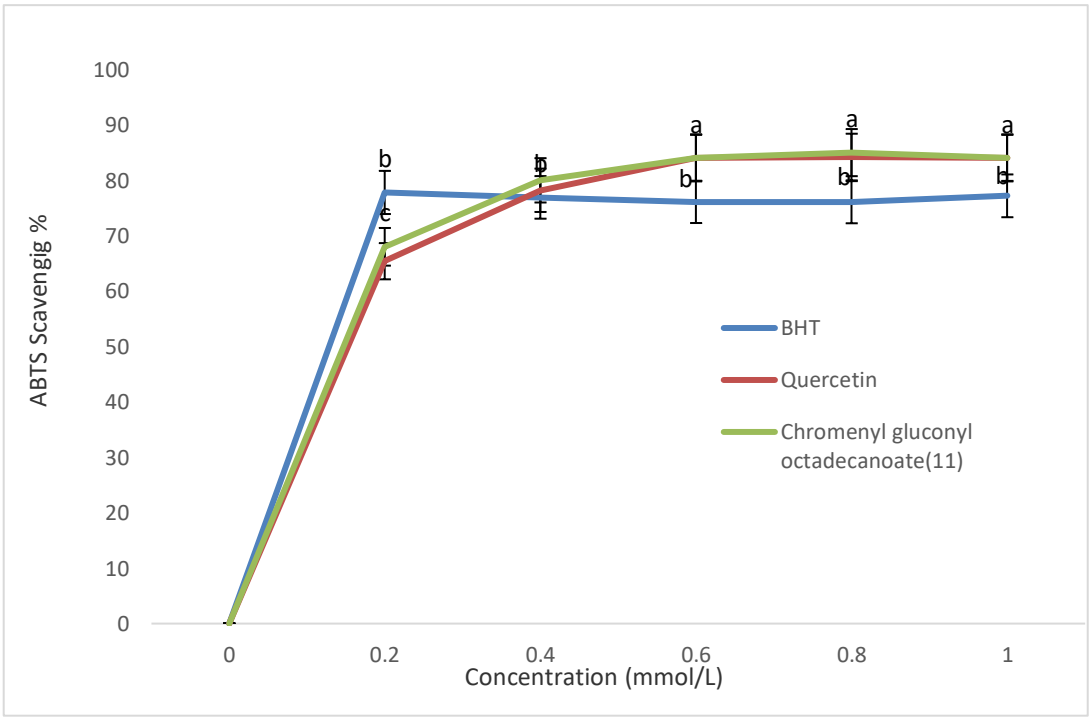


Fig. 2**:** ABTS free radical scavenging activity of different concentrations (0-1 mmol/L) of (Quercetin, Chromenylgluconyloctadecanoate and BHT: butyl hydroxyl toluene).
(Data are expressed as mean ± SD values given to represent the means of three determinations.)

3.6.3 Lipid oxidation in sunflower oil

The oxidation stability of refined sunflower oil was enhanced by the addition of chromenylgluconyloctadecanoate (**11)** (0.4 mmol/L) much more than that occurred by the addition of the same concentration of TBHQ (tert-butylhydroquinone). The induction period of pure refined sunflower oil was 5.32 h. and it was increased to 10.83 h. upon adding TBHQ (0.4mmol/L), while it reaches 19.88 h. by adding chromenylgluconyloctadecanoate **(11)** (0.4 mmol/L) as in figure (3).

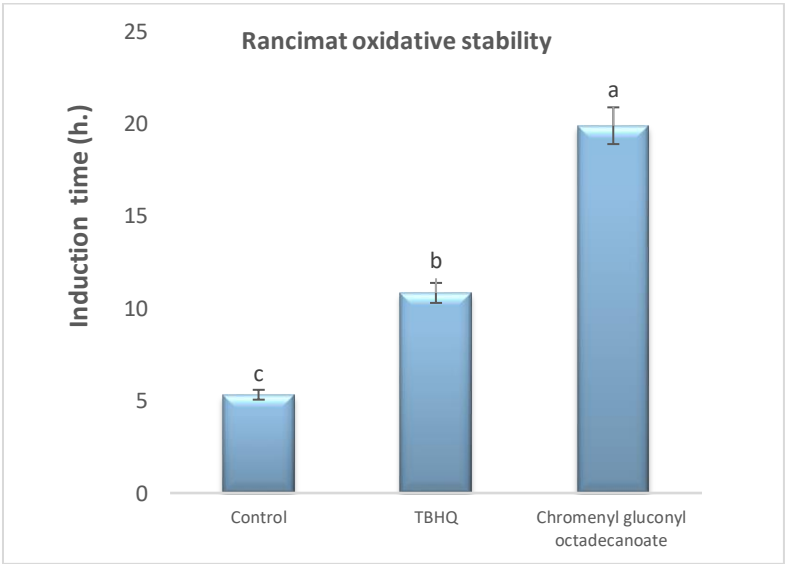


Fig. 3**:** Rancimat oxidative stability index at 110°C of sunflower oil with 0.4 mmol/L of different antioxidant treatments(Quercetin, Chromenylgluconyloctadecanoate and TBHQ: tert-Butylhydroquinone).
(Data are expressed as mean ± SD values given to represent the means of three determinations.)

