Synthesis and Application of a New Amphiphilic Antioxidant

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Abstract: A new amphiphilic antioxidant (tannyl stearate) derived from reaction of tannic acid with stearic acid was synthesized in order to improve tannic acid solubility in lipid materials. This reaction gives many products having different degree of esterification (tannyl mono, di, tri, tetra, penta, hexa, hepta…… stearate) which were separated using silica gel column chromatography and tentative identification was carried out using thin layer chromatography (TLC). The intrinsic viscosities (\(\eta\)) were used to differentiate between the different molecular weight of the produced esters. Tannyl penta stearate is assumed to be the most suitable amphiphilic antioxidant derivative, where those derivatives with less degree of esterification would be less soluble in fat, and those of higher degree of esterification would exhaust more hydroxyl group that cause decreases of antioxidant activity. The structure of tannyl penta stearate was approved depending on its chemical analysis and spectral data (IR, \(\text{H}^1\text{NMR}\)). The emulsification power of tannyl penta stearate was then determined according to method described by El-Sukkary et al., in order to prove its amphiphilic property. Then tannyl penta stearate was tested for its antioxidant and radical scavenging activities in three different manners, those are, lipid oxidation in sunflower oil using Rancimat, (DPPH) free radical scavenging and total antioxidant activity. {Pure tannic acid (T), butylhydroxyanisol (BHA) and butylhydroxytoluene (BHT) were used as reference antioxidant radical saving compounds}. Then tannyl penta stearate was added to sunflower oil, frying process was carried out and all physicochemical parameters of the oil were considered, and compared to other reference antioxidant in order to study the effect of this new antioxidant toward oil stability. Acute oral toxicity of the tannyl penta stearate was carried out using albino mice of 21–25 g body weight to determine its safety according to the method described by Goodman et al. Also liver and kidney functions of those mice were checked. Thus it could be concluded that the addition of tannyl penta stearate to frying oils offers a good protection against oxidation. The effectiveness of tannyl penta stearate as lipid antioxidant has been attributed mainly to its stability at high temperature. And according to acute lethal toxicity test tannyl penta stearate was found to be a safe compound that can be used as food additive.

Key words: tannic acid, stearic acid, antioxidant, DPPH, Rancimat, polymer content

1 INTRODUCTION

Tannic acid is a natural product that can be used as dietary supplement, food additive and flavoring agent, it considered to be safe for the human uses. It is included in the European Union list of food flavorings, and the use of tannic acid up to the proposed maximum level of 15 mg/kg complete feed is safe for all animals. Thus the newly synthesized antioxidant is assumed to be safe for human usage.

Antioxidant is a compound that has ability to inhibit the oxidation of other substance by capturing electrons or hydrogen. Usually, oxidation reaction produces free radicals, which consequently cause chain reactions. Antioxidants can capture the produced free radicals, thus, they could inhibit the chain reactions, and retard other oxidation processes. It means that: antioxidants are going to oxidize themselves, so they are generally reducing agents.

It’s well known that, antioxidants compounds can highly protect human body, through preventing radicals formation, which causes damage to DNA, lipids, protein and other biomolecules, so they can hinder the proceed of many diseases as well as lipid oxidation. Thus they are
widely used in dietary supplements and diseases preventing.

Biologically antioxidants were used in prohibiting the oxidation of unsaturated fats, which is the main factor for rancidity, and as dietary supplements. Usually, the used antioxidants are BHA (butylated hydroxy anisole), BHT (butylated hydroxy toluene), propyl gallate (PG) and tert-butylhydroquinone (TBHQ). But recently, those antioxidants were questioned due to their toxicity.

Tannic acid, a natural plant antioxidant, is a glucose derivative at which hydroxyl groups are substituted with galloyl residues (as show in Fig. 1). Thus it can be considered as a polyphenolic compound, and consequently, it has high ability to capture the free radical through both of its hydroxyl groups and its benzene rings.

Similar to all polyphenols, tannic acid possess antioxidant activity, but its reaction mechanism is still incompletely studied, and still needed for a supplementary investigation.

