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Synthesis and Biological Activities of 3,5-Disubstituted-4-Hydroxycinnamic Acids Linked to a Functionalized Coumarin

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ABSTRACT

As part of our interest in the coumarin chemical nucleus, a series of 3,5-disubstituted-4-hydroxycinnamic acids to which 6-hydroxy-7-methoxy-4-methyl-3-isopropylcoumarin is grafted was synthesized via a straightforward synthetic method. Chemical identities of the synthesized conjugates were specified by analyzing their FTIR, ^{1}H-NMR, and ^{13}C-NMR spectra. Three biological activities were investigated for the final products along with that of the functionalized coumarin itself, which are: antioxidant, exploratory antitumor, and antimicrobial activities. The first activity was screened via DPPH test, while the second one was tested by a well-documented MTT assay against the following cell lines: SKG, AMN3, MCF-7, and HeLa. Third activity was assessed by an agar disk diffusion method versus four standard bacterial strains and two standard fungal strains. The test pathogens included *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Aspergillus niger*, and *Candida albicans*. The outcomes assumed from the antioxidant activity assay indicated that **M2** has a better activity and **M5** has less activity than the other synthesized conjugates, the same outcome is documented from studying the exploratory antitumor activity. Concerning the antimicrobial activity, the prepared conjugates, as well as the functionalized coumarin, showed an acceptable activity with an ascendant effect attributed to **M1**. Based on these findings, it is concluded that the synthesized conjugates **M1** and **M2**may be good candidates as antimicrobial and antitumor agents, respectively. Also, these conjugates may provide new scaffolds for the development of modern therapeutic agents.

Keywords: Hydroxycinnamic acid, Coumarin, Antitumor, Antioxidant, Antibacterial, Antifungal.

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INTRODUCTION

One of the advanced approaches for the development of new therapeutic agents is to build a novel scaffold by linking two or more bioactive compounds in a single entity. This approach is generally targeting complex diseases, especially those related to carcinogenesis, oxidative stress, and infection (1). Many chemical and enzymatic techniques can be used to afford covalently-linked conjugates(2,3). However, chemical conjugation is currently preferred because of its simplicity, flexibility, and monetary value(4).

For centuries, it has been realized that nature is an inexhaustible source of products that characterized by their chemical variety and biochemical particularity(5).Despite their structural intricacy, a vast number of naturally-derived products have been extensively investigated and they exhibited potential biological activities (6). It is reported that the chemical integration of these products, especially those of low molecular weights, may result in conjugates with improved mutual or additive pharmacological activities (7,8).

Since its original isolation by Vogelin 1820, coumarin and its derivatives have enticed many researchers to explore their various biological activities(9). Among them; the antitumor(10), antioxidant(11), anticholinesterase(12), and antimicrobial (13)activities of many coumarins have been extensively studied and the results showed that many of them have potential therapeutic effects. Based on a literature review initiated in November 2019, a functionalized coumarin of the chemical name 6-hydroxy-7-methoxy-4-methyl-3-isopropylcoumarin attracted the attention as a promising scaffold for the development of antitumor agents. This is because it exhibited excellent antitumor activity against many cancer cell lines(14–16).

Hydroxycinnamic acids are secondary metabolites that are highly distributed in the plant kingdom. These acids, such as *p*-coumaric, sinapic, caffeic and ferulic acids, account for around one-third of the total phenolic components found in the human diet(17,18). Hydroxycinnamic acids and their derivatives have appealed a great interest because they exhibit a significant antioxidant capacity(19,20)as well as other biological activities such as antimicrobial (21,22) and antitumor (23)effects. In one of our previous studies, many hydroxycinnamic acid derivatives were synthesized and investigated as antitumor agents. Only six derivatives of them showed a promising activity versus four common cancer cell lines(24); this encouraged the team to initiate this investigation.

The aim of this work is to synthesize new coumarin conjugates with improved biological activities. These conjugates were prepared by linking 6-hydroxy-7-methoxy-4-methyl-3-isopropylcoumarin to different 3,5-disubstituted-4-hydroxycinnamic acid derivatives. The investigated biological activities included the antioxidant capacity marked via DPPH, the exploratory antitumor activity estimated by MTT test contra four cancer cell lines, and the antimicrobial activity contra four standard bacterial strains and two standard fungal strains utilizing agar disc diffusion method.

