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Research Article

Synthesis of Kojic Ester Derivatives as Potential Antibacterial Agent

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The search for lead product with beneficial pharmacological properties has become a great challenge and costly. Extraction and synthetic modification of bioactive compounds from natural resources has gained great attention and is cost effective. In this study, kojic acid was produced from fungal fermentation, using sago waste as substrate, and chemically incorporated with chalcones and azobenzene to form a series of kojic ester derivatives and evaluated for antibacterial activities. Kojic ester bearing halogenated chalcone demonstrated active inhibition against *Staphylococcus aureus* compared to that of standard ampicillin. The inhibition increased as the electronegativity of halogens decreased, while incorporation of azobenzene derivatives on kojic acid backbone demonstrated fair antibacterial activity against *Escherichia coli* with minimum inhibitory concentration (MIC) of 190–330 ppm. The presence of C=C and N=N reactive moieties in both chalcone and azo molecules contributed to the potential biological activities of the kojic acid ester.

1. Introduction

Kojic acid is a natural pyrone produced from fungal fermentation from various types of carbon substrates in the presence of fungi [1, 2]. Kojic acid and its derivatives have drawn attention and were studied intensively due to its significant biological activities in medicine and pharmacological field such as antifungal, antibiotic [3, 4], anti-inflammatory and analgesic [5], and antibacterial [6] properties.

Many recent studies reported on chemical modification of kojic acid for various applications such as antibacterial activity [7] and dye sensitized solar cell (DSSC) [8]. Synthesis of bioactive molecules employing kojic acid as a precursor via esterification of available hydroxyl groups has been widely reported to overcome the drawbacks of its hydrophilic properties in various applications [9–12]. Esterification of kojic acid, for instance, is able to enhance the hydrophobic properties which in turn improved its performance as tyrosinase inhibitor and antioxidant [13, 14].

Incorporation of kojic acid into other biological active compounds has also been reported with enhanced biological activities [15, 16]. Chalcones are an example of biological active compound which is derived from natural sources such as fruits, vegetables, spices, tea, and soy-based foodstuff [17, 18] and used as an intermediate precursor of flavonoids and isoflavonoids [19]. Chalcone is also commonly prepared via Claisen Schmidt condensation. The synthesis of chalcone has drawn much attentions due to the presence of its α,β-unsaturated ketone moieties which contributes to various biological activities such as antimalaria [20], anticancer [21], antiprotozoal, anti-inflammatory, antibacterial, antifilarial, anticonvulsant [22], antifungal, insect antifeedant, antimutagenic [23], and antioxidant [24, 25]. The presence of halogen substituents particularly at the *para* position of chalcone backbone has been reported with excellent biological activities such as antibacterial [26] and anti-inflammatory [27], while azobenzene bearing aryl/alkyl and N=N reactive moieties have been extensively reported due to its excellent antibacterial [28, 29], antioxidant [30], anti-inflammatory [31], antifungal [32], and antitumor [33] properties. Azobenzene is also recognized for its medicinal properties such as antineoplastic [34], antidiabetic [35], and antiseptic [36].

Herein, we report on the isolation of kojic acid from sago waste and utilized it as a precursor for the synthesis

of kojic acid ester bearing chalcone **3a–c** and azobenzene **5a–d** moieties. These compounds were screened for potential antibacterial activities against *E. coli* and *S. aureus*, and the effect of different moieties towards biological activities of kojic acid was studied.

2. Experimental

All the reagents were used as received without any further purification. Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were recorded with KBr on Perkin Elmer 1605 FTIR spectrophotometer. The ^{1}H NMR were recorded in $CDCl_3$ or DMSO-d_6 on JEOL ECA 500 spectrometer at 500 MHz using TMS as internal standard. The elemental carbon hydrogen nitrogen sulphur (CHNS) elemental analyses were performed using Thermo Scientific™ FLASH 2000 CHNS/O Analyzer.

