



Bioorganic synthesis, characterization and evaluation of a natural phenolic lipid

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ABSTRACT

The first synthesis of a phenolic natural monoacylglycerol (1- [11-(ferulyloxy) undecanoyl] glycerol) was carried out by bioorganic synthesis starting from ferulic acid. The synthetic route of the target lipidic compound was designed involving a chemo-enzymatic approach using immobilized *Candida antarctica* lipase as biocatalyst in two of the steps conducted in organic medium. The prepared lipidic compound was characterized by using spectral data and evaluated for antimicrobial, antioxidant and cytotoxic studies to examine its potential. The synthesized compound showed moderate antimicrobial activity and showed very good antioxidant activity in DPPH radical scavenging assay and also in oxidation inhibition in soybean oil by differential scanning calorimetry. The cytotoxic studies of the synthetic lipid showed promising activity against A549 and HeLa cancer cell lines with IC₅₀ values of 9.102 and 9.886 μM respectively. The prepared compound can be useful in designing novel phenolic lipids with potential applications in cosmetic and biomedical fields.

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1. Introduction

Natural products are a source of many complex compounds which are ingredients in many pharmaceuticals, agrochemicals, nutraceuticals and bioactives [1]. Among the arsenal of several complex natural products, lipids which are one of the important biomolecules are of interest due to their multiple applications ranging from food, pharma, biomedical and oleochemical applications [2]. The fatty acids which are the building blocks of lipids are of great importance as they serve as substrate for the production of many bioactive lipid derivatives with potential in biomedical applications [3]. Plants and animals are the main sources of natural lipids which are composed of a mixture of several simple and complex lipids. Recently, a lipidic compound was isolated from a plant source where it was found that the compound had phenolic substituents along with polar hydroxyl functional groups with an aliphatic chain. The compound was identified as 2, 3-dihydroxypropyl 11-[(2E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoyl] oxy} undecanoate or 1- [11-(ferulyloxy) undecanoyl] glycerol, which is a naturally occurring lipidic derivative of ferulic acid [4]. The molecular structure of this

lipid shows that it is a monoacylglycerol derivative of ferulic acid. This lipid was also isolated recently from the grains of *Echinochloa utilis* where the authors reported that the compound exhibited potent NO inhibitory activity against lipopolysaccharide (LPS)-induced NO release with beneficial effects against inflammation [5].

The structure of the compound shows that it has both lipidic nature and also possess phenolic moiety which can have potential application as a cosmetic agent. Literature reports suggest that any compound which is intended to be used as a cosmeceutical ingredient should have some properties such as anti-oxidant, anti-inflammatory, skin whitening, anti-aging, anti-wrinkling, photo protective activity for its potential use in cosmetics [6]. It is reported that ferulic acid based structured lipids like ferulylated acylglycerols are potent UV absorbing agents and antioxidants that can be used in food and cosmetics [7]. Based on the structure of the compound, we anticipate that it could have promising potential in cosmetic and biomedical fields and can be explored further. Also several research groups have been working on the modification of natural phenolics to improve their biological activity and antioxidant potential [8]. The research interest in antioxidants which are found in natural sources has been increasing due to their health beneficial properties. This has prompted us to synthesize and evaluate the compound for antimicrobial, anti-oxidant and cytotoxicity properties to examine its potential. For the synthesis

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of the target compound we have designed reactions where enzyme catalyzed transformations can be employed. The unique characteristics of enzymes like milder reaction conditions, better selectivity have made them as routine catalysts in organic synthesis. These characteristics of the biocatalyst provide organic chemists an opportunity to avoid or at least reduce the usage of toxic reagents which are used in a multi step organic synthesis [9,10]. Also there has been an increased interest in enzymatic transformations conducted in non-conventional organic medium such as organic solvents as solubility of many organic compounds is facilitated in solvents rather than water [11]. Hence, the aim of the work was to synthesize the target compound employing a bioorganic route and evaluate its preliminary activities.

2. Materials and methods

2.1. General information

Solketal (DL – 1,2 – Isopropylidinediglycerol), ferulic acid, vinyl acetate and 11-bromoundecanoic acid were purchased from Sigma. Potassium hydroxide, mercuric acetate, sulphuric acid, hydrochloric acid, silica gel for column chromatography were purchased from SRL chemicals, merck, Avera, Advent and Acme Synthetic Chemicals, Mumbai, India respectively. All solvents were procured from Merck and were of highest grade of purity. Immobilized lipase from *Candida antarctica* (CAL-B) was procured from Novozymes Bagsvaerd, Denmark).