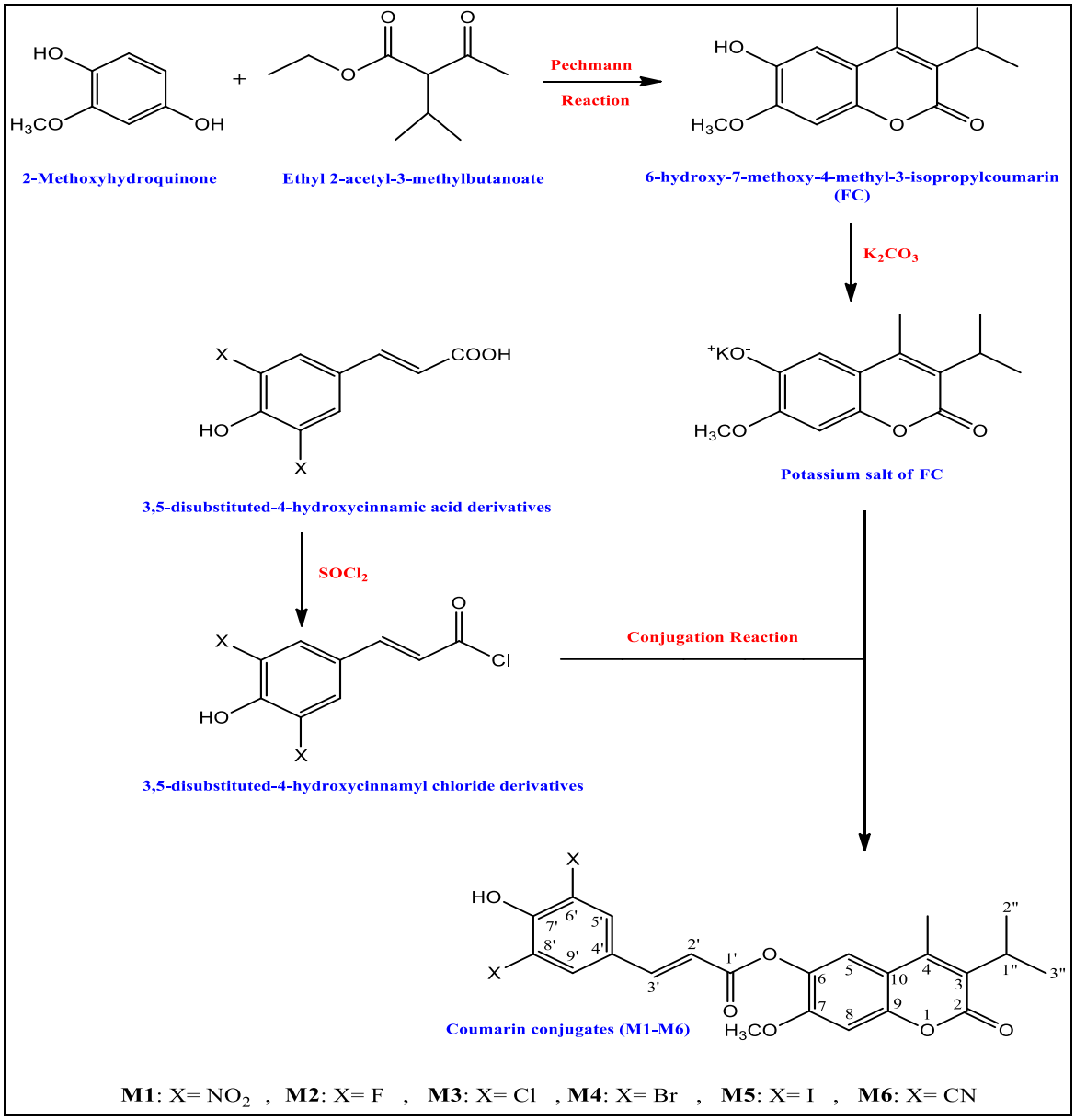
MATERIALS AND METHODS

Testing agents and chemicals utilized in the present study were acquired from well-documented international sources. Melting points of the synthesized conjugates have assessed

utilizing the CIA 9300 melting point instrument and these points were corrected. The ascendingly operated TLC using the previously coated silica gel plates (Merck) and eluted by a mobile phase composed from MeOH and $CHCl_3$(3:7)was performed to define the R_f values of conjugates. The wavelengths of maximum absorption and IR spectra of the prepared conjugates were scanned on Varian and Bruker-Alpha ATR spectrophotometers, respectively. Bruker (300 MHz) instrument was used to report the ^1H NMR and ^{13}C NMR spectra of the conjugates utilizing DMSO-*d6* as a solvent.

Synthesis of the coumarin conjugates

The steps of the chemical synthesis of coumarin conjugates (M1-M6) are displayed in Scheme 1.



Scheme 1: The synthetic steps of the coumarin conjugates (M1-M6).

Synthesis of 3,5-disubstituted-4-hydroxycinnamic acid derivatives

These derivatives were synthesized based on the article reported by *Mustafa* (2019) with no major modifications. The selected substitutions included six electron-withdrawal groups, which are NO_2, CN, F, Cl, Br, and I (24).

Synthesis of 6-hydroxy-7-methoxy-4-methyl-3-isopropylcoumarin (Functionalized coumarin, FC)

In a conical flask, the mixture of 2-methoxyhydroquinone (1.4 g, 10 mmol), ethyl 2-acetyl-3-methylbutanoate (5 mmol, 1.79 ml), and $BiCl_3$ (4 mmol, 1.26 g) was radiated for 90 min at 50°C in an ultrasonic water bath (40 kHz, 350 W, Power sonic410, Korea). To facilitate the reaction and enhance the solubility of inorganic catalyst, HCl (40 ml, 0.1 N) was added to the reaction mixture. The solid raw product was filtered, washed with cold H_2O (20 ml×3), and recrystallized from a mixture of $CHCl_3$:MeOH (5). Physical

properties and spectral data of this derivative were closely related to those reported by *Kawase et al* (2005)(15).

Synthesis of coumarin conjugates (M1-M6)

The suspension of FC (1.42 g, 5 mmol) and anhydrousK_2CO_3 (1.38 g, 5 mmol) in 20 ml ethanol was stirred at room temperature (RT) for 1 hr and subsequently concentrated under reduced pressure. The crude was filtered, dissolved in ethyl acetate (30 ml) and filtered. Under vacuum, the filtrate was evaporated to yield a potassium salt of FC(10).