2 MATERIALS AND METHODS

2.1 INSTRUMENTS

Melting point was measured by a Gallenkamp electro-thermal melting point apparatus. The infrared spectrum was recorded for potassium bromide on a Pye Unicam Sp 3-300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. The NMR spectrum was recorded on a Varian VX-300 NMR spectrometer. "H spectrum was run at 300 MHz. Chemical shifts are quoted in δ and were related to that of the solvents. The mass spectra were recorded on a Shimadzu GCMS-QP-1000EX. Elemental analyses were carried out at the Micro-analytical Center of Cairo University.

The intrinsic viscosities (η) of the prepared compounds were measured using a capillary viscometer.

The oil oxidative stability was estimated by measuring the oxidation induction time, on a Rancimat apparatus (Metrohm CH series 679). Air (20 L/h) was bubbled through the oil (5.0 g) heated at 100 ± 2°C, with the volatile compounds being collected in water, and the increasing water conductivity was continually measured. The time taken to reach the conductivity inflection was recorded.

2.2 Materials

Commercially tannic acid was purchased from El-Gomhoria Trade Pharmaceuticals & Chemicals Company, Cairo, Egypt. BHA (butyl-hydroxy anisole), BHT (butylhydroxytoluene), TBHQ (tert.butylhydroquinine), L-ascorbic acid and linoleic acid were purchased from Merck. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), sodium phosphate buffer, tween 20 and absolute ethanol, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphates (ALP), creatinine and urea kites were purchased from sigma (sigma-aldrich GmbH, sternheim, Germany). All other chemicals used were of analytical grade and were obtained from merck and sigma (sigma-aldrich GmbH, sternheim, Germany). Animals were obtained from animal house – National research centre.

2.3 METHODS

2.3.1 Synthesis of Tannyl stearate

Tannic acid (1,2,3,4,6-Pentakis-O-|3,4-dihydroxy-5-[[(3,4,5-trihydroxybenzoyl)oxy]benzoyl|β-D-glucopyranose.

Fig. 1 Tannic acid or 1,2,3,4,6-Pentakis-O-[3,4-dihydroxy-5-[(3,4,5-trihydroxybenzoyl)oxy]benzoyl|β-D-glucopyranose.
TLC.

2.3.3 TLC detection

Silica gel plates were activated at 120°C for 1 h before used. A few microliters of each fraction collected from column chromatography were loaded to the marked points about 10 mm from the bottom of silica plate. The plates were developed in petroleum ether: diethyl ether: acetic acid (80:90:1, v/v/v) at room temperature, and the separated spots were visualized by iodine fume. Samples of the same rate of flow (Rf value) were collected together in one category.

2.3.4 Viscometric measurements

Each category was dried individually under vacuum, then the intrinsic viscosities (η) were measured for each category in distilled water at 25°C using a capillary viscometer at surfactant concentrations in the range 0.005–5.0 g L⁻¹.

The molecular weights (Mw) were calculated as described by R.X. Yan using equation: η = 3.38 × 10⁻³ M⁻¹.

2.3.5 Elemental analysis and spectral data of tannyl stearates

Structures of tannyl stearates were confirmed according to their elemental analysis and spectral data that are described below.

Tannyl mono stearates

\[ \text{Yield (2.66 g, 2.1%), } ^1\text{H NMR (CDCl}_3) : \delta 0.89 (t, J = 7.1 \text{ Hz, 3H}), 1.25 (m, 26H), 1.34 (m, 2H), 1.55 (m, 2H), 2.22 (t, J = 7.1 Hz, 2H), 4.34 (dd, 2H), 4.64 (q, J = 9.9 Hz, 1H), 4.80 (q, J = 9.8 Hz, 1H), 5.1 (s, 20H), 5.23 (q, J = 10.0 Hz, 2H), 6.62 (q, J = 10.1 Hz, 1H), 7.1 (s, 4H), 7.2 (dd, J = 9.4 Hz, 5H), 7.25 (dd, J = 8.9 Hz, 8H), 7.36 (s, 3H). \]

\[ ^{13}\text{C NMR (DMSO – d}_6) : 51.4, 22.6, 25.6, 29.6, 35.5, 66.3, 71.2, 97.2, 110.2, 114.8, 115.5, 125.0, 131.0, 135.0, 141.0, 146.7, 153.7, 166.7, 173.3. \]

For C₁₈H₃₀O₆₇, Calcd: C, 65.73; H, 7.38%. Found: C, 66.01; H, 7.17%.