*2.1. Fermentation of Kojic Acid (**1**).* Sago waste was used as the substrate for kojic acid fermentation. Prior to fermentation, sago waste (5 g) was dried, ground, and sieved. The medium was supplemented with 3% (w/v) urea and 10% (w/v) mineral salts solution containing KH_2PO_4, (w/v) $MgSO_4{\cdot}7H_2O$, and yeast extract. Mixed strains of *Aspergillus flavus NSH9* and *A. flavus Link 44-1* were added for fermentation process. The culture was incubated at 30 ± 2°C in static condition for 20 days. The slurry suspension culture (40 mL) was extracted with ethyl acetate (2 × 15 mL). The organic layer was evaporated in vacuo to form crude brown solid and recrystallized from ethanol to afford **1** (0.15 g, 30%) as a yellowish needle like solid; m.p. 149-150°C [Reported 151–154°C [37]]; ν_{max} (KBr/cm^{-1}) 3173 (OH), 2919 (C=C), 1661 (C=O), 1228 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.04 (1H, s, OH-5), 8.02 (s, 1H, H-6), 6.34 (1H, s, H-3), 5.67 (1H, s, OH-7), 4.29 (1H, s, H-7); ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.4 (C-4), 168.6 (C-2), 146.2 (C-5), 139.8 (C-6), 110.4 (C-3), 60.0 (C-7).

*2.2. General Method for Synthesis of 4-Carboxyl Chalcone (**2a–c**).* Acetophenone (1.2 g, 0.1 mmol) was added to 4-carboxybenzaldehyde derivatives (0.15 g, 0.1 mmol) in methanol (50 mL). KOH (1.7 g, 0.3 mmol) in methanol was added and the mixture was stirred at room temperature for 4 h under nitrogen atmosphere. The precipitate formed was filtered and recrystalized from ethanol to afford title compound.

*4-[(E)-3-Oxo-3-phenyl-prop-1-enyl]benzoic Acid (**2a**).* Compound **2a** (2.32 g, 92%) as a yellowish powder. m.p. 223-224°C; (Found: C, 76.61; H, 4.37%. $C_{16}H_{12}O_3$ Requires C, 76.2; H, 4.8%); ν_{max} (KBr/cm^{-1}) 3059 (C=CH), 1677 (C=O), 1608 (COO), 1578 (aromatic); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.17 (2H, d, J = 7.45 Hz, H-3), 8.05 (1H, d, J = 16.05 Hz, H-β), 8.02 (2H, d, J = 8.06 Hz, H-8), 7.99 (2H, d, J = 8.00 Hz, H-2), 7.78 (1H, d, J = 15.45 Hz, H-α); 7.69 (1H, t, J = 7.73 Hz, H-10), 7.59 (2H, t, J = 7.75 Hz, H-9).

*4-[(E)-3-(4-Bromophenyl)-3-oxo-prop-1-enyl]benzoic Acid (**2b**).* Compound **2b** (2.65 g, 88%) as a yellowish powder; m.p. 246-247°C; (Found: C, 58.44; H, 3.65%. $C_{16}H_{11}BrO_3$ Requires C, 58.0; H, 3.3%); ν_{max} (KBr/cm^{-1}) 3064 (C=CH), 1663 (C=O), 1607 (COO), 1583 (aromatic); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.13 (2H, d, J = 8.00 Hz, H-8), 8.05 (1H, d, J = 15.45 Hz, H-β), 8.02 (2H, d, J = 8.00 Hz, H-3), 7.98 (2H, d, J = 8.60 Hz, H-9), 7.79 (1H, d, J = 15.45 Hz, H-α), 7.79 (2H, d, J = 8.00 Hz, H-2).

*4-[(E)-3-(4-Chlorophenyl)-3-oxo-prop-1-enyl]benzoic Acid (**2c**).* Compound **2c** (2.12 g, 74%) as a yellowish powder. m.p. 259-260°C; (Found: C, 67.09; H, 3.27%. $C_{16}H_{11}ClO_3$ Requires C, 67.0; H, 3.9%); ν_{max} (KBr/cm^{-1}) 2943 (C=CH), 1678 (C=O), 1612 (COO), 1577 (aromatic); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.20 (2H, d, J = 8.40 Hz, H-8), 8.00 (1H, d, J = 16.05 Hz, H-β), 7.93 (2H, d, J = 8.40 Hz, H-3), 7.90 (2H, d, J = 8.40 Hz, H-9), 7.78 (1H, d, J = 16.05 Hz, H-α), 7.65 (2H, d, J = 7.65 Hz, H-2).