To the cold solution of 3,5-disubstituted-4-hydroxycinnamic acid derivative (5 mmol) in 30 ml dry ether, a solution of newly distilled thionyl chloride (6 mmol, 0.9 ml) in 10 ml dry ether was dropwise added under dry atmospheric conditions with stirring. For 1 hr, the reaction mixture was heated at 40°C and then refluxed for 3 hr. The excess of thionyl chloride, as well as the solvent, were evaporated under vacuum. The crude product of acyl chloride was dissolved in 20 ml dry ether and dropwise added to the suspension of the potassium salt of FC in 30 ml dry ether. The reaction mixture was refluxed for 3 hr and the progress of the reaction was checked by TLC using ethyl acetate: ether (1:1) mixture as a mobile phase. The reaction was quenched by the addition of H_2O (2×25 ml). The ether layer was dried over anhydrous sodium sulfate and evaporated under vacuum (25).

Physical attributes and structural qualification of coumarin conjugates (M1-M6)

(E)-3-isopropyl-7-methoxy-4-methylcoumarin-6-yl-(7'-hydroxy-6',8'-dinitro) cinnamate (M1):Light yellow crystals($CHCl_3$: EtOH); 68% Yield; mp 137-140°C; UV (EtOH) λ_{max}423; R_f 0.43; IR ν_{max} 3344, 3042, 2910, 1718, 1611, 1540, 1469, 1322, 1230, 1026 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6): δ= 8.65 (2H, H-5', H-9', s), 8.07 (1H, H-3', d, *J*= 15 Hz), 7.52 (1H, H-5, s), 6.82 (1H, H-8, s), 6.60 (1H, OH-7', s), 6.52 (1H, H-2', d, *J*= 15 Hz), 3.91 (3H, OCH_3-7, s), 2.73 (1H, H-1", m, *J*= 6 Hz), 1.90 (3H, CH_3-4, s), 1.36 (6H, H-2", H-3", d, *J*= 6 Hz) ppm;^{13}C-NMR (75 MHz, DMSO-d_6): δ= 162.9 (C-1', C), 160.3 (C-2, C), 154.4 (C-7, C), 150.4 (C-7', C), 148.7 (C-9, C), 145.7 (C-3', CH), 144.9 (C-4, C), 136.0 (C-6, C), 136.9 (C-6', C-8', C), 135.2 (C-5', C-9', CH), 130.2 (C-4', C), 128.1 (C-3, C), 121.1 (C-5, CH), 120.6 (C-10, C), 119.3 (C-2', CH), 110.2 (C-8, CH), 48.6 (OCH_3-7, CH_3), 23.4 (C-1'', CH), 21.4 (C-2", C-3", CH_3), 19.7 (CH_3-4, CH_3) ppm.

(E)-3-isopropyl-7-methoxy-4-methylcoumarin-6-yl-(7'-hydroxy-6',8'-difluoro-) cinnamate (M2): White crystals ($CHCl_3$: EtOH); 82% Yield; mp 124-127°C; UV (EtOH) λ_{max}314; R_f 0.52; IR ν_{max} 3341, 3048, 2912, 1713, 1616, 1538, 1309, 1230, 1035 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.86 (1H, H-3', d, *J*= 15 Hz), 7.55 (1H, H-5, s), 6.80 (1H, H-8, s), 6.68 (2H, H-5', H-9', s), 6.48 (1H,OH-7', s), 6.32 (1H, H-2', d, *J*= 15 Hz), 3.96 (3H, OCH_3-7, s), 2.72 (1H, H-1'', m, *J*= 6 Hz), 1.93 (3H, CH_3-4, s), 1.32 (6H, H-2", H-3", d, *J*= 6 Hz) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 165.2 (C-1', C), 161.8 (C-2, C), 155.5 (C-7, C), 151.3 (C-6', C-8', C), 148.3 (C-9, C), 146.1 (C-3', CH), 144.1 (C-4, C), 136.1 (C-6, C), 134.2 (C-7', C), 131.3 (C-4', C), 129.9 (C-3, C), 121.5 (C-5, CH), 120.0 (C-10, C), 118.6 (C-2', CH), 116.6 (C-5', C-9', CH), 108.4 (C-8, CH), 50.1 (OCH_3-7, CH_3), 22.6 (C-1'', CH), 21.4 (C-2", C-3", CH_3), 20.7 (CH_3-4, CH_3) ppm.

(E)-3-isopropyl-7-methoxy-4-methylcoumarin-6-yl-(7'-hydroxy-6',8'-dichloro) cinnamate (M3):Off-white powder ($CHCl_3$: EtOH); 70% Yield; mp 130-133°C; UV (EtOH) λ_{max}322; R_f 0.50; IR ν_{max} 3335, 3042, 2897, 1718, 1620, 1540, 1311, 1092, 1030 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.82 (1H, H-3', d, *J*= 15 Hz), 7.50 (1H, H-5, s), 7.09 (2H, H-5', H-9', s), 6.83 (1H, H-8, s), 6.53 (1H, OH-7', s), 6.28 (1H, H-2', d, *J*= 15 Hz), 3.82 (3H, OCH_3-7, s), 2.66 (1H, H-1", m, *J*= 6 Hz), 1.84 (3H, CH_3-4, s), 1.39 (6H, H-2", H-3", d, *J*= 6 Hz) ppm;^{13}C-NMR (75 MHz, DMSO-d_6): δ= 166.3 (C-1', C), 160.4 (C-2, C), 155.8 (C-7', C), 152.0 (C-7, C), 149.9 (C-9, C), 146.2 (C-3', CH), 142.4 (C-4, C), 138.5 (C-6, C), 132.5 (C-5', C-9', CH), 130.9 (C-4', C), 128.1 (C-3, C), 122.8 (C-6', C-8', C), 120.2 (C-5, CH), 118.8 (C-10, C), 117.2 (C-2', CH), 111.7 (C-8, CH), 50.5 (OCH_3-7, CH_3), 22.8 (C-1", CH), 21.0 (C-2", C-3", CH_3), 18.9 (CH_3-4, CH_3) ppm.