Tannyl di stearates

\[ \text{Yield (3.46 g, 3.1%), } ^1\text{H NMR (CDCl}_3) : \delta 0.91 (t, J = 6.9 \text{ Hz, 6H}), 1.26 (m, 52H), 1.36 (m, 4H), 1.57 (m, 4H), 2.25 (t, J = 7.0 Hz, 4H), 4.36 (dd, 2H), 4.66 (q, J = 10.1 Hz, 1H), 4.81 (q, J = 9.8 Hz, 1H), 4.96 (s, 20H), 5.23 (q, J = 10.0 Hz, 2H), 6.64 (q, J = 10.1 Hz, 1H), 6.98 (s, 4H), 7.19 (dd, J = 9.3 Hz, 5H), 7.27 (dd, J = 8.8 Hz, 8H), 7.35 (s, 3H). \]

\[ ^{13}\text{C NMR (DMSO – d}_6) : 51.4, 23.2, 25.0, 30.1, 33.3, 65.7, 70.2, 97.7, 109.8, 113.8, 115.9, 125.0, 131.0, 134.9, 142.2, 145.8, 155.0, 166.0, 174.2. \]

For C₁₈H₃₀O₆₇, Calcd: C, 60.21; H, 5.41%. Found: C, 60.40; H, 5.33%.

Tannyl tri stearates

\[ \text{Yield (6.5 g, 5.2%), } ^1\text{H NMR (CDCl}_3) : \delta 0.89 (t, J = 6.9 \text{ Hz, 9H}), 1.26 (m, 78H), 1.34 (m, 6H), 1.58 (m, 6H), 2.25 (t, J = 7.2 Hz, 6H), 4.34 (dd, 2H), 4.67 (q, J = 10.0 Hz, 1H), 4.79 (q, J = 10.0 Hz, 1H), 5.11 (s, 30H), 5.25 (q, J = 10.0 Hz, 2H), 6.64 (q, J = 9.9 Hz, 1H), 7.2 (s, 4H), 7.16 (dd, J = 9.3 Hz, 5H), 7.26 (dd, J = 8.8 Hz, 8H), 7.36 (s, 3H). \]

\[ ^{13}\text{C NMR (DMSO – d}_6) : 51.4, 23.2, 24.8, 29.6, 33.6, 67.0, 71.0, 97.5, 110.1, 114.0, 115.5, 124.2, 130.3, 135.1, 141.5, 146.2, 155.4, 167.4, 174.5. \]

For \( \text{C}_{138}\text{H}_{260}\text{O}_{49} \), Calcd: C, 62.44; H, 6.21%. Found: C, 62.38; H, 6.33%.

2.3.6 Emulsification power of tannyl penta stearate

Tannyl penta stearate 10 mL (0.1 wt%) was placed in a 100 mL cylinder and then 10 mL of the paraffin oil was added. The cylinder was shaken vigorously for 10 min and then allowed to settle. The time required to separate 9 mL of pure surfactant solution was recorded (average of three
2.3.7 Lipid oxidation in sunflower oil

Rancimat 673 (Metrohm Co., Herisau, Switzerland), was used to determine the oxidative stability of sunflower oil free from any antioxidant addition, sunflower oil with TBHQ (200 ppm) and sunflower oil with tannyl penta stearate (200 ppm) according to Rancimat method Gutierrez37. Sample of tannyl penta stearate (200 ppm) and TBHQ (200 ppm) were added individually to the sunflower oil (without any external additives) and compared to a third oil sample free from any antioxidant. A stream of air at a flow rate of 10 ± 0.2 L/h was bubbled through the oil at 110 ± 0.2°C. The volatile degradation products were trapped in distilled water in a second vessel, causing an increase of water conductivity. The IP was measured as the intersection of the tangent lines using the software provided with the instrument.