*2.3. General Method for Synthesis of Kojic Acid-Chalcone Derivatives (**3a–c**).* Chalcone **2a–c** (1.26 g, 0.05 mmol) was added to a solution of **1** (0.71 g, 0.05 mmol), DMAP (0.12 g, 1 mmol), and DCC (1.03 g, 0.05 mmol) in DCM (30 mL). The reaction mixture was stirred at 0°C for 4 h, washed with acetic acid (1 M, 2 × 20 mL), followed by Na_2CO_3 solution (1 M, 2 × 20 mL), and dried over magnesium sulphate. The crude product obtained was purified by column chromatography over silica gel (eluting with 1 : 2 EA/Hex) to afford title compound.

*[6-(Hydroxymethyl)-4-oxo-pyran-3-yl] 4-[(E)-3-oxo-3-phenyl-prop-1-enyl] Benzoate (**3a**).* Compound **3a** (0.4 g, 23%) as a white powder. m.p. 162-163°C; (Found: C, 69.56; H, 4.25%. $C_{22}H_{16}O_6$ Requires C, 70.2; H, 4.3%); ν_{max} (KBr/cm^{-1}) 2987 (C=CH), 1730 (C=O ester), 1665 (C=O), 1644 (C=O), 1605 (aromatic), 1201 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.69 (1H, s, H-6), 8.11 (7H, m, H-9, H-10, H-15, H-β), 7.79 (2H, d, J = 16.05 Hz, H-α), 7.67 (1H, m, H-17), 7.59 (2H, m, H-16), 6.65 (1H, s, H-3), 5.02 (2H, s, H-7).

*[6-(Hydroxymethyl)-4-oxo-pyran-3-yl]4-[(E)-3-(4-bromophenyl)-3-oxo-prop-1-enyl] Benzoate (**3b**).* Compound **3b** (0.4 g, 19%) as a white powder. m.p. 194-195°C; (Found: C, 58.37; H, 3.24%. $C_{22}H_{15}BrO_6$ Requires C, 58.0; H, 3.3%); ν_{max} (KBr/cm^{-1}) 2926 (C=CH), 1697 (C=O ester), 1651 (C=O), 1623 (C=O), 1570 (aromatic), 1242 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.67 (1H, s, H-6), 8.13 (2H, d, J = 8.6 Hz, H-15), 8.12 (2H, d, J 8.00 Hz, H-10), 8.04 (1H, d, J = 15.45 Hz, H-β), 7.99 (2H, d, J = 8.00 Hz, H-16), 8.00 (1H, d, J = 12.6 Hz, H-α), 7.80 (2H, d, J = 8.55 Hz, H-9), 6.50 (1H, s, H-3), 4.40 (2H, s, H-7).

*[6-(Hydroxymethyl)-4-oxo-pyran-3-yl]4-[(E)-3-(4-chlorophenyl)-3-oxo-prop-1-enyl]benzoate (**3c**).* Compound **3c** (0.5 g, 25%) as a white powder. m.p. 202-203°C; (Found: C, 63.54; H, 3.31%. $C_{22}H_{15}ClO_6$ Requires C, 64.3; H, 3.7%); ν_{max} (KBr/cm^{-1}) 2927 (C=CH), 1731 (C=O ester), 1666 (C=O), 1643 (C=O), 1607 (aromatic), 1239 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.67 (1H, s, H-6), 8.13 (4H, t, J = 7.73 Hz, H-15, H-10), 8.04 (1H, d, J = 14.9 Hz, H-β), 7.99 (2H,

d, J = 8.0 Hz, H-16), 7.86 (1H, d, J = 11.45 Hz, H-α), 7.79 (2H, d, J = 7.45 Hz, H-9), 6.50 (1H, s, H-3), 4.39 (2H, s, H-7).