(E)-3-isopropyl-7-methoxy-4-methylcoumarin-6-yl-(7'-hydroxy-6',8'-dibromo) cinnamate (M4):Off-white solid($CHCl_3$: EtOH); 65% Yield; mp 142-145°C; UV (EtOH) λ_{max}332; R_f 0.56; IR ν_{max} 3338, 3061, 2884, 1721, 1619, 1535, 1312,1048, 1021 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.78 (1H, H-3', d, *J*= 15 Hz), 7.57 (1H, H-5, s), 7.31 (2H, H-5', H-9', s), 6.77 (1H, H-8, s), 6.55 (1H, OH-7', s), 6.30 (1H, H-2', d, *J*= 15 Hz), 3.82 (3H, OCH_3-7, s), 2.79 (1H, H-1", m, *J*= 6 Hz), 1.99 (3H, CH_3-4, s), 1.37 (6H, H-2", H-3", d, *J*= 6 Hz) ppm;^{13}C-NMR (75 MHz, DMSO-d_6): δ= 168.0 (C-1', C), 164.3 (C-2, C), 160.4 (C-7', C), 154.8 (C-7, C), 148.4 (C-9, C), 146.0 (C-3', CH), 143.2 (C-4, C), 136.3 (C-5', C-9', CH), 133.6 (C-6, C), 130.1 (C-4', C), 126.4 (C-3, C), 121.8 (C-5, CH), 118.2 (C-10, C), 115.3 (C-2', CH), 112.9 (C-6', C-8', C), 109.2 (C-8, CH), 47.5 (OCH_3-7, CH_3), 22.3 (C-1", CH), 21.1 (C-2", C-3", CH_3), 20.0 (CH_3-4, CH_3) ppm.

(E)-3-isopropyl-7-methoxy-4-methylcoumarin-6-yl-(7'-hydroxy-6',8'-diiodo) cinnamate (M5):Whitesolid ($CHCl_3$: EtOH); 62% Yield; mp 115-117°C; UV (EtOH) λ_{max}307; R_f 0.59; IR ν_{max} 3336, 3056, 2920, 1717, 1624, 1542, 1314, 1039, 1003 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.82 (1H, H-3', d, *J*= 15 Hz), 7.57 (2H, H-5', H-9', s), 7.42 (1H, H-5, s), 6.78 (1H, H-8, s), 6.44 (1H, OH-7', s), 6.34 (1H, H-2', d, *J*= 15 Hz), 3.87 (3H, OCH_3-7, s), 2.78 (1H, H-1", m, *J*= 6 Hz), 1.90 (3H, CH_3-4, s), 1.35 (6H, H-2", H-3", d, *J*= 6 Hz) ppm;^{13}C-NMR (75 MHz, DMSO-d_6): δ= 178.0 (C-7', C), 166.4 (C-1', C), 163.2 (C-2, C), 153.7 (C-7, C), 147.1 (C-9, C), 145.4 (C-3', CH), 143.2 (C-4, C), 141.8 (C-5', C-9', CH), 135.7 (C-6, C), 132.2 (C-4', C), 127.5 (C-3, C), 123.3 (C-5, CH), 120.1 (C-10, C), 118.8 (C-2', CH), 107.3 (C-8, CH), 86.6 (C-6', C-8', C), 52.5 (OCH_3-7, CH_3), 22.5 (C-1", CH), 21.9 (C-2", C-3", CH_3), 18.9(CH_3-4, CH_3) ppm.

(E)-3-isopropyl-7-methoxy-4-methylcoumarin-6-yl-(7'-hydroxy-6',8'-dicyano) cinnamate (M6): Tancrystals ($CHCl_3$: EtOH); 57% Yield; mp 152-155°C; UV (EtOH) λ_{max}351; R_f 0.43; IR ν_{max} 3340, 3061, 2888, 2238, 1721, 1628, 1540, 1311, 1037 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.90 (1H, H-3', d, *J*= 15 Hz), 7.72 (2H, H-5', H-9', s), 7.46 (1H, H-5, s), 6.78 (1H, H-8, s), 6.51 (1H, OH-7', s), 6.36 (1H, d, H-2', *J*= 15 Hz), 3.84 (3H, OCH_3-7, s), 2.68 (1H, H-1", m,

J= 6 Hz), 1.99 (3H, CH_3-4, s), 1.37 (6H, H-2", H-3", d, J= 6 Hz) ppm;^{13}C-NMR (75 MHz, DMSO-d_6): δ= 167.9 (C-7', C), 164.6 (C-1', C), 162.4 (C-2, C), 155.7 (C-7, C), 148.9 (C-9, C), 147.0 (C-3', CH), 143.3 (C-4, C), 141.8 (C-5', C-9', CH), 134.8 (C-6, C), 131.4 (C-3, C), 129.5 (C-4', C), 124.1 (C-5, CH), 120.6 (C-10, C), 117.5 (C-2', CH), 112.4 (CN-6', C), 111.2 (C-8, CH), 100.8 (C-6', C-8', C), 50.8 (OCH_3-7, CH_3), 22.4 (C-1", CH), 21.8 (C-2", C-3", CH_3), 17.2 (CH_3-4, CH_3) ppm.