The proton NMR spectra were recorded on Varian 500/300 and 125/75 MHz instrument and TMS was used as an internal standard. Mass spectra were recorded using electron spray ionization on Waters e2695 Separators module (Waters, Milford, MA, USA) mass spectrometer. Infra red (IR) spectra were recorded using a Perkin-Elmer Fourier transform (FT)-IR Spectrum BX instrument (Model: Spectrum BX; Connecticut, USA). All the synthesized compounds were purified by silica gel (60–120 mesh) column chromatography (Acme Synthetic Chemicals, Mumbai, India) and were identified by thin-layer chromatography (TLC). TLC was performed on precoated silica gel 60 F₂₅₄ from Merck (Darmstadt, Germany).

2.2. Synthesis

2.2.1. Preparation of vinyl ferulate (2)

Vinyl ferulate was synthesized from ferulic acid and vinyl acetate following a reported protocol with slight modifications [12]. To a mixture of ferulic acid (1.5 g, 7.73 mmol) and vinyl acetate (11.4 mL, 123.68 mmol) in tetrahydrofuran (20 mL), mercury acetate (4%, w/w) was added and the mixture was stirred for 30 min under nitrogen atmosphere. After 30 min, 2–4 μ L of sulphuric acid was added and the mixture was stirred overnight at 40 °C. After 12 h, excess of sodium acetate (20 mg) was added followed by the removal of vinyl acetate and solvent. The remaining crude reaction product was purified by column chromatography (hexane and ethyl acetate (92:8 v/v) to obtain pure vinyl ferulate in 1.2 g (70.6%) at R_f 0.87 on TLC run on hexane: ethyl acetate (80:20; v/v).

¹H NMR (300 MHz, CDCl₃) δ 3.93 (s, CH₃, 3 H), 4.62 (dd, 7.78 Hz, 1.52 Hz, = CH, 1 H), 4.94 (dd, 15.56 Hz, 1.67 Hz, = CH, 1 H), 5.94 (s, OH, 1 H), 6.28 (d, 15.86, = CH, 1 H), 6.92 (d, 8.08 Hz, = CH, 1 H), 7.04 (d, ArH, 1 H), 7.08–7.11 (m, ArH, 2 H), 7.43–7.45 (m, = CH, 1 H), 7.70 (d, 15.86, = CH, 1 H); IR (cm⁻¹): 1718.58 cm⁻¹, 3021.04 cm⁻¹, 3417.78 cm⁻¹; MS: M-1 = 219.22.

2.2.2. Preparation of 11-hydroxyundecanoic acid (4)

11-hydroxyundecanoic acid prepared from 11-bromoundecanoic acid employing a reported protocol [13]. Briefly, a mixture of 11-bromoundecanoic acid (2 g, 7.54 mmol) and a solution of 2 M aq

potassium hydroxide solution (150 mL) were stirred at 100 °C for 16 h. The progress of the reaction was monitored by TLC using hexane and ethyl acetate (70:30; v/v). After complete conversion, the temperature was brought to about 25 °C and the pH was adjusted to 1 by adding concentrated hydrochloric acid. The resulting precipitate was filtered and dried under reduced pressure to obtain 11-hydroxyundecanoic acid as a white solid in 1.45 g (95%).

mp: 65–66 °C.

¹H NMR (300 MHz, CDCl₃) δ 1.28 (m, (CH₂)₆, 12 H), 1.62 (m, (CH₂)₂, 4 H), 2.34 (t, J = 7.6 Hz, CH₂, 2H), 3.63 (t, J = 6.8 Hz, CH₂, 2 H).

IR (cm⁻¹): 1216.38 cm⁻¹, 3022.17 cm⁻¹, 3400.55 cm⁻¹.