*2.4. Synthesis of Azobenzene Derivatives (**4a–d**).* HCl (8 M, 100 mL) was added drop wise to a solution of 4-aminoethylbenzoate (5 g, 30.0 mmol) in methanol (50 mL) in an ice bath at 0–5°C. Sodium nitrite (3.1 g, 45.0 mmol) in distilled water (10 mL) and phenol (3.4 g, 36 mmol) in methanol (50 mL) were added drop wise to the reaction mixture. NaOH solution (1 M, 100 mL) was then added drop wise to pH 8.5–9.5. The reaction mixture was stirred for 4 hr under nitrogen atmosphere. Methanol (50 mL) was added and acidified with HCl (8 M, 50 mL) to form precipitate. The solid was filtered, washed, and recrystallized from ethanol. KOH (0.13 g, 2.4 mmol) was added to the solid (0.2 g, 0.8 mmol) in methanol (50 mL) and heated under reflux for 4 h under nitrogen atmosphere. Cold water (30 mL) was added to the reaction mixture, acidified with acetic acid (8 M), and filtered and recrystallized from ethanol to give azobenzene intermediate. Azobenzene (1.5 g, 5 mmol) was added to a solution of bromoalkene derivatives, t- BuOK (0.62 g, 5.0 mmol) and KI (20 mg) in acetone (100 mL), and heated at reflux for 48 h under nitrogen atmosphere. DCM (50 mL) and water (30 mL) were added and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL) and the combined organic layers were dried and evaporated under reduced pressure and recrystallized from ethanol to give title compound **4a–d** as colorless crystals. FTIR and NMR data were consistent with the reported literature [8].

*2.5. Synthesis of Kojic Acid-Azobenzene (**5a–d**).* Thionyl chloride (0.036 g, 0.3 mmol) was added to a solution of **1** (0.014 g, 0.1 mmol) in DMF (20 mL) and heated under reflux for 4 h. The reaction mixture was extracted with DCM (2 × 15 mL). The organic layer was dried, filtered, and concentrated under reduced pressure to give kojyl chloride as yellowish needle solid (0.012 g, 70%). Azo intermediate was added to a solution of kojyl chloride prepared (0.1 g, 0.6 mmol) in DMF (30 mL) with TEA (0.015 g, 0.15 mmol) in dried acetone (20 mL) and heated under refluxed for 24 h. The reaction mixture was washed with dilute HCl (1 M, 2 × 20 mL). The organic layer was dried, filtered, and concentrated under reduced pressure. Crude product was recrystallized from ethanol to afford **5b–e** as colorless crystals. FTIR and NMR data were consistent with the reported literature [8].

2.6. Antibacterial Screening

2.6.1. Turbidimetric Kinetic Method. The antibacterial activities of synthesized **4a–d** and **5a–d** were screened against *Escherichia coli* using turbidimetric kinetic method. *Escherichia coli* were cultured on LB plate agar for 24 h at 37°C and a single colony was transferred to the media containing nutrient broth prepared. The inoculum was allowed to grow at 37°C with 250 rpm stirring overnight. Inoculum (0.2 mL) was added to 10 mL of culture medium which had been added with different concentrations (50 ppm, 80 ppm, and 100 ppm) of respective compounds in DMSO. Three replicates of each concentration were prepared. The control used was the medium broth of inoculums added with DMSO with no active compound. The mixture was shaken at 230 rpm at 37°C. The transmittances (T) of the aliquots were taken every 1 h interval for six continuous hours using UV-Visible Spectrophotometer Optima SP-300. The antibacterial activity was determined from the graph of In Nt versus time plotted. The In Nt values represent the number of colony forming units (units/mL) which follows the equation of In Nt = 27.1 − 8.56 T [38, 39]