Biological investigations

Free radical scavenging capacity

Screening the antioxidant activity of the synthesized conjugates was performed using a well-documented DPPH (1,1-diphenyl-2-picryl-hydrazyl) test. In abbreviation, utilizing a methanolic solution (1 mM) of the investigated compound, the following serial concentrations were prepared: 200, 100, 50, 25, 12.5 μM. To a marked tube, the test methanolic solution (DPPH, 0.5 ml, 0.1 mM) and the tested methanolic solution (1.5 ml of the elected sample's concentration) were mixed and brooded at RT in a dark place for half an hour. The absorbance at a specific wavelength (517 nm) was followed spectroscopically versus a positive control composed from MeOH (1.5 ml) and DPPH (0.5 ml). The percentage of scavenging capacity (SC) was calculated utilizing the coming numerical rule:

$SC\ \% = (A_C - A_S/A_C) \times 100$

The abbreviations A_C and A_S refer to the absorbances of positive control and sample, respectively(26).

Exploratory cytotoxic activity

In a hub containing 96 holes, elected cell line was disseminated attaining 10,000 cells for an individual hole. After 24 hr, the holes were handled singly by a selected concentration of the screened compounds. The coming concentrations (200, 100, 50, 25, 12.5, 6.25 μg/ml) were applied and previously prepared from a standard (1mM) solution. Assay was conducted later on72 hr of handling by dismissing the medium, providing the test solution (MTT, 26μl, 3.24 mM), and subsequently brooding the handled cells at 37 °C for 1.5 hr. The absorbances of the handled hole (As) and unhandled hole (Ac) were measured via microplate reader aligned at492 nm. The percentage of growth inhibition (GI) was verified using the coming numerical role:

$GI\ \% = (A_U - A_T)/A_U \times 100$

The abbreviations A_U and A_T refer to the absorbances of unhandled and handled holes, respectively (27).

Studying the antimicrobial effects

Analysis of the antibacterial potential

The test bacterial strain was pre-cultured for 16 hr at 37°Cin a nutrient broth (5 ml) and its turbidity was revised by normal saline to 0.5 McFarland standard affording a net inoculum of 1.5 × 10^8 CFU/ml. Whattman's No. 3 filter papers were used to prepared disks of 2 mm in diameter which steeped with the DSMO solution (10 μl, 20 mg/ml)of each tested product. In a sterile environment, the pre-cultured bacterial broth (100μl) and molten agar (20 ml) were mixed and poured into cell-culture dishes. Once the mixture was solidified, the sterile forceps used to seed the prepared disks on agar. The zones of bacterial growth inhibition were detected next to the incubation period of 24 hr at 37°C, measured by Mitutoyo digital vernier caliper series 500 (USA), and reported in millimeters(21). The activity index (A_I) of the synthesized conjugates was determined utilizing the following numerical statement: A_I= Inhibition zone of product/ Inhibition zone of positive control(28).

Analysis of antifungal activity

The followed procedure was similar to that applied in the analysis of the antibacterial activity but with three important modifications including the use of Potato dextrose broth as a pre-culturing medium, Potato dextrose agar as a culturing medium, and Petri dishes was incubated at 30°C for 48 hr (29).

RESULTS AND DISCUSSION

Design of the synthetic pathway

The synthetic project of this work (as displayed in Scheme 1) started from the preparation of FC via a Pechmann condensation of 2-methoxyhydroquinoneandethyl 2-acetyl-3-methylbutanoate in the presence of Lewis acid. This coumarin was then treated with $K_2CO_3$to heighten the nucleophilic capability of phenolic hydroxyl group through its conversion into phenoxide. This attacks the carbonyl carbon of the prepared 3,5-disubstituted-4-hydroxycinnamyl chloride derivatives affording the final conjugates (M1-M6)(25). It is proposed that the nucleophilicity of the phenolic hydroxyl of 3,5-disubstituted-4-hydroxycinnamic acids is too weak to result in a self-condensation since there are two electron-withdrawing groups ortho to it(30). According to the spectroscopic data of the synthesized conjugates and their similitude to those found in the published reports (15,24), it is concluded that the structures depicted in Figure 1 represent those belonged to the synthesized conjugates.