2.3.8 DPPH Free radical scavenging activity

The hydrogen donating capacity of the tannyl penta stearate was quantified in terms of its ability to scavenge the stable free radical, DPPH (1.1-Diphenyl-2-picrylhydrazyl), and it was compared to that of tannic acid, BHA and BHT. DPPH radical scavenging activity of the antioxidant compounds was measured according to the method of Blois38. Briefly, 1 mL of variable concentrations of each series (10, 20, 30, 40 μg/mL EtOH) was added to 1 mL of a DPPH solution (0.2 mM in ethanol) as the free radical source and kept for 30 min at room temperature. The decrease in the solution absorbance, due to proton donating activity of the added antioxidant was measured at 517 nm using portable hyper-spectrometer (Spectronic 21D, Milton roy boulder, Colorado, USA). L-Ascorbic acid was used as blank. The percentage of DPPH radical scavenging activity was calculated using the following formula:

\[ \text{DPPH radical scavenging activity(\%)} = \left( \frac{A_0 - A_i}{A_0} \right) \times 100 \]

Where \( A_0 \) was the absorbance of the blank and \( A_i \) was the absorbance of the sample or standard.

2.3.9 Uses of ferric thiocyanate method (FTC) for determination of total antioxidant activity

The antioxidant activities of tannyl penta stearate and standards (Tannic acid, BHA and BHT) were determined according to the ferric thiocyanate method39 as described by Gulcin40. For stock solutions, 10 mg of tannyl penta stearate was dissolved in 10 mL distilled water. Then, the solution was diluted to 15 μg/mL concentration of tannyl penta stearate in 2.5 mL of sodium phosphate buffer (0.04 M, pH 7.0), then it was added to 2.5 mL of linoleic acid emulsion in sodium phosphate buffer (0.04 M, pH 7.0). Therefore, 5 mL of the linoleic acid emulsion was prepared by mixing and homogenizing 15.5 μL of linoleic acid, 17.5 mg/g of tween-20 as emulsifier, and 5 mL phosphate buffer (pH 7.0). (In this step reaction could be proceed without emulsifier, but it was added in order to get the same experimental condition for all samples, as it was compared to tannic acid, BHA and BHT).

On the other hand, the control (5 mL) was composed of 2.5 mL of linoleic acid, emulsion and 2.5 mL 0.04 M sodium phosphate buffer (pH 7.0). The mixed solution (5 mL) was incubated at 37°C. The peroxide level was determined by reading the absorbance at 500 nm using portable hyper-spectrometer (Spectronic 21D, Milton roy boulder, Colorado, USA) after reaction with FeCl₃ (3.5%) and thiocyanate (30%) at intervals during incubation. During the linoleic acid peroxidation, peroxides are formed and that leads to oxidation of Fe³⁺ to Fe⁴⁺. The latter ions form a complex with ammonium thiocyanate and this complex has a maximum absorbance at 500 nm. This step was repeated every 10 h. High absorbance indicates high linoleic acid emulsion peroxidation. The solutions without antioxidant were used as blank samples. Total antioxidant activity determination was performed triplicate. The inhibition percentage of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

\[ \text{Inhibition of Lipid peroxidation(\%)} = \left( \frac{(A_0 - A_i)/A_0} {A_0} \right) \times 100 \]

Where \( A_0 \) was the absorbance of the blank which contains only linoleic acid emulsion and sodium phosphate buffer, and \( A_i \) was the absorbance of the sample or standard.

All analyses were performed in triplicate. The data were recorded as mean values.

2.3.10 Physicochemical properties of frying oil

Acidity, peroxide value, iodine value, and oxidized fatty acid content were determined according to the method described by A. O. A. C.41. Polar components in oil was measured by column chromatography according to the method described by Wallking and Wessels42. Polymer content was analyzed according to the method of Peled et al.43.

2.3.11 Acute toxicity test

Albino mice of 22-25 g weight were housed individually under standard conditions (12 h light/dark cycle; 25 ± 3°C temperature; 35–60 relative humidity). Thirty animals were divided into six groups (5 animals/group), one group was used as control and were fed on standard mice feed only, and the others were given a mixture of oral doses of tannyl penta stearate with different concentrations and standard mice feed according to Goodman et al.44.