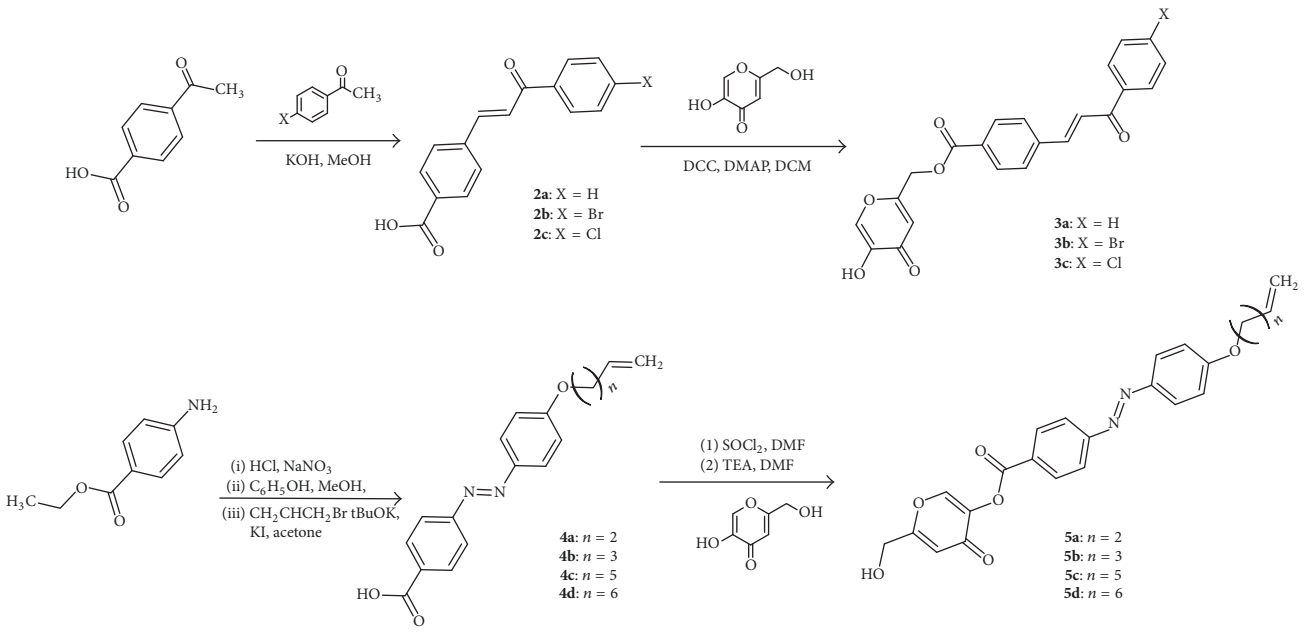
2.6.2. Via Kirby Bauer Disc Diffusion Method. The antibacterial activities of the synthesized **2a–c** and **3a–c** were evaluated against *Escherichia coli* and *Staphylococcus aureus* using Kirby Bauer Disc Diffusion method. *E. coli* and *S. aureus* were used as inoculums and cultured in Mueller-Hinton Broth (MHB) and incubated at 37.5°C with constant shaking at 150 rpm for 18 h. The bacteria suspension prepared was inoculated onto the entire surface of a Mueller-Hinton Agar (MHA) plate with sterilised cotton tipped swab to form an even lawn. Sterilised filter paper disc impregnated with 10 μL of the compound in DMSO was placed on the bacteria surface of MHA plate using a sterilised forcep. The plates were incubated at 37.5°C for 24 h. The zone of inhibition was measured in millimetres (mm) to estimate the potency of the tested compound.

3. Results and Discussion

3.1. Preparation of Kojic Acid via Fermentation. Kojic acid **1** was prepared via fermentation of sago hampas employing mixed strains of *Aspergillus flavus NSH9* and *Aspergillus flavus Link 44-1. Aspergillus flavus* was used for a higher yield of **1**[15]. The optimum parameters were from a combination of 3% (w/v) urea and 10% mineral salts solution containing KH_2PO_4, (w/v) $MgSO_4{\cdot}7H_2O$ and yeast. KH_2PO_4 and $MgSO_4$ supported the growth of bacteria strain and boosted the production of kojic acid [40]. Yeast is a nitrogen source for metabolic activation to enhance kojic acid production. Its high levels of essential components such as vitamins and oligoelements support growth of bacteria and fermentation process [3].