3.7 Evaluation of the prepared compound as a surfactant

Due to the intermolecular van der Waals force, liquid molecules are attracted to gather or to other molecules in contact with them (solid or gas), thus it causes a decrease in the liquid surface area, which is accompanied by an increase in surface tension. When a surfactant molecule is added, it takes place between the surface film-forming the liquid molecules, through the formation of a new attraction force between the surfactant and the liquid molecules, and consequently, decreases the intermolecular attraction force between liquid molecules, so, the surface area increases and the surface tension decreases.

3.7.1 Oil–water interfacial tension γo/w

Measurement of the surface tension between oil and water must be performed using pure oil, as the presence of any polar moiety within the oil leads to a decrease in the surface tension as a result of the force of attraction between the polar and the water molecules[40].
So, the fatty acids composition and physicochemical characteristics of the purified sunflower oil are illustrated in Tables (4 &5).

Table (4): Fatty acid composition of the purified Sunflower Oil

Fatty acid	**Relative Amount (%)**
Palmitic acid ($C_{16:0}$)	7.3±0.03
Palmitoleic acid ($C_{16:1}$)	0.32±0.003
Stearic acid ($C_{18:0}$)	3.44±0.05
Oleic acid ($C_{18:1}$)	33.93±0.51
Lenoleic acid ($C_{18:2}$)	52.27±0.42
Lenolenic acid ($C_{18:3}$)	0.36±0.007
Arachidic acid ($C_{20:0}$)	0.33±0.005
Eicosenoic acid ($C_{20:1}$)	0.31±0.003
Behenic acid ($C_{22:0}$)	0.87±0.005
Lignoceric acid ($C_{24:0}$)	0.34±0.007
Others	0.53
Saturated fatty acid (%)	12.28
unsaturated fatty acid (%)	87.19

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

Table (5): Physicochemical properties of Sunflower oil.

Parameter	Sunflower oil	
	Before purification	After purification
Refractive index at 25°C	1.4665^{a}±0.01	1.4666^{a}±0.04
Acid value (as oleic acid)	0.64^{a}±0.01	0.01^{b}±0.002
Peroxide value (meq./kg oil)	3.37^{a}±0.03	3.35^{a}±0.02
Iodine value (Hanus)	136.4^{a}±1.22	136.3^{a}±1.31
Saponification number	201.037^{a}±2.95	193.8^{a}±1.94
Polar contents (%)	1.1^{a}±0.04	0.0^{b}±0.0

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

The newly prepared chromenylgluconyloctadecanoate was used as an emulsifier to reduce the interfacial tension between water and oil and was compared with the well-known surfactant soy lecithin. And there was a significant difference between them where chromenylgluconyloctadecanoate lowered the interfacial tension much more than the soy lecithin did as in Figure (4). This may be related to the high polarity of chromenylgluconyloctadecanoate, which makes it more easily attracted to water molecules[41].