2.3.12 Liver and kidney function tests

Two weeks after the feeding experiment, liver and kidney function of those Albino mice were tested in order to improve the safety of tannyl penta stearate as a food additive.

Thus alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphates (ALP), creatinine and urea were determined spectrophotometrically according to Young, D.S45 and Burtis et al.46.

Statistical significance between the control and experimental data were subjected to one way analysis of variance
3 Results and Discussion

3.1 Structure

Treatment of tannic acid (Fig. 1) with stearic acid in diethyl ether/ethanol and in the presence of dehydrating agent, afforded a series of the corresponding esters of different degree of esterification (tannyl mono, di, tri, tetra, penta, hexa, hepta······stearate) via water elimination.

3.2 Silica gel low-pressure column chromatography

Those esters were separated from each other by means of silica gel column chromatography according to their molecular weight. Where the individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute one at a time. During the entire chromatography process the eluent is collected in a series of fractions.

3.3 TLC Detection

The mobile phase could draw up the components via capillary action. When different analytes ascend the TLC plate at different rates, separation is achieved. Tentative identification for each fraction was carried out with TLC where each compound has its own RF value (Table 1). The spots were visualized by iodine fume.

3.4 Viscometric measurements:

All of the isolated structures were confirmed according to their intrinsic viscosities which are correlated to their molecular weight and according to the equation \( \eta = 3.38 \times 10^{-5} M^{0.43} \) which was described by R.X. Yan as in Table 2.

3.5 Elemental analysis and spectral data of tannyl penta stearate

The structure of tannyl penta stearate (Fig. 2) which is the desirable compound was then further confirmed according to its elemental analysis and spectral data, where its IR spectrum revealed bands at \( \nu \) 3432, 3062, 2848, 1730 cm\(^{-1}\), due to \( \text{OH} \), \( \text{C–H aromatic} \), \( \text{CH}_2 \) and \( \text{C’O} \) functions, respectively. The \(^1\text{H} \) NMR spectrum of the same product revealed the appearance of signals at \( \delta \) 0.89-1.32 and 5.0 due to aliphatic protons and OH functions respectively.

According to these analysis and spectral data, tannyl

<table>
<thead>
<tr>
<th>Table 1</th>
<th>RF value of tannyl stearate esters.</th>
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<tbody>
<tr>
<td>Compound</td>
<td>RF value</td>
</tr>
<tr>
<td>Tannyl mono stearate</td>
<td>0.44</td>
</tr>
<tr>
<td>Tannyl di stearate</td>
<td>0.41</td>
</tr>
<tr>
<td>Tannyl tri stearate</td>
<td>0.39</td>
</tr>
<tr>
<td>Tannyl tetra stearate</td>
<td>0.37</td>
</tr>
<tr>
<td>Tannyl penta stearate</td>
<td>0.34</td>
</tr>
<tr>
<td>Tannyl hexa stearate</td>
<td>0.32</td>
</tr>
<tr>
<td>Tannyl hepta stearate</td>
<td>0.29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Expected molecular weight (M Wt E) and average molecular weight obtained from viscosity measurements (M Wt V).</th>
</tr>
</thead>
<tbody>
<tr>
<td>compound</td>
<td>M Wt E, gmol(^{-1})</td>
</tr>
<tr>
<td>Tannyl mono stearate</td>
<td>1967</td>
</tr>
<tr>
<td>Tannyl di stearate</td>
<td>2234</td>
</tr>
<tr>
<td>Tannyl tri stearate</td>
<td>2500</td>
</tr>
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<td>2767</td>
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</tr>
<tr>
<td>Tannyl hexa stearate</td>
<td>3299</td>
</tr>
<tr>
<td>Tannyl hepta stearate</td>
<td>3566</td>
</tr>
</tbody>
</table>
penta stearate may found in so many isomeric structures. But all of those isomers are expected to pose the same physical properties.