3.2. Synthesis of Kojic Acid Ester Derivatives. Kojic ester **2a–c** was prepared via Claisen Schmidt condensation followed by Steglich esterification with isolated kojic acid to yield **3a–c**, whereas kojic ester **(5a–d)** was prepared via kojyl chloride [11] followed by addition of **4a–d** to afford **5a–d** (70–87%). The overall synthesis is shown in Scheme 1. Structures of the compounds synthesized were confirmed by elemental analysis, IR spectra, and ^{1}H NMR spectra.

The IR spectra for **2a–c** showed absorptions band at 3064–2943 cm^{-1} and 1678–1663 cm^{-1} attributed to C=C and C=O group of chalcone linkage. A sharp band at 1612–1607 cm^{-1} indicated the presence of carboxyl group. Absorption peaks at 1583–1577 cm^{-1} revealed the presence of aromatic group. The ^{1}H NMR showed aromatic protons of **2a** at δ 8.17–7.59 ppm as doublet and triplet while aromatic protons of **2b-c** appeared at δ 8.20–7.65 ppm as four doublets with coupling constant 8–8.6 Hz. The peaks of CH_α = CH_β were observed at δ 7.79–7.78 and 8.05–8.00 ppm as



Scheme 1: Synthesis of kojic esters **(3a–c)** and **(5a–d)**.

two doublets with J_{ab} 15.5–16.1 Hz, which indicated *trans* configuration.

Formation of **3a–c** was confirmed by FTIR and ^{1}H NMR spectroscopies. The IR spectrum for **3a–c** showed appearance of C=O ester absorption band at 1731–1697 cm^{-1}. The IR spectrum revealed two strong bands at 1666–1651 cm^{-1} and 1644–1623 cm^{-1} attributed to ν(C=O) of chalcone and kojic acid, respectively. The absorption frequency at 1605–1570 cm^{-1} was attributed to aromatic group while ν(C-O) was represented by absorption band at 1242–1201 cm^{-1}. The ^{1}H NMR showed that all corresponding peaks indicated the formation of **3a–c**. The existence of kojic acid moieties in **3a–c** was proven by appearance of two kojic acid olefinic proton at δ 8.69–8.67 and 6.65–6.50 ppm as singlet. The oxymethyl protons of kojic acid were represented by peaks at δ 5.02–4.39 ppm.

The NMR spectra of kojic ester (**5a–d**) via formation of kojyl chloride revealed the presence of peak attributed to oxymethyl proton (OCH_2) at δ 5.26–5.24 ppm as singlet, which indicated 7-*O*-substituent **1** [41]. In comparison to 5-*O*-substituted **3a–c**, 7-*O*-substituted **5a–d** showed shifted H-6 olefinic proton signals to upfield and oxymethyl proton signals to downfield. Carbonyl group of ketone is more deshielded than carbonyl group of ester due to deshielding effect of pyrone ring [42].

3.3. Antibacterial Activities of Kojic Ester Derivatives. The kojic ester derivatives **3a–c** and **5a–d** were evaluated for antibacterial activities against gram negative strain *E. coli* at 37°C via turbidimetric method at three different concentrations, 50 ppm, 80 ppm, and 100 ppm, and compared to chalcones (**2a–c**) and azobenzene (**4a–d**) derivatives. Due to solubility limitation of **2a–c** and **3a–d** in organic solvent, turbidimetric method was only applied on **4a–d** and **5a–d**. Negative control, which is comprised of solvent and inoculums, showed significant increase in growth of *E. coli*. The antibacterial assay was expressed by plotting graph of In N_t versus time. The In N_t value indicated number of colony forming (growth of *E. coli*) units/mL which followed the expression of In N_t = 27.1 − 8.56 T [39].