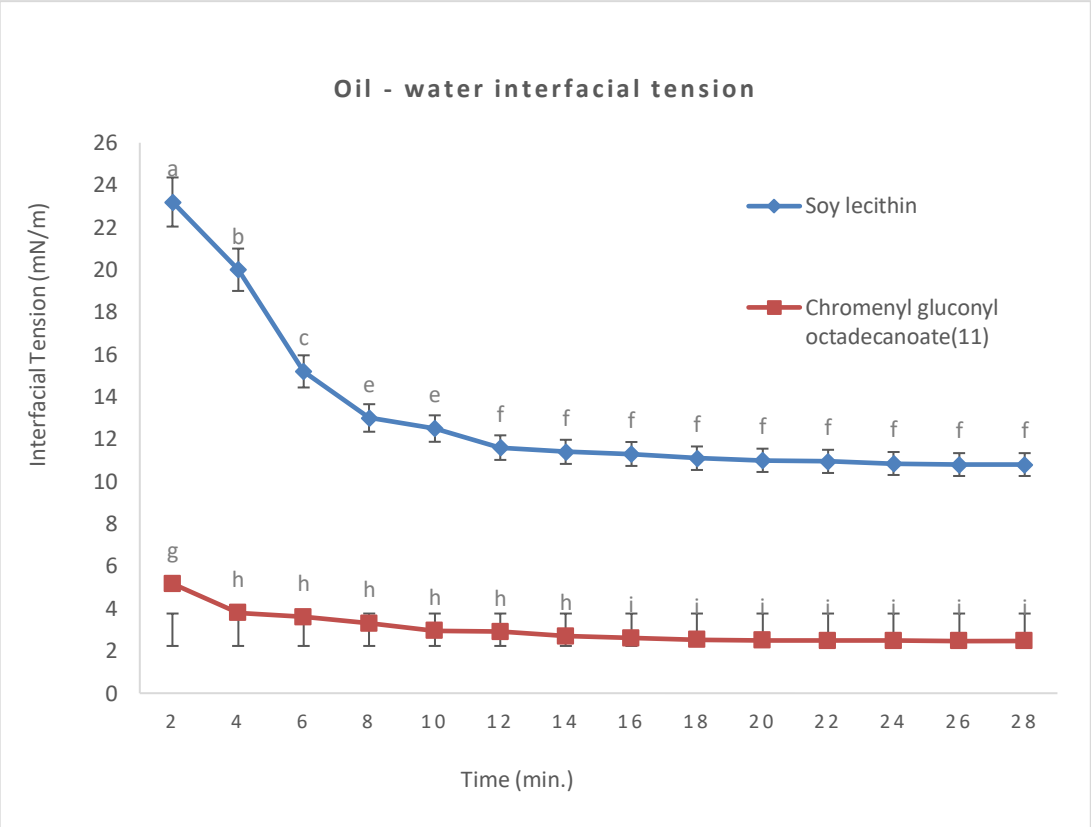


Fig.4. Interfacial tension decay profiles at 20 °C of 0.01 wt. % individual aqueous solutions of soy lecithin and chromenylgluconyloctadecanoate**(11)**.
Data are expressed as mean ± SD values given to represent the means of three determinations.

3.7.2 Determination of critical micelle concentration (CMC) by air-water surface tension method

Surfactant molecules still diffuse between the molecules of the water surface film till the saturation point, at which all intermolecular spaces of the water surface film are occupied by the surfactant molecules. Then, any more surfactant molecules will find no place to diffuse in, so they go to the bulk solution, forming colonies known as micelles.

The surfactant concentration at this saturation point is the critical micelle concentration (CMC), which is a characteristic of the surfactant in a given solvent at a given temperature. It appeared as a breakpoint in plots of surface tension versus surfactant concentration at a known temperature.

The effect of chromenylgluconyloctadecanoate as a surfactant on the air-water surface tension was studied and compared to that of soy lecithin as shown in Figure (5). The newly prepared chromenylgluconyloctadecanoate was found to significantly lower surface tension compared to lecithin.

This is likely due to the surfactant's larger molecular size, which allows for fewer molecules of the newly prepared compound to diffuse through the molecules that make up the water surface film and, as a result, lower critical micelle concentrations[42], where those were found to be (0.06mmol /L) and (0.1 mmol /L) due to chromenylgluconyloctadecanoate and lecithin respectively.

The results were significantly different. However, because it has more polar functions than soy lecithin, it is easier to diffuse between the molecules that make up the water surface film. This lowers the attraction force between them, increasing surface area and decreasing surface tension.