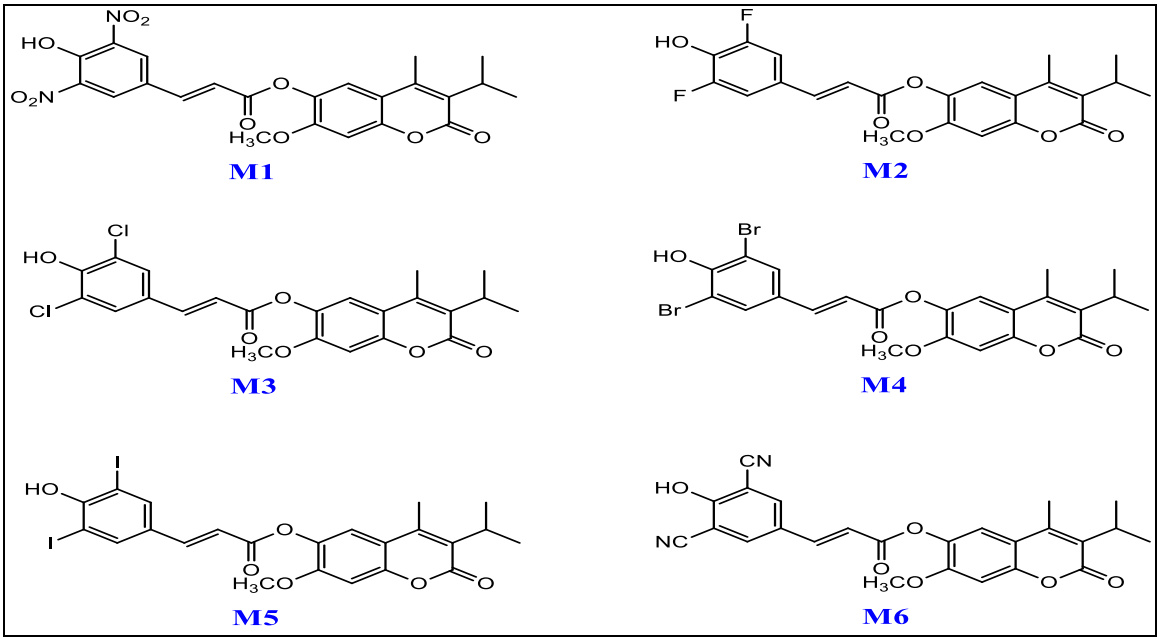


Figure 1: The chemical architectures of the synthesized conjugates.

Biological investigations

The effects of conjugating FC with 3,5-disubstituted-4-hydroxycinnamic acids and the effects of different substitutions found in the resultant conjugates were inspected via the in vitro biological activities. This inspection was carried out by comparing the exploratory antitumor, antioxidant and antimicrobial activities of the synthesized conjugates with those of the FC and reference compounds.

Antioxidant capacity

There are many reports investigated the antioxidant capacity of the natural and synthetic phenolic compounds(31). Most of these studies have attributed this effect to the capability of such compounds to donate their phenolic hydrogen in an H-transfer mechanism acting as chain-breaking antioxidants(32, 33).

The data presented in Table 1 and their presentation found in Figure 2 described that the SC_{50}, a concentration of the product demanded to capture fifty percentage of DPPH free radicals, values of M2 and M5 have attained the attention. M2 exhibited the best antiradical activity among the other synthesized conjugates and the FC. This may be due to that the presence of fluorides ortho to the phenolic hydroxyl group that may enhance the ability of this conjugate to donate its hydrogen atom from the aforementioned group (34). M5 exhibited the least antiradical activity and this is maybe due to the large atomic size of iodide which may hinder the donation of hydrogen from the phenolic hydroxyl group (35).

In general, the antiradical activities of the synthesized conjugates are better than that of FC, this can be attributed to the extended conjugated system which results from linking the FC and the utilized hydroxycinnamic acid derivatives (36).

Table 1: Results of examining the antioxidant and anticancer activities of the synthesized products and references.

Compound Name	Antioxidant activity SC_{50}± SD	Exploratory antitumor activity IC_{50} ± SD			
	DPPH free radicals scavenging capacity	SKG	AMN3	MCF-7	HeLa
R*	45.36 ± 0.72	22.17 ± 0.98	24.64 ± 1.20	12.86 ± 1.00	13.44 ± 0.92
FC	89.04 ± 1.32	43.56 ± 1.80	49.90 ± 1.80	39.17 ± 0.82	35.11 ± 1.78
M1	67.97 ± 1.10	140.60 ± 1.20	149.67 ± 1.90	137.10 ± 2.10	120.87 ± 0.98
M2	48.12 ± 0.90	20.56 ± 1.45	19.72 ± 1.35	12.71 ± 1.56	12.40 ± 1.85
M3	55.38 ± 0.90	82.84 ± 1.45	90.61 ± 1.85	78.12 ± 1.10	72.98 ± 1.95
M4	78.12 ± 1.05	92.58 ± 1.35	94.56 ± 0.90	90.45 ± 1.78	90.21 ± 1.45
M5	91.45 ± 1.43	148.29 ± 1.35	156.87 ± 1.82	137.35 ± 1.08	122.69 ± 1.10
M6	67.64 ± 1.20	60.35 ± 1.90	82.14 ± 1.72	66.69 ± 1.72	59.03 ± 0.90

* The reference (R) is either ascorbic acid for the antioxidant activity or 5-fluorouracil for antitumor activity.SD is calculated for three independent trials. $SC_{50}$and $IC_{50}$are expressed in μM.

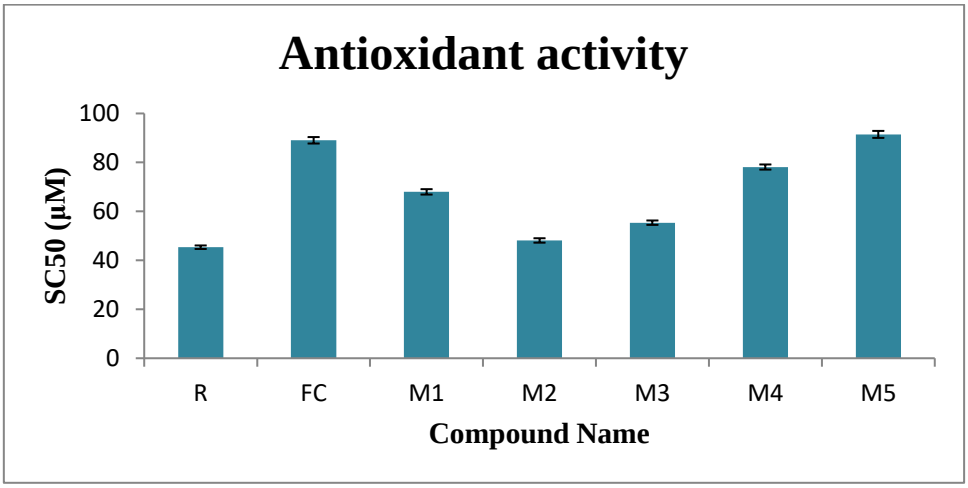


Figure 2: Graph of the antioxidant activity outcomes, which were assumed from DPPH check.