Table 1: MIC for **4a–d** and **5a–d**.

Compounds	MIC (ppm)
4a	>400
4b	>400
4c	>400
4d	>400
5a	200
5b	190
5c	330
5d	330

Kojic ester **4a–d** exhibited weak inhibition while **5a–d** exhibited good inhibition against *E. coli*. The inhibition of **5a–d** is concentration dependent where 100 ppm > 80 ppm > 50 ppm. The minimum inhibitory concentration (MIC) of **4a–d** and **5a–d** was determined by extrapolating the concentration to zero growth rate of *E. coli*. The MIC of **4a–d** and **5a–d** is summarized in Table 1. Kojic ester moieties in azobenzene are envisaged to increase the lipophilic properties in **5a–d** to interact and destruct the bacterial cell membrane [43]. The presence of C=O and N=N moieties in kojic ester offers more reactive sites that easily protonated under acidic condition and reacted with the bacterial surfaces which further enhance the biological activity [44]. Compound **5a-b** showed better activity than **5c-d** due to the increase of

Table 2: Inhibition zones of **2a–c** and **3a–c**.

Compounds	Zone of inhibition (mm)	
	E. coli	*S. aureus*
2a	-	16.0
2b	-	13.0
2c	-	8.0
3a	-	8.0
3b	-	11.0
3c	-	13.0
DMSO	-	-
Kojic acid	-	-
Ampicillin	16.0	11.0

chain length after the cutoff effect associated with decrease in solubility.

Antibacterial activity of kojic esters **2a–c** and **3a–c** bearing chalcone moieties was conducted by measuring the zone of inhibition on agar plates (Kirby Bauer Disc Diffusion method) against *S. aureus* and *E. coli* bacterial strain due to solubility limitation in DMSO. Ampicillin was used as reference standard and DMSO as negative control.

The zone inhibition of **2a–c** and **3a–c** is shown in Table 2. Kojic acid showed no inhibition against both *S. aureus* and *E. coli,* while both series of **2a–c** and **3a–c** showed no inhibition against *E. coli* compared to standard ampicillin. Compounds **2a–c** and **3a–c**, however, showed active inhibition against *S. aureus* with **2c** and **3a**-**b** being inhibited better than ampicillin. The selective antibacterial activity of **2a–c** and **3a–c** against gram positive bacterial strain is due to the significance different in the membrane composition and architecture of gram positive and gram negative organism [45]. *S. aureus* has simpler mesh like cell membrane compared to *E. coli* which eases the penetration of compound [46].

Antibacterial activities of **2a–c** increased with the decrease in electronegativity of substituent on chalcone. Electronegativity in decreasing order is as follows: Cl (8 mm) > Br (13 mm) > H (16 mm). Polarized ability of antibacterial agent increases with the decreased in electronegativity which reasoned to enhance activity of acting antibacterial agent [47].

The antibacterial activities of **3a–c**, however, showed an opposite trend. The inhibition was increased with the increased in electronegativity. The incorporation of kojic acid transformed the lipophilic properties of the compound. A good balance between hydrophilic and hydrophobic properties of compound makes good antibacterial agent [48]. Compound **3c** with chlorine substituents showed the biggest zone of inhibition with 13 mm while **3a** with hydrogen showed the least zone of inhibition (8 mm).

4. Conclusions

A series of kojic esters **2a–c** and **3a–c** has been successfully synthesized and exhibited good antibacterial activity towards gram positive *S. aureus* bacteria strain. Antibacterial activities of **2a–c** increased with the decrease in electronegativity of halogen substituent on chalcone with **2c** showing the highest inhibition, better than the ampicillin reference standard. Lipophilic properties of kojic ester bearing chalcone derivatives **3a**-**b** inhibited better antibacterial activity. Kojic ester **5a–d** bearing azobenzene derivatives showed good inhibition against *E. Coli* compared to **4a–d**. The presence of C=O ester, N=N, and alkene chains has increased the lipophilic properties of of **5a–d** and disrupts the bacteria cell walls.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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