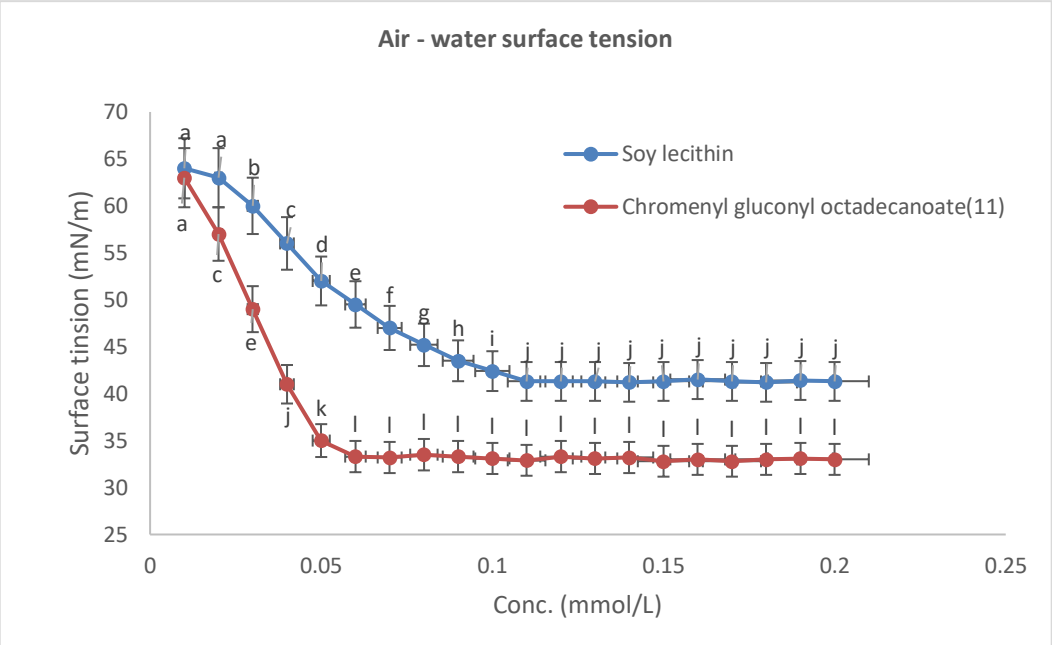


Fig. 5. Surface tension versus concentration plots at 25 °C of soy lecithin and chromenylgluconyloctadecanoate**(11)** solutions
Data are expressed as mean ± SD values given to represent the means of three determinations.

3.7.3 Emulsion stability

A stable dispersion of two or more immiscible liquids kept in suspension by an emulsifier is known as an emulsion. Its resistance to alterations in its physical characteristics over time is referred to as its stability[43,44]. Thus, the ability of the newly prepared chromenylgluconyloctadecanoate to stabilize the emulsion was compared with that of soy lecithin, and the results showed that the emulsion stability due to the presence of the newly prepared chromenylgluconyloctadecanoate was significantly ($P \leq 0.05$) higher than that related to soy lecithin up to 16 h., after that there was no significant difference up to 24 h. as illustrated in figure (6).

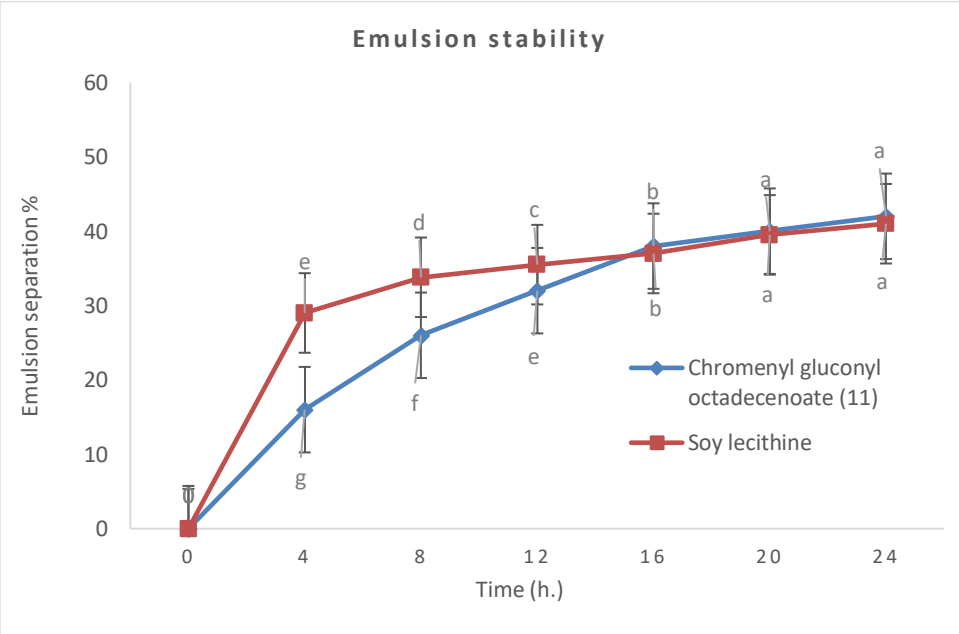


Fig. 6. w/o emulsion stability at 25 ◦C with 0.5% w/v of chromenylgluconyloctadecanoate**(11)** and soy lecithin
Data are expressed as mean ± SD values given to represent the means of three determinations.

3.8 Acute toxicity testand liver and kidney function tests:

The newly prepared chromenylgluconyloctadecanoate was examined for its safety through acute toxicity tests and liver as well as kidney function tests as illustrated in tables (6 & 7)

Table (6) Acute oral lethal toxicity of chromenylgluconyloctadecanoate

Groups	**Dose (mmol/kg)**	**No. of animals/group**	**No. of dead animals**
1	2	5	0
2	4	5	0
3	6	5	0
4	8	5	0
5	10	5	0

Table (7) Liver and kidney function tests of rats fed on chromenylgluconyloctadecanoateat different concentrations