MS: M-1 = 201.33

2.2.3. Preparation of (E)-11-(3-(4-hydroxy-3-methoxyphenyl)acryloyloxy) undecanoic acid (5)

Vinyl ferulate (900 mg; 4.09 mmol) and 11-hydroxyundecanoic acid (1.03 g; 5.11 mmol; 1.25 eq) were solubilized in 10 mL of hexane and 2-butanone (2:1; v/v) and to this mixture was added immobilized lipase from *Candida antarctica* (15% to wt of substrates). The reaction mixture was stirred at 60 °C while monitoring the progress by TLC using hexane and ethyl acetate (80:20; v/v). Product formation was observed from the third day onwards and another 5% of lipase was added to the reaction mixture. After maximum conversion, the reaction mixture was diluted with ethyl acetate and the lipase was washed several times with the solvent and separated by filtration. Solvent was evaporated and the crude product was purified by column chromatography (hexane and ethyl acetate (90:10; v/v) to obtain the ferulic acid ester of hydroxy undecanoic acid in 570 mg (37%).

¹H NMR (300 MHz, CDCl₃): δ 1.29 (s, (CH₂)₆, 12 H), 1.63–1.71 (m, (CH₂)₂, 4 H), 2.35 (t, J = 7.5 Hz, CH₂, 2H), 3.65 (t, –OCH₂, J = 6.6 Hz, 2 H), 3.94 (s, –OCH₃, 3 H), 6.26 (d, J = 15.9 Hz, = CH, 1 H), 6.93 (d, J = 8.1, 5.5 Hz, ArH, 1 H), 7.13–7.02 (m, ArH, 2 H), 7.58 (d, J = 15.9 Hz, = CH, 1 H); IR (cm⁻¹): 1742.56 cm⁻¹, 2938.90 cm⁻¹, 2985.89 cm⁻¹, 3460.28 cm⁻¹

MS: M-1 = 377.34.

2.2.4. Synthesis of (E)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 11-(3-(4-hydroxy-3-methoxyphenyl)acryloyloxy)undecanoate (7)

Ferulic acid ester of 11-hydroxyundecanoic acid (570 mg; 1.5 mmol) and solketal (250 mg; 1.96 mmol; 1.3 eq) were solubilised in a mixture of 10 mL of hexane: 2-butanone (2:1; v/v). To this mixture, immobilized lipase from *Candida antarctica* (10% to wt of substrates) was added and the reaction mixture was magnetically stirred at 60 °C. After maximum conversion, the reaction mixture was filtered to separate the lipase, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulphate to obtain the crude product. The crude product was purified by column chromatography (hexane and ethyl acetate (92:8; v/v) to obtain pure acetone in 250 mg (34% yield).

¹H NMR (300 MHz, CDCl₃): δ 1.29 (s, (CH₂)₉, 18 H), 1.67–1.71 (m, (CH₂)₂, 4 H), 2.30 (t, J = 7.5 Hz, CH₂, 2 H), 3.62–3.66 (m, CH₂, –OCH₂, 4 H), 3.93 (s, –OCH₃, 3 H), 4.05 (t, J = 6.7 Hz, CH, 1 H), 4.19 (t, J = 6.7 H, CH₂, 2 H), 5.85 (s, OH, 1 H), 6.29 (d, J = 15.9 Hz, (= CH), 1 H), 6.89 (d, ArH, 1 H), 7.01–7.08 (m, ArH, 2 H), 7.61 (d, J = 15.9 Hz, (= CH), 1 H); IR (cm⁻¹): 1216.08 cm⁻¹, 1708.62 cm⁻¹, 2858.29 cm⁻¹, 2928.19, 3021.31 cm⁻¹, 3389.21 cm⁻¹; MS: = M + 23 = 515.28

2.2.5. Synthesis of 1- [11-(ferulyloxy) undecanoyl] glycerol (8)

A mixture of acetone (7) of previous step (130 mg, 0.003 mol) dissolved in methanol (10 mL) was placed in ice bath and to this cooled solution was added 2 M aqueous hydrochloric acid (0.5 mL, 1.5 eq) in one portion. After few minutes, the ice bath was removed and the reaction mixture was stirred for about 4 h at room

temperature (20 °C) while monitoring by TLC using hexane and ethyl acetate (60:40; v/v). When the conversion was maximum (3 h), diethyl ether (20 mL) was added to the reaction mixture and washed with 10 mL of saturated aqueous sodium bicarbonate and extracted with diethyl ether (3 x 25 mL). The organic phases containing the product were dried over sodium sulphate and concentrated to obtain the crude product which was purified by column chromatography using chloroform to obtain the final product in 65% yield (130 mg).