3.6 Emulsification power of tannyl penta stearate
The emulsion formation of paraffin oil in water using the prepared tannyl penta stearate solution was measured as an indication for the emulsifying tendency of these surfactants. The synthesized compound showed emulsification power for paraffin oil equal to 1834 s at room temperature. This result could be attributed to the presence of five hydrophobic fatty chains which increase the hydrophobicity of the molecules and consequently increases its tendency for migration from the aqueous medium to the oil medium and thus increases its emulsifying power.

3.7 Lipid oxidation in sunflower oil
As shown in Fig. 3, sunflower oil without additives had an induction period (IP) of 6 h, addition of tannyl penta stearate (200 ppm) increased the induction period to 23.75 h. On the other hand, TBHQ was added to sunflower oil at the same concentration, and the induction period was increased to 12.85 h. Thus tannyl stearate can be used as phenolic antioxidant that can easily soluble in both hydrophobic and hydrophilic mediums.

3.8 DPPH radical scavenging activity
The violet stable DPPH (1,1-Diphenyl picryl hydrazyl) has a characteristic absorption at 517 nm. Antioxidant can scavenging this radical, by its reduction, through hydrogen donating to form the yellow DPPH molecule (1,1-Diphenyl picryl hydrazine) and, thus, the decreases in absorbance was associated with scavenging of DPPH by antioxidant. This decrease in absorbance is an indicator for the antiradical power of an antioxidant.

The scavenge effect of tannyl penta stearate and standard on the DPPH decreased in order of tannic acid ≥ tannyl penta stearate > B.H.A > B.H.T, as shown in Fig. 4. These results indicate that tannyl stearate can be used as radical inhibitor or scavenger to limit the free radical damage of any system.

3.9 Uses of ferric thiocyanate method (FTC) for determination of total antioxidant activity
Antioxidant was investigated for its power to retard and inhibit linoleic acid oxidation.
As it’s known, lipid oxidation is a free radical chain reaction. On the other hand, it’s correlated with free radicals formations which are associated with many biological and industrial damage. During the early stage of this reaction, peroxide molecules are formed, and in the presence of FeCl₂ and thiocyanate peroxides leads to oxidation of Fe²⁺ to Fe³⁺. The latter ions form a complex with ammonium thiocyanate that has a characteristic absorbance at 500 nm.
Antioxidant can scavenge the peroxide molecules, and consequently decreases the absorbance at 500 nm.
Total antioxidant activity of tannyl penta stearate, tannic acid, BHA and BHT was determined by the ferric thiocyanate method in the linoleic acid system. Tannyl penta stearate showed effective antioxidant activity in this system. The effect of tannyl penta stearate on lipid peroxidation of linoleic acid emulsion is shown in Fig. 5. These results showed that tannyl penta stearate has influential and sturdy antioxidant efficiency by this method.

3.10 Physico-chemical properties of frying oil
3.10.1 Changes in acidity
The increase in levels of acidity in oil systems with antioxidant was in the order to tannyl penta stearate < BHT < control, as shown in Fig. 6, the higher acidity of the control oil system compared to systems with antioxidants is due to the presence of the phenolic antioxidants that inhibit oxi-
Synthesis and application of a new amphiphilic antioxidant

Fig. 5 Total antioxidant activities of T_S, tannic acid, BHA and BHT at the same concentration (15 μg/mL) as determined by the thiocyanate method. (T_S: tannyl penta stearate, T: tannic acid, BHA: butylhydroxyanisole, BHT: butylhydroxytoluene).

3.10.2 Changes in iodine value
Changes in iodine value during frying process in all systems are given in Fig. 7. A significantly larger change in iodine value in the control compared to the other systems indicated that the rate of oxidation of unsaturated fatty acids was reduced in the presence of antioxidants. The changes in iodine value also confirm that tannyl penta stearate was more effective in protecting oxidation of unsaturated fatty acids than BHT.

3.10.3 Changes in oxidized fatty acids
The changes in oxidized fatty acids of all systems are presented in Fig. 8. The results showed that control had a consistently higher level of oxidized fatty acids after frying process. The increases of oxidized fatty acids were in the following order: control > BHT > tannyl penta stearate.