Exploratory antitumor activity

The synthesized conjugates and FC itself were screened for their exploratory antitumor activity versus four common human cancer cell lines including SKG (esophageal), AMN3 (murine mammary adenocarcinoma), MCF-7 (breast), and HeLa (cervix). This screening was performed via MTT check utilizing six sequent diluted concentrations of the screened compounds, as well as the cytotoxic drug 5-fluorouracil and the solvent DMSO as positive and negative standards, respectively.

The results shown in Table 1 and displayed in Figure 3 exhibited three interesting concerns; first is the synthesized conjugates except M2 revealed IC_{50} values greater than that of FC but less than that of positive control. Second concern is the outcomes of exploratory anticancer activity are robustly associated with those assumed from assaying the antiradical potential. This concern is correlated with many studies that attributed the antitumor activity of various compounds to their free radical scavenging activity (37–39). The third concern is the IC_{50} values of M2 versus the test cancer cell lines are less than those of the other conjugates, FC, and positive control. This exceptional activity of M2 may be related to the presence of fluorides in the chemical structure of this conjugate. It is hypothesized that these substituents may improve the physicochemical properties of the conjugate M2 by enhancing its aqueous solubility and cellular uptake (40).

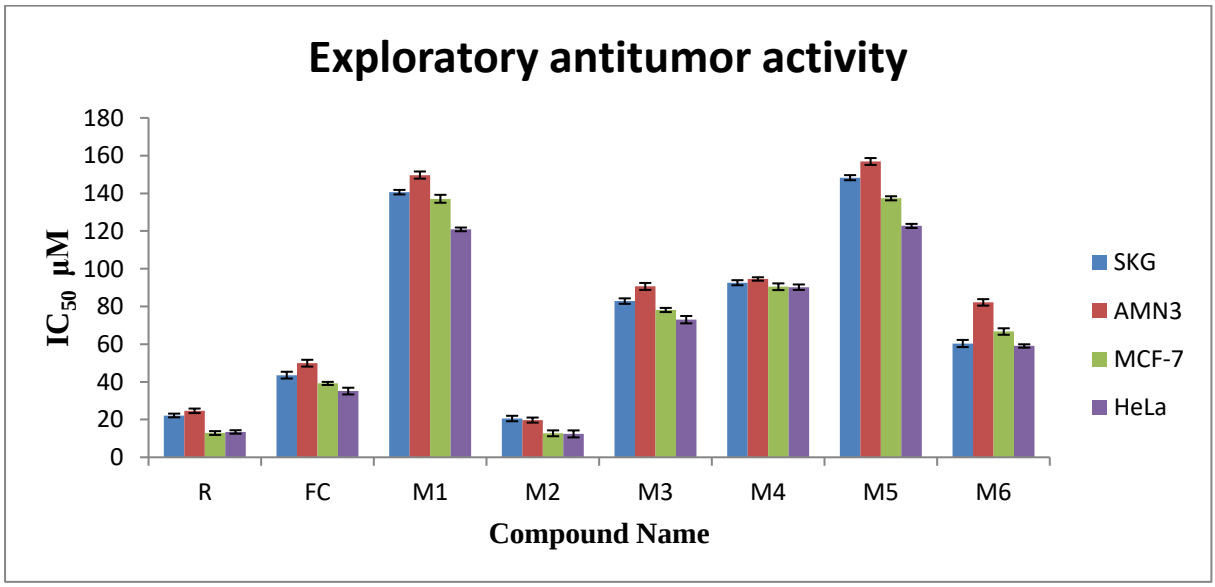


Figure 3: Graph of the exploratory anticancer outcomes, which were assumed from MTT check.

Antimicrobial activity

The antimicrobial activity of the synthesized conjugates was investigated against six standard pathogens via an agar disk diffusion method utilizing DMSO as a negative control, and Ciprofloxacin (for bacterial) or Nystatin (for fungi) as a positive control. The test bacteria were *Escherichia coli*,

Klebsiella pneumonia, Pseudomonas aeruginosa, and *Haemophilus influenzae* while the test fungi were *Aspergillus niger*, and *Candida albicans*.

The outcomes shown in Table 2 and their presentation found in Figure 4 reported two interesting issues; first is the prepared conjugates and FC itself have an acceptable antimicrobial activity but less than those of the references. The second issue is the M1 has the best antimicrobial activity among the synthesized conjugates and FC. It is hypothesized that the nitro groups found in this conjugate could improve its antimicrobial activity. It is demonstrated that the inclusion of the nitro group in the chemical structures of many antimicrobial agents has improved their activity. This may be attributed to the capability of this chemical group to modify many electronic and physicochemical properties of the compounds bearing this group (41, 42).