¹H NMR (500 MHz, CDCl₃): δ 1.28 (s, (CH₂)₆, 12 H), 1.63–1.72 (m, (CH₂)₂, 4 H), 2.35 (t, J = 7.5 Hz, CH₂, 2 H), 3.5–3.72 (m, CH₂, –OCH₂, CH, 3 H), 3.93 (s, –OCH₃, 3 H), 4.11–4.22 (m, CH₂, CH₂, 4 H), 5.91 (s, OH, 1 H), 6.29 (d, J = 15.9 Hz, (=CH), 1 H), 6.90 (d, ArH, 1 H), 7.01–7.08 (m, ArH, 2 H), 7.61 (d, J = 15.9 Hz, =CH, 1 H); ¹³C NMR (125 MHz, CDCl₃): δ 24.91 (s, CH₂), 25.96 (s, CH₂), 29.30–29.42 (m, (CH₂)₆), 55.98 (s, –OCH₃), 63.37 (s, CH₂), 64.62 (s, CH₂), 65.21 (s, CH₂), 70.29 (s, CH), 109.33 (s, ArCH), 114.73 (s, =CH), 115.66 (s, ArCH), 123.07 (s, ArCH), 127.06 (s, ArC), 144.71 (s, =CH), 146.85 (s, ArC), 147.93 (s, ArC), 167.46 (s, C = O), 174.33 (s, C = O).

IR (cm⁻¹): 1731.21 cm⁻¹, 2256.08 cm⁻¹, 3020.16 cm⁻¹, 3424.75 cm⁻¹; MS: M + 23 = 475.34

HRMS: M + 23 = observed mass: 475.2281; calculated mass: 475.2308

2.3. Antioxidant activity

DPPH radical scavenging activity: The preliminary antioxidant activity of the synthetic lipid was measured by the DPPH radical scavenging activity following a reported protocol with small modification [14]. 200 μL of synthesized lipid (1 mM and 2 mM concentration) was added to a 0.1 mM methanolic DPPH radical solution (2 mL) in a screw cap tube and the final volume was made up to 3 mL with methanol. This solution was kept in dark for about 40 min and the absorbance was measured at 517 nm. Methanol was used as blank and standard antioxidants like BHT and alpha tocopherol at 1 mM concentration were used as positive control. The DPPH radical scavenging activity was determined by the formula: [(Ac – As) / Ac] × 100; where Ac and As are the absorbance of the control and sample respectively. The assay was conducted 3 times and the results are presented as average of three measurements.

Oxidation stability by differential scanning calorimetry: Differential scanning calorimeter (DSC) was used to determine the oxidative onset temperature (OT) according to the method described by ASTM (Standard Test Method for Oxidation Onset Temperature of Hydrocarbons by Differential Scanning Calorimetry. E2009 – 08). DSC was studied using Q-100 thermal analyzer from M/s TA instruments under continuous oxygen atmosphere at a flow of 50 mL min⁻¹. Soybean oil sample with and without test compounds (4.0–5.0 mg) was placed in an open aluminium pan under constant flow of oxygen and an empty pan was used as reference. The temperature was raised from 25 °C to 300 °C at a heating rate of 10 °C min⁻¹. Samples of soybean oil as control and soybean oil with synthetic lipid, BHT and alpha tocopherol as reference compounds were analyzed for oxidative onset temperature. The DSC analysis was determined for 3 times and the results are presented as average of three measurements.

2.4. Antimicrobial evaluation

In vitro antibacterial activity of the newly synthesized compounds was studied against the bacterial strains, Gram-positive organisms: *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 9041), *Bacillus subtilis* (MTCC 441), Gram-negative organisms viz *Escherichia coli* (MTCC 443),

Pseudomonas aeruginosa (MTCC 741), *Klebsiella pneumonia* (MTCC 618) by Agar Well Diffusion Method [15]. The minimum inhibitory concentrations (MIC) of various synthetic compounds were tested against by Microdilution method recommended by CLSI Standard Protocol [16].

2.5. Cytotoxicity studies (MTT assay)

The cell viability (MTT test) of the synthetic lipid was assessed following our earlier published literature and compared with Doxorubicin as reference compound [17].