Parameter Groups	ALT (U\L)	AST (U\L)	ALP (U\L)	Urea (mg\dL)	Creatinine (mg\dL)
Control	$20.83^{e}\pm 0.12$	0.21 ± 34.84^{d}	1.08 ± 137.22^{e}	0.32 ± 33.17^{f}	0.006 ± 0.74^{d}
3 mmol/Kg	0.13 ± 20.97^{d}	0.18 ± 34.96^{c}	0.91 ± 137.98^{d}	0.35 ± 33.54^{e}	0.006 ± 0.74^{d}
6 mmol/Kg	0.15 ± 20.99^{d}	0.20 ± 35.00^{c}	1.37 ± 138.87^{c}	0.24 ± 33.93^{d}	0.003 ± 0.75^{c}
9 mmol/Kg	0.13 ± 21.13^{c}	0.23 ± 35.35^{b}	0.98 ± 139.92^{b}	0.50 ± 34.32^{c}	0.004 ± 0.75^{c}
12 mmol/Kg	0.17 ± 21.33^{b}	0.22 ± 35.72^{b}	1.21 ± 140.89^{a}	0.22 ± 34.78^{b}	0.005 ± 0.76^{b}
15 mmol/Kg	0.15 ± 21.59^{a}	0.23 ± 36.02^{a}	1.05 ± 141.79^{a}	0.31 ± 35.05^{a}	0.006 ± 0.77^{a}

(Data are expressed as mean ± SD values given to represent the means of three determinations)
For each column, the common averages in the same letter have no significant differences between them

Table (7) shows that various concentrations of chromenylgluconyloctadecanoate caused statistically significant differences in ALT, AST, ALP, and urea enzymes. However, creatinine enzyme levels did not differ significantly. Despite their significant differences, these enzymes are still within the safe range.

3.9 Uses of Chromenylgluconyloctadecanoate (11) as an antioxidant emulsifier for the production of blended fat spread

The hydrophilic-lipophilic balance (HLB) is a measure of a compound's degree of hydrophilicity or lipophilicity. This value provides information about the surfactant's characteristics. The HLB value of Chromenylgluconyloctadecanoate was determined to be 8.3 using the Griffin equation, indicating its suitability as a water-in-oil emulsifier. Additionally, with an HLB value falling within the range of 7-9, it can also be utilized as a spreading agent[33]. This makes it a promising emulsifier for the production of blended fat spread.

3.9.1 Fatty acid profiles, quality parameters and oxidation stability of anhydrous milk fat, sunflower oil, palm kernel oil and blended fats spread

Blended fats are primarily created to lower costs, reduce fat intake, and decrease levels of saturated fatty acids, while still maintaining quality. Thus, when the new blended fat spread was produced, the percentage of saturated and unsaturated fatty acids changed from 71.46% and 27.66 % in the anhydrous milk fat to 43.5% and 55.94% in the produced blended fat spread, respectively. As a result, the produced blended fat spread is a healthier product[45]. The sample contains seventeen different fatty acids, with the most dominant unsaturated fatty acid being linoleic acid (32.39 %) followed by oleic acid (21.9 %), The most dominant saturated fatty acids were palmitic acid (14.9 %) followed by lauric acid (12.49 %), myristic acid (6.35 %) and stearic acid (5.15 %) as illustrated in table (8).

Table (8): Fatty acid composition of anhydrous milk fat, sunflower oil, palm kernel oil and blended fat spread

	Fatty acid	**Anhydrous milk fat**	**Sunflower oil**	**Palm kernel oil**	**Blended fat spread**
Saturated fatty acids	Butyric acid($C_{4:0}$)	$3.60^{a}\pm0.04$	-----	-----	$0.9^{b}\pm0.004$
	Caproic acid($C_{6:0}$)	$1.64^{a}\pm0.02$	-----	$0.29^{b}\pm0.003$	$0.48^{b}\pm0.002$
	Caprylic acid($C_{8:0}$)	$0.82^{b}\pm0.04$	-----	$3.81^{a}\pm0.02$	$1.16^{b}\pm0.007$
	Capric acid($C_{10:0}$)	$1.83^{b}\pm0.01$	-----	$3.64^{a}\pm0.04$	$1.36^{b}\pm0.018$
	Lauric acid($C_{12:0}$)	$2.85^{c}\pm0.04$	-----	$47.12^{a}\pm0.1$	$12.49^{b}\pm0.13$