Table 2: Results of the antimicrobial activities for the prepared products and references.

Microorganisms	R*	FC	M1	M2	M3	M4	M5	M6
Escherichia coli ATCC 25922	32.63± 0.89	12.38 ± 1.35	28.01 ± 1.35	23.14 ± 1.45	26.32 ± 1.20	10.16± 1.25	10.32 ± 1.10	18.33 ± 1.90
Klebsiella pneumonia ATCC 700603	31.47 ± 1.00	23.49 ± 1.45	24.33 ± 1.20	24.36 ± 1.35	24.76 ± 1.45	22.12 ± 1.10	22.43 ± 0.89	22.15 ± 1.10
Pseudomonas aeruginosa ATCC 27853	35.32 ± 0.78	12.77 ± 0.90	22.89 ± 1.85	20.12 ± 1.90	20.43 ± 1.00	11.72 ± 1.25	11.58 ± 1.30	17.22 ± 1.35
Haemophilus influenzae ATCC 49247	27.46 ± 1.23	17.34 ± 1.80	22.16 ± 1.35	18.88 ± 1.85	20.78 ± 1.45	15.19 ± 1.75	15.46 ± 1.85	14.98 ± 1.80
Aspergillus niger ATCC 16888	12.22 ± 0.98	10.12 ± 1.35	11.04 ± 0.98	10.08 ± 0.98	10.76 ± 1.35	08.16 ± 1.45	07.95 ± 1.20	11.37 ± 1.45
Candida albicans ATCC 10231	19.08 ± 0.69	10.05 ± 1.10	13.86 ± 0.90	10.67 ± 1.35	10.16 ± 1.90	10.02 ± 1.20	09.41 ± 1.00	10.42 ± 1.20

* The reference (R) is either Ciprofloxacin (10 µg/disk) for the antibacterial activity or Nystatin (100 units/disk) for the antifungal activity. The results represent the growth inhibition zones expressed in mm ± SD, which is calculated for three independent trials.

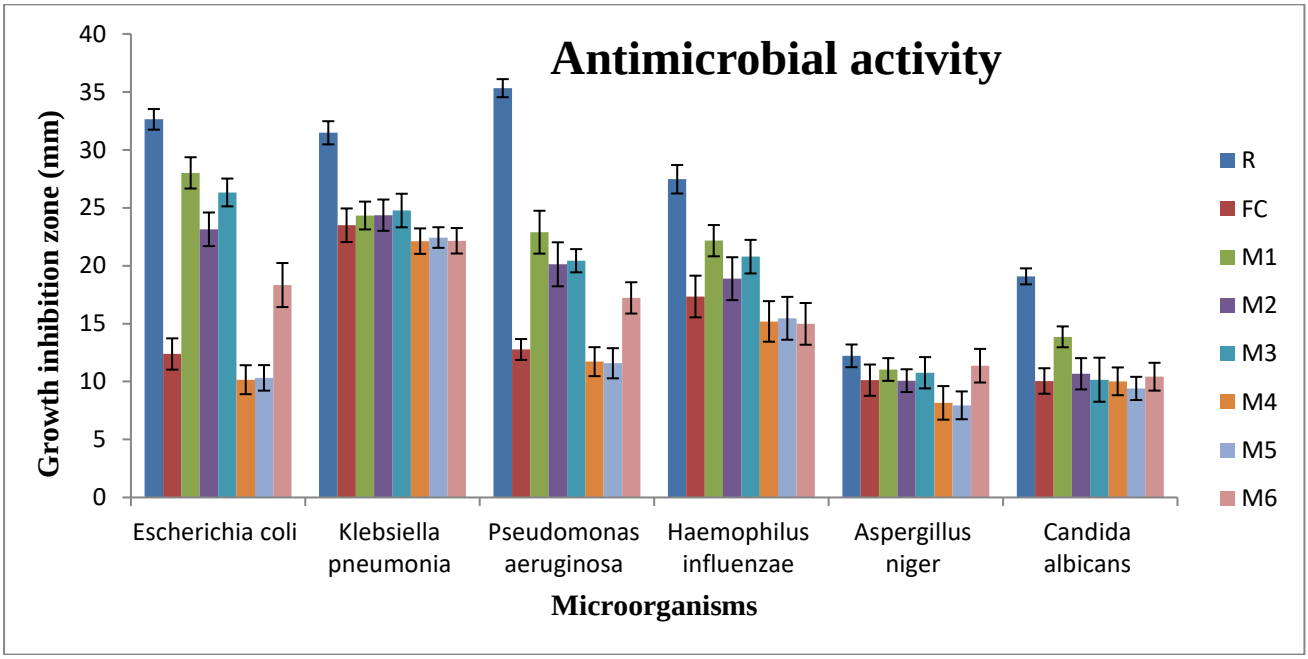


Figure 4: Graph of the antimicrobial activity outcomes, which were assumed by agar disk diffusion method.

CONCLUSIONS

This work described the preparation of six conjugates by grafting FC to a series of 3,5-disubstituted-4-hydroxycinnamic acids. From the applied biological studies, it is concluded three interesting issues; the first is the prepared conjugates have a good antiradical and antitumor activities with a superior activity contributed to M2. The second issue reveals that there is a potential positive correlation between the aforementioned activities suggesting that the possible mechanism of antitumor activity of these conjugates may be related to their antiradical activity. The last issue is the synthesized

conjugates possess a notable antimicrobial activity against the test pathogens with a superior action contributed to the conjugate M1.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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