3.10.4 Changes in peroxide values
Changes in peroxide values during frying process are presented in Fig. 9. The results of this study showed that, in system 1 (control), the formation of peroxides seemed to increase rapidly from the beginning frying. Sunflower oil with the addition of antioxidants had significantly lower than those of the control, throughout the duration of the study. However, the peroxide value of tannyl penta stearate system was significantly lower than the peroxide value of BHT system. In general, the oxidative stability order was tannyl penta stearate > BHT > control.
3.10.5 Changes in polar components

The changes in polar components during frying process in all systems are given in Fig. 10. The obtained data showed that the polar components of all systems increased after frying process. The rate of formation of polar components was faster in the oil system without antioxidants than in oil systems with antioxidants. BHT and tannyl penta stearate showed significantly lower formation of polar components than control. Tannyl penta stearate showed significantly less formation of polar components compared to BHT.

3.10.6 Change in polymer content

The changes in polymer contents of all systems are presented in Fig. 11. And it was noted that the rate of polymer formation was faster in the oil system without antioxidants than in oil systems with antioxidants. BHT and tannyl penta stearate showed significantly lower formation in polymer content than control. Tannyl penta stearate showed less formation of polymer content compared to BHT.

3.10.7 Acute toxicity:

Acute lethal toxicity test (Table 3) revealed that Tannyl penta stearate showed complete safety up to 13 g/kg mice weight.

3.10.8 Liver and kidney function tests

Table 4 shows sera AST activity of rats fed on tannyl penta stearate at different concentrations (5, 7, 9, 11, and 13 g/L). They were slight non-significant increase in the activity of AST during the whole experiment for rats fed on tannyl penta stearate. The data in Table 3 for rat sera activities of ALT and ALP on investigated tannyl penta stearate indicate similar results for AST enzyme activity. Table 3 shows the changes of urea and creatinine contents of rats fed on tannyl penta stearate at different concentration. The results show that the administration of tannyl penta stearate induced very little change on sera levels of urea and creatinine during the whole experiment.

### Table 3: Acute oral lethal toxicity of tannyl penta stearate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (g/kg)</th>
<th>No. of animals/group</th>
<th>No. of dead animals</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 4: Liver and kidney function tests of rats fed on tannyl penta stearate at different concentrations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.48 ± 0.02</td>
<td>36.350 ± 0.003</td>
<td>136.82 ± 0.32</td>
<td>33.94 ± 0.02</td>
<td>0.756 ± 0.051</td>
</tr>
<tr>
<td>5g/L</td>
<td>21.56 ± 0.02</td>
<td>36.354 ± 0.010</td>
<td>136.54 ± 0.05</td>
<td>33.88 ± 0.02</td>
<td>0.720 ± 0.003</td>
</tr>
<tr>
<td>7g/L</td>
<td>21.72 ± 0.04</td>
<td>36.326 ± 0.005</td>
<td>136.04 ± 0.23</td>
<td>33.96 ± 0.02</td>
<td>0.736 ± 0.008</td>
</tr>
<tr>
<td>9g/L</td>
<td>21.84 ± 0.08</td>
<td>36.326 ± 0.005</td>
<td>135.78 ± 0.18</td>
<td>33.96 ± 0.05</td>
<td>0.734 ± 0.002</td>
</tr>
<tr>
<td>11g/L</td>
<td>22.92 ± 0.29</td>
<td>36.288 ± 0.006</td>
<td>136.02 ± 0.07</td>
<td>33.88 ± 0.02</td>
<td>0.738 ± 0.002</td>
</tr>
<tr>
<td>13g/L</td>
<td>22.9 ± 0.04</td>
<td>36.256 ± 0.011</td>
<td>135.08 ± 0.05</td>
<td>33.98 ± 0.02</td>
<td>0.748 ± 0.002</td>
</tr>
</tbody>
</table>
Synthesis and application of a new amphiphilic antioxidant

References
4) EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Scientific Opinion on the safety and efficacy of tannic acid when used as feed flavouring for all animal species. EFSA J. 12 (10), 3828 (2014).
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