2.6. Statistical analysis

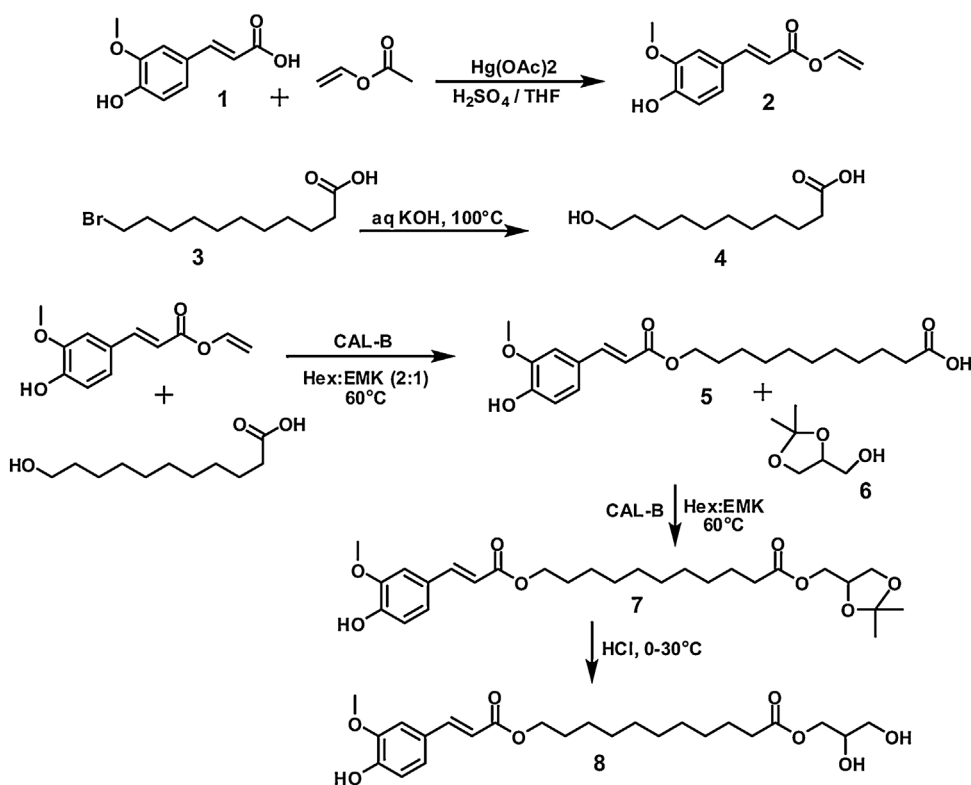
Statistical analysis was performed using unpaired Student's *t*-test followed by one-way ANOVA by Dunnett's test for antioxidant activity and Tukey's multiple comparison tests for cytotoxicity activity using Graph Pad Prism version 6.04 for Windows (Graph Pad Software, San Diego, CA).

3. Results and discussion

3.1. Synthesis

In this work, we demonstrated a chemo-enzymatic approach towards the synthesis of a natural phenolic lipid which can have potential applications in food, cosmetic and pharma industries. The bioorganic synthetic route followed is shown in Scheme 1. The synthetic route for the target compound involved preparation of vinyl ferulate (**2**) which was carried out by the vinyl exchange between ferulic acid (**1**) and vinyl acetate which yielded vinyl ferulate in 70% yield as one of the coupling partner. The structure of vinyl ferulate was confirmed by spectral analysis and also by comparing with the earlier reported literature values.

The transesterification between vinyl ferulate (**2**) and 11-hydroxy undecanoic acid (**4**) was catalyzed by immobilized lipase from *Candida antarctica* which resulted in desired ferulic acid ester of 11-hydroxyundecanoic acid product (**5**) in 37% yield during twelve days of reaction time period. It was found that product formation was observed from third day of the reaction and reached maximum at a reaction time of twelve days. It is well known that the vinyl group will influence the enzyme catalyzed step towards product formation instead of reverse reaction [18]. The transesterification reaction was carried out in organic media consisting of hexane and 2-butanone mixture which was reported to be the suitable solvent for enzymatic esterification reactions in organic media [19]. In the present study, we found that a mixture of 2:1 ratio of hexane and 2-butanone (v/v) was better as the two substrates were solubilised in this mixture. In addition, this solvent mixture was also reported to be non-toxic which further influences the lipase catalyzed reactions [20]. The product of this transesterification reaction had both ester and acid functionalities and hence we employed a second lipase catalyzed esterification step between the acid group of fatty acid (**5**) and solketal (**6**), which had primary hydroxyl functionality to obtain the corresponding acetonide ester (**7**) in 34% yield. The acetonide ester compound was finally deprotected using aqueous hydrochloric acid to yield the desired target compound, 1- [11-(ferulyloxy) undecanoyl] glycerol (**8**) in 65% yield. The products of each step in the synthesis were purified by silica gel column chromatography and the purified compounds were characterized by spectral data. The spectral analysis was compared with the reported literature data and the structure of the final product was confirmed with HRMS analysis also. The HRMS data showed a strong peak which corresponded to [M + Na] at *m/z* 475.2281 which further confirmed the structure of the product.