	Myristic acid($C_{14:0}$)	10.20^{b}±0.05	0.03^{d}±0.002	15.21^{a}±0.06	6.35^{c}±0.05
	Palmitic acid ($C_{16:0}$)	38.90^{a}±0.2	6.61^{d}±0.03	7.71^{d}±0.02	14.9^{b}±0.08
	Stearic acid($C_{18:0}$)	11.62^{a}±0.05	3.09^{c}±0.05	2.80^{c}±0.002	5.15^{b}±0.08
	Arachidic acid ($C_{20:0}$)	----	0.31^{a}±0.004	-----	0.15^{a}±0.006
	Behenic acid($C_{22:0}$)	-----	0.82^{a}±0.006	-----	0.41^{a}±0.007
	Lignoceric acid ($C_{24:0}$)	-----	0.31^{a}±0.005	-----	0.15^{a}±0.005
Total saturated		71.46^{b}	11.17^{d}	80.58^{a}	43.50^{c}
Mono-unsaturated fatty acids	Myristolic acid($C_{14:1}$)	1.11^{a}±0. 02	-----	-----	0.28^{b}±0.007
	Palmitoleic acid ($C_{16:1}$)	1.41^{a}±0.01	0.34^{b}±0.002	-----	0.52^{b}±0.02
	Oleic acid($C_{18:1}$)	22.82^{b}±0.08	25.05^{a}±0.34	15.01^{c}±0.02	21.9^{b}±0.12
	Eicosenoic acid ($C_{20:1}$)	-----	0.28^{a}±0.004	-----	0.57^{a}±0.002
Polyunsaturated fatty acids	Lenoleic acid ($C_{18:2}$)	1.80^{c}±0.05	62.08^{a}±0.22	3.61^{c}±0.003	32.39^{b}±0.15
	Lenolenic acid ($C_{18:3}$)	0.52^{a}±0.02	0.31^{a}±0.005	-----	0.28^{a}±0.007
Total unsaturated		27.66^{c}	88.06^{a}	18.62^{d}	55.94^{b}
	Others	0.88	0.77	0.80	0.56

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

The chemical characteristics of anhydrous milk fat, sunflower oil, palm kernel oil, and the blended fat spread produced in this study are all within the permissible values for human consumption according to the Egyptian Standard specifications (2005) for edible fats and oils[46].

The measured parameters of the blended fat produced fell within the range of those found in anhydrous milkfat, palm kernel oil, and sunflower oil, as shown in Table (9) as this product is a physical mixture of these ingredients. Based on the refractive index (1.4619), acid value (0.71 mg KOH/g oil), and peroxide value (0.29 mEq/Kg oil) of the fat spread, it is most likely that rancidity will develop more quickly than it will in anhydrous milk fat and palm kernel oil but less quickly than in sunflower oil. The fact that every sample was chilled right away following preparation forms the basis of this evaluation. Conversely, the blended fat spread made with chromenylgluconyloctadecanoate was compared to a sample supplemented with soy lecithin, anhydrous milk fat, sunflower oil, and palm kernel oil in terms of their rancimat oxidative stability. The purpose of this comparison was to obtain a comprehensive understanding of the resistance against chemical deterioration over extended periods of storage. The oxidative stability of the blended fat spread was significantly enhanced by the addition of chromenylgluconyloctadecanoate, as demonstrated in Figure (7).

Table (9): Physicochemical properties of anhydrous milk fat, sunflower oil, palm kernel oil andblended fat spread (all samples were refrigerated immediately after preparation)

Parameter	**Anhydrous milk fat**	**Sunflower oil**	**Palm kernel oil**	**Blended fat spread**
Refractive index at 25ºC	1.4562^{b}±0.021	1.4739^{a}±0.003	1.412^{c}±0.011	1.4619^{b}±0.007
Acid value (mg KOH/g oil)	0.20^{c}±0.007	0.74^{b}±0.009	1.21^{a}±0.021	0.71^{b}±0.021
Peroxide value (mEq/Kg oil)	0.39^{b}±0.020	0.23^{d}±0.021	0.44^{a}±0.062	0.29^{c}±0.016

Iodine value (g I_2/ 100g oil)	33.42^{c}±0.011	133.31^{a}±1.22	16.11^{d}±0.33	100.76^{b}±1.62
Saponification number (mg KOH/g oil)	227.53^{a}±2.021	188.24^{c}±3.054	208.03^{b}±2.43	197.11^{c}±1.34
Polar contents (%)	0.00	0.00	0.00	0.00