Scheme 1. Bioorganic synthetic route.

3.2. Biological evaluation of the prepared compound

3.2.1. Antioxidant activity

The antioxidant activity of the synthetic lipid was measured using preliminary DPPH radical scavenging activity and also by measuring oxidative stability using DSC in a lipid medium and compared to BHT and alpha tocopherol as reference compounds in respective assays. The DPPH radical scavenging activity of the prepared lipid showed very good antioxidant activity compared to commercially available BHT. At 0.5 mM concentration BHT exhibited 17.61% FRSA (Free radical scavenging activity) and synthetic lipid showed an FRSA of 33.10% and the activity was increased as the concentration was increased which is shown in Table 1 and Fig. 1. However, the activity was lower when compared to ferulic acid at all the tested concentrations. The antioxidant activity of phenolic compounds was reported to be dependent on factors like structure of the compound, position of the substituents on the phenolic ring, and also the medium in which the activity is determined [21]. These factors could be one of the reasons for the difference in antioxidant activity observed even though the number of phenolic hydroxyls is same in all the three tested compounds. The compound 5 which is the unbound lipid, *i.e.* 11-feruloyl undecanoic acid was also screened for DPPH scavenging

activity. The antioxidant activity in DPPH radical scavenging assay was in the following order: ferulic acid > synthetic lipid > 11-feruloyl undecanoic acid > BHT.

The antioxidant capacity of the prepared lipid was also examined by its oxidation stability potential in soybean oil by determination of oxidative onset temperature (OT) using DSC. Soybean oil was chosen as it contains higher amounts of unsaturated fatty acids along with linolenic acid. DSC is reported to be a reliable method for determining the oxidative stability of oils as it is convenient, simple and rapid technique [22]. Oil was extracted from soybeans and used for DSC study both as such and also in the presence of test compounds. The results of the DSC analysis are shown in Fig. 2.

Table 1
DPPH radical scavenging assay.

| Test compound | 0.5M | 1M | 2M |
|-----------------------------|-----------------|-------------------|-----------------|
| Ferulic acid | 36.34 ± 0.32*** | 62.0 ± 0.25*** | 81.53 ± 0.14*** |
| 11-feruloyl undecanoic acid | 27.30 ± 0.52*** | 49.09 ± 0.12 (ns) | 68.61 ± 0.14*** |
| Synthetic lipid | 33.10 ± 0.98*** | 49.70 ± 0.63*** | 72.67 ± 0.29*** |
| BHT | 17.61 ± 0.77 | 37.4 ± 0.88 | 48.33 ± 0.55 |

Solvent: methanol; ±: standard deviation of mean; ns: none significant; *** results found significantly different from BHT at P < 0.001 (Student's *t*-test, followed by One-way ANOVA).

% FRSA at different molar concentration of compound

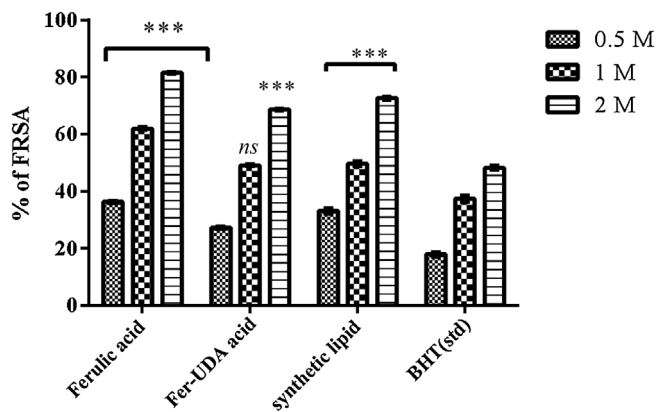


Fig. 1. DPPH radical scavenging assay of test compounds. *** results found significantly higher from BHT at P < 0.001 (Student's *t*-test, followed by One-way ANOVA).