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

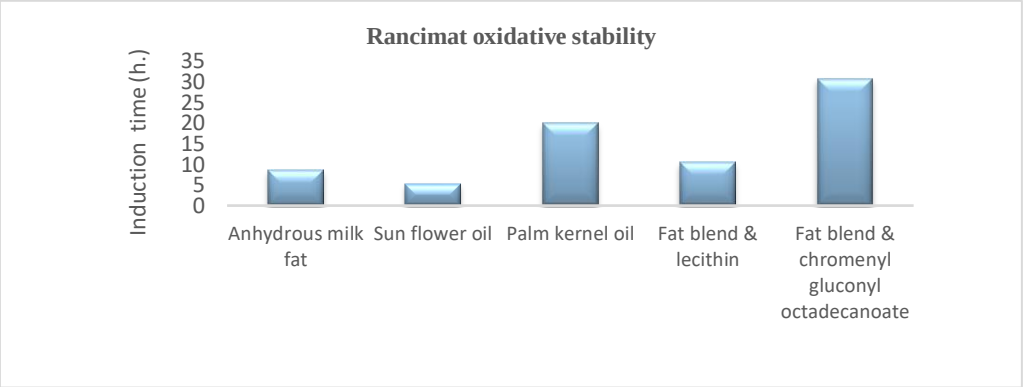


Fig. 7**:** Rancimat oxidative stability index at 110°C of anhydrous milk fat, sunflower oil, and palm kernel oil, blended fat spread supplemented with soy lecithin and blended fat spread supplemented with chromenylgluconyloctadecanoate (Data are expressed as mean ± SD values given to represent the means of three determinations.)

3.9.2 Colour Analysis

Food colour is usually an important indicator of food quality. *L** (lightness; 100 = white, 0 = black), *a** (a positive number indicates red and a negative number indicates green), and *b** (a positive number indicates yellow and a negative number indicates blue) are used for the determination of fat spread colour. The L* of any suspension is related to the number and size of suspended particles. The surface of a suspended drop refracts the incident light rays in many directions, and the interference between the refracted rays causes the white colour to form. Thus, the greater the surface areas due to the number and/or size of suspended droplets, the greater the whiteness of the suspension and vice versa. Colour measurements of two prepared blended fat spread samples are illustrated in Table (10). The L* value of the prepared sample which was supplemented with chromenylgluconyloctadecanoatewas higher than that of the control sample (supplemented with soy lecithin) and showed a statistically significant difference. ($P \leq 0.05$). This is caused by the larger refractive surface area due to the larger number and/or size of the suspended droplets, which means the higher efficiency of chromenylgluconyloctadecanoate to suspend water droplets in the oil. Moreover, there were non-significant differences ($P > 0.05$) between the a* values of the two prepared samples. While the b* values showed significant differences ($P \leq 0.05$) between the two prepared samples and this may be related to the yellow colour of chromenylgluconyloctadecanoate.

Table (10): Colour measurements of the preparedBlended fat spread

	L*	a*	b*
Blended fat spread with Chromenylgluconyloctadecanoate (11)	93.12^{a}±0.84	5.77^{a}±0.28	3.12^{a}±0.11
Blende fat spread with soy lecithin	81.54^{b}±1.20	5.65^{a}±0.31	2.34^{b}±0.07

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

3.9.3 Textural evaluation

There is a strong correlation between the texture profile of food and how it feels in our mouths. The intermolecular forces that exist between molecules in the dispersed phase and between those molecules and the molecules in the continuous phase are closely related to this profile in emulsions.

Thus, the hardness, spreadability, cohesiveness, and adhesiveness of the prepared blended fat spread were evaluated and compared to a control sample. Hardness is measured as the maximum positive peak in the time force curve and is defined as the maximal force needed to compress food between the tongue and palate to a specific deformation or penetration.

The generated blended fat spread supplemented with Chromenylgluconyloctadecanoate as an emulsifier had considerably ($P \leq 0.05$) higher hardness at 5°C and 22°C than the control sample. This is indicated in Table 11. This has to do with how the newly manufactured emulsifier causes oil droplets to agglomerate. Spread ability, which is shown as the area of the maximum positive peak in the time force curve, is closely correlated with hardness. The force needed to spread the blended fat spread that was made with Chromenylgluconyloctadecanoate at 5°C and 22°C is shown in Table 11; the difference between the sample and the control was not statistically significant ($P > 0.05$). Adhesiveness is measured as the negative peak area and is defined as the effort needed to remove food that sticks to the mouth during normal swallowing. Similar to Table 11, the produced blended fat spread containing chromenylgluconyloctadecanoate had a significantly ($P \leq 0.05$) lower adhesiveness than the control sample. Furthermore, cohesiveness is defined as the minimum negative peak and denotes the strength of the internal bonds that comprise the food's body. When chromenylgluconyloctadecanoate was added to the prepared blended fat spread, its cohesiveness increased significantly ($P \leq 0.05$) compared to the control sample. This is because the molecules are bound together more tightly as a result of increased intermolecular attraction forces, which results in the bulk property of liquid resisting separation[47,48]. The term "viscosity" describes a fluid's resistance to flowing, which is impacted by forces between molecules. Higher viscosity results from stronger forces. Table 11 demonstrates that the blended fat spread supplemented with chromenylgluconyloctadecanoate had a viscosity that was significantly ($P \leq 0.05$) higher than the control sample. This is because stronger intermolecular forces were produced by the addition of the newly prepared emulsifier.

Table (11): Texture profile analysis of the prepared blended fat spread.