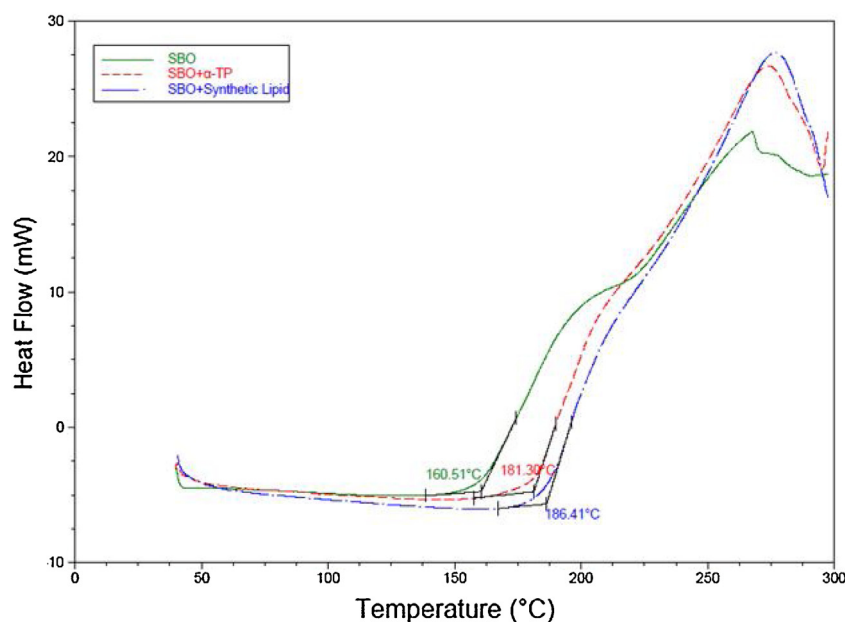


Fig. 2. DSC profiles of test compounds.

Table 2

Antimicrobial activity of the synthesized lipid.

| Bacterial sps | Zone of inhibition (150 µg /mL) | | MIC (Serial dilution 150 µg /mL to 1.17 µg /mL) | |
|-----------------------------------|---------------------------------|--------------|---|--------------|
| | Synthetic lipid | Streptomycin | Synthetic lipid | Streptomycin |
| <i>Staphylococcus epidermidis</i> | – | 25 | <150 | 2.34 |
| <i>Bacillus subtilis</i> | 13 | 24 | 37.5 | 4.68 |
| <i>Staphylococcus aureus</i> | 12 | 23 | 37.5 | 2.34 |
| <i>Escherichia coli</i> | 13 | 23 | 18.75 | 4.68 |
| <i>Pseudomonas aeruginosa</i> | 10 | 28 | 75 | 1.17 |
| <i>Klebsiella pneumoniae</i> | 11 | 26 | 75 | 4.68 |

In DSC study, soybean oil was found to have oxidative onset temperature of 160.51 °C which increased in the presence of antioxidant compounds at 1% concentration. The oxidative onset temperatures in the presence of the synthetic lipid and alpha tocopherol were found to be 186.41 °C and 181.30 °C respectively. The results indicated that the synthetic lipid has better potential in delaying the oxidative deterioration of soybean oil compared to alpha tocopherol.

3.2.2. Antimicrobial activity

The synthesized lipid along with the ferulic acid and the unbound lipid were screened for antimicrobial activity against three Gram positive and three Gram negative bacterial strains to know whether it can be a potent antimicrobial agent. Antimicrobial activity was measured by both zone of inhibition and also by determination of minimum inhibitory concentrations (MIC) and the results are presented in Table 2. MIC was determined only for the compounds which exhibited activity by zone of inhibition method. It was found that the synthetic lipid exhibited antimicrobial activity against both Gram positive and Gram negative bacterial strains. Ferulic acid and the unbound lipid did not exhibit antimicrobial activity. Among the tested strains, the synthetic lipid exhibited highest activity against *Escherichia coli* with an MIC value of 18.75 µg/mL followed by *Bacillus subtilis* and *Staphylococcus aureus*. However, the antimicrobial activity was lower compared to standard antibiotic Streptomycin for all the bacterial strains in the present study.