	Hardness(N)		Spread ability (N.S)		Cohesiveness (N) at 5°C	Adhesiveness (N.S) at 5°C	Viscosity (Pa s)
	5°C	25°C	5°C	25°C			
Blended fat spread with Chromenylgluconyloctadecanoate (11)	57.36 ^{a} ±1.21	3.64 ^{a} ±0.32	332.54 ^{a} ±1.22	42.66 ^{a} ±1.41	0.408 ^{a} ±0.020	8.70 ^{b} ±0.35	282 ^{a} ±4.23
Blende fat spread with lecithin	44.52 ^{b} ±1.30	2.58 ^{b} ±0.20	326.77 ^{a} ±1.20	39.62 ^{a} ±1.322	0.279 ^{b} ±0.018	9,22 ^{a} ±0.28	246 ^{b} ±4.92

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

3.9.4 Solid fat content (SFC)

The percentage of fat that is solid at a particular temperature is known as the solid fat content. This measurement is a crucial determinant of the texture, appearance, flavour, melting rate, and shelf life of plastic fats. and fat products' stability[49].According to Figure (8), the manufactured blended fat spread supplemented with chromenylgluconyloctadecanoate had SFC values of 19.6% at 20 °C, which is more than 10% and hence avoids oiling off [50].

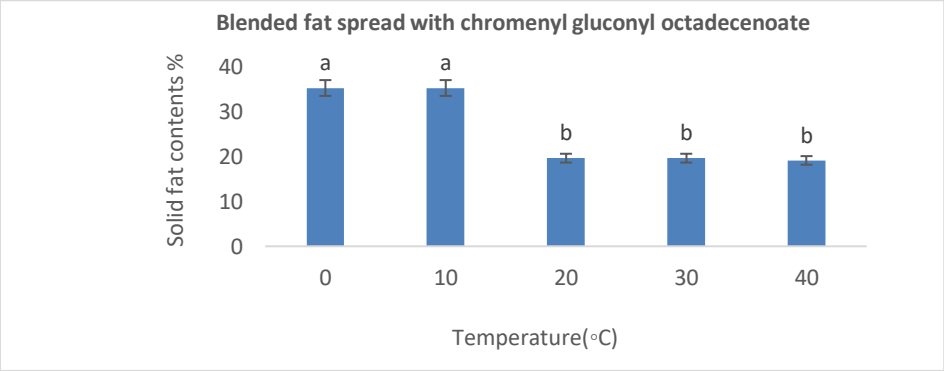


Fig (8): Solid fat contents (%) of the prepared blended fat spread
Data are expressed as mean ± SD values given to represent the means of three determinations.

3.10 Sensory evaluation

A sensory analysis of a commercial blended fat spread and a blended fat spread that had been supplemented with soy lecithin and chromenylgluconyloctadecanoate revealed that the properties of the blended fat spread had improved when chromenylgluconyloctadecanoate was used as an emulsifier during production, as illustrated in Table 12.

Table (12): Sensory evaluation of the prepared blended fat spread supplemented with Chromenylgluconyloctadecanoate, soy lecithin and a commercial one

	Blended fat spread supplemented with Chromenylgluconyloctadecanoate(11)	Blended fat spread supplemented with lecithin	Commercial blended fat spread
Colour	9.0±0.33^{b}	8.9±0.430^{b}	9.3±0.370^{a}
Appearance	8.9±0.31^{a}	8.6±0.25^{b}	9.0±0.21^{a}
Odour	9.4±.10^{a}	9.4±0.16^{a}	8.7±.32^{b}
Spreadability	9.5±0.30^{a}	8.0±0.23^{b}	7.2±0.27^{c}
Texture	8.9±0.28^{a}	8.2±0.27^{c}	8.4±0.31^{b}
Flavour	9.3±0.35^{a}	9.2±0.40^{a}	8.6±0.24^{b}
Overall acceptability	9.1	8.7	8.5

4. Conclusion

The emulsification activity of the newly prepared Chromenylgluconyloctadecanoate (11) was found to be significantly higher than that of soy lecithin. This could be attributed to the non-cyclic structure of the hydrophilic moiety, which reduces restrictions on the OH functions, making it easier for hydrogen bonds to form. Ultimately, this allows for polar molecules to attach more easily. Furthermore, due to the presence of the flavonoid moiety, there was a significantly higher difference in antioxidant activity compared to TBHQ, and no significant difference compared to BHT. The combination of two naturally occurring compounds and the results of an acute toxicity test have shown promise for the use of this newly synthesised antioxidant emulsifier as a food additive. As an example, this antioxidant emulsifier was used to prepare a blended fat spread (35% H_2O), and it significantly improved its properties.

5. Conflicts of interest

The authors declare no competing interests.

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