3.2.3. Cytotoxicity studies

The prepared phenolic lipid derivative along with the ferulic acid and the unbound lipid were studied for its cytotoxicity against a panel of 7 cell lines with Doxorubicin as standard reference compound. The results of the cytotoxicity studies are shown in Table 3. It was observed that the synthesized compound showed promising cytotoxicity effect against A549 and HeLa cell lines with IC₅₀ values of 9.102 and 9.866 µM respectively. It exhibited

Table 3

In vitro cytotoxicity test of compounds against cancerous and non-cancerous cell lines.

| Cell line | IC ₅₀ values (µM) | |
|-----------|------------------------------|---------------|
| | Synthetic lipid | Doxorubicin |
| A549 | 9.102 ± 0.075*** | 0.55 ± 0.16 |
| DU145 | 10.464 ± 0.572*** | 0.363 ± 0.01 |
| HeLa | 9.866 ± 0.294*** | 0.8 ± 0.71 |
| HepG2 | 10.282 ± 0.272*** | 0.72 ± 0.012 |
| MCF7 | 11.501 ± 0.045*** | 2.0 ± 0.81 |
| B16 | 19.035 ± 0.257*** | 0.7 ± 0.56 |
| HEK | 56.180 ± 0.738 | 0.010 ± 0.001 |

Cell lines: A549 Homo sapiens lung carcinoma (ATCC® CCL-185™); DU145 Homo sapiens prostate carcinoma (ATCC® HTB-81™); HeLa Homo sapiens cervix adenocarcinoma (ATCC® CCL-2.1™); Hep G2 liver hepatocellular carcinoma (ATCC® HB-8065™); MCF7 human breast adenocarcinoma ((ATCC® HTB-22™); B16-F10 mouse skin melanoma (ATCC® CRL 6475™); HEK-293 Homo sapiens embryonic kidney cells (ATCC® CRL-1573™); ±: standard deviation of mean; *** P < 0.001 is significantly different of cytotoxicity activity in cancer cell lines from normal cell lines of synthetic lipid (Student's t-test, followed by One-way ANOVA).

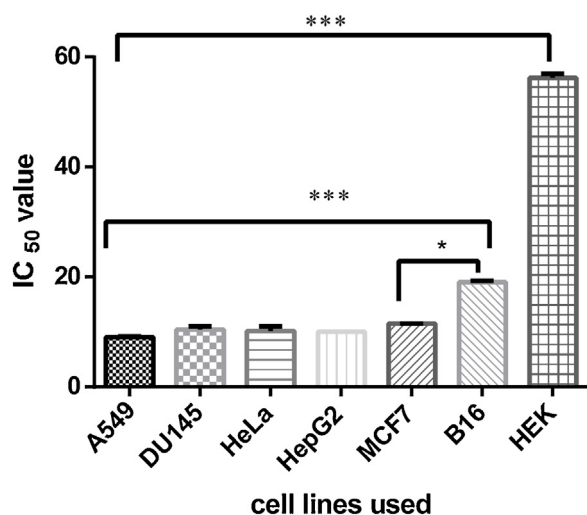


Fig. 3. Multiple comparisons of cytotoxicity effects of synthesized lipid among cancer and normal cell lines. * $P < 0.05$; *** $P < 0.001$ is significantly different among the cell lines used (Tukey's Multiple comparison test).

moderate activity against HepG2, DU145, MCF7 and B16 cell lines where the IC₅₀ values were in the range of 10.464–19.035 μM. The prepared compound was least active against HEK cell lines with an IC₅₀ value of 56.180 μM. The mean differences of IC₅₀ value of synthesized lipid among the cell lines used (Tukey's multiple comparison test), showed that there was a statistically significant lower IC₅₀ value observed among all the cancer cell lines compared to normal cell line (HEK) ($P < 0.001$). Whereas, the mean differences in cytotoxicity of synthesized lipid was compared among the cancer cell lines, a significant lower IC₅₀ was found in all the cancer cell lines when compared to B16 cell line ($P < 0.001$) as shown in Fig. 3. It was observed that ferulic acid and the unbound lipid did not show activity against the tested cell lines. However, it was found that the prepared compound was less effective compared to the reference drug Doxorubicin against all cell lines.

4. Conclusions

Bioorganic synthesis of a natural phenolic lipid derivative was carried out for the first time following a chemo-enzymatic approach. Immobilized *Candida antarctica* lipase was employed as a biocatalyst in organic media for two esterification reactions. The synthesized lipid was characterized by spectral analysis and the product was screened for antioxidant, antimicrobial and cytotoxic activities. The synthesized compound has shown promising antioxidant and cytotoxic activity and moderate antimicrobial activity. Further studies on the related derivatives of the phenolic lipid with applications could be promising as the compounds could have potential in biomedical fields.

Declaration of Competing Interest

We declare that we have no conflict of interests.

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