Original Article

Effects of Structural Manipulation on the Bioactivity of some Coumarin-Based Products

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Abstract
Coumarin (2H-1-benzopyran-2-one) is a plant-derived natural product known for its pharmacological properties, such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, antiinflammatory, antidiabetic, oxidation, and neuroprotective. Two coumarin-based products were identified in the seeds of two apple phenotypes commonly known as Granny Smith and Red Delicious. This study aimed to evaluate the chemical manipulation of these coumarin-based products to more lipophilic semisynthetic compounds and trace the role of the phenolic hydroxyl group in the bioactivity of the parent natural products. The bioactivity evaluation included studying the potentials of the natural- and semisynthetic-coumarins as antioxidant, antineoplastic, antifungal, and antibacterial agents. At the first step, the antiradical potential of these products was evaluated versus the free radicals of hydroxyl and DPPH. The second potential was investigated utilizing an MTT-based photo assay versus several cancer-line cells, including SK-OV-3, MCF-7, KYSE-30, LC540, HeLa, AR-42J, AB12, and AMN3. The third and fourth potentials were recognized by conducting a disc-diffusion method against six infective bacterial strains and three fungal strains. The test bacteria were Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Haemophilus influenzae, Salmonella typhi, and Pseudomonas aeruginosa. On the other hand, the test fungi included Aspergillus flavus, Candida albicans, and Aspergillus niger. The results arising from these biopotentials revealed that the investigated functional group exerted a positive impact on the antiradical and antineoplastic potentials of the natural derivatives; however, they had a negative consequence on their antimicrobial potentials.

Keywords: Natural coumarins, Semisynthetic derivatives, Antiradical, Antineoplastic, Antibacterial, Antifungal, Structural manipulation

1. Introduction
Nature has been the primary source of bioactive products with a wide range of biologically biased behaviors due to the differences in their chemical properties and targeted biomolecules since ancient times (1). Investigation of the chemical properties of the isolated natural products and evaluation of their useful pharmacological processes may help speed up the drug development process (2-4).

Natural products with a coumarin backbone in their chemical structures have attracted a lot of interest, some of which are focused on their biomedical applications (5-11). Antimicrobial (12), antiradical (13), antineoplastic (14), anticholinesterase (15), and antidiabetic (16) effects are examples of these applications. Natural coumarins are isolated from varied sources involving the plant kingdom, where they have been detected in different plant materials (17).
The structural alteration of the natural bioactive products may reveal many new pharmaceutical-related applications, such as identifying the biotargets (18), binding interactions (19), and mode of action of these products (20). In addition, such chemical modification may minimize the mounting resistance versus the marketed drugs, alter their metabolic pathways, and transform the complex structure to a simple and easily prepared derivative with similar or improved bioactivity (20, 21).

The ultimate goal of structural alteration regarding natural products is to improve their drug-like properties. This is commonly accomplished by deleting, incorporating, or replacing specific functional groups to investigate their impact on the biophysicochemical and biomedical properties. Many natural pharmacophores with a phenolic hydroxyl group in their chemical backbones, which may exert a charming effect on the bioactivity, showed promising antiradical, antineoplastic, and antimicrobial activities.

This study aimed to identify the role of the phenolic hydroxyl group found in the chemical framework of two coumarin-based products in their bioactivity. These products have originally been isolated and characterized from the seeds of two apple phenotypes recognized as Granny Smith and Red Delicious. The aim was satisfying by transforming the investigated group phenotype to a more lipophilic one. Moreover, the antiradical, antineoplastic, and antimicrobial potentials of the original products and their semisynthetic compounds were estimated and compared in this study.

2. Materials and Methods

For this study, the biological and chemical reagents were procured from reputable international suppliers, including Bio-World, Scharlau, CHEM-LAB, Haihang, and Sigma-Aldrich. The seeded spots on the coated plates with silica gel were mobilized by an eluted system consisting of CH$_2$Cl$_2$: EtOH (3:1). An open-capillary method was utilized to specify the melting points (MP) of the derived compounds, while their infrared spectra obtained from the Bruker ATR photometer were examined for the presence of unique functional groups. The maximum absorption ($\lambda_{\text{max}}$) scores of the investigated natural products and semisynthetic compounds at varied wavelengths were specified by the apparatus named UVD-2950 (LABOMED). The chemical shifts for the protons (300 MHz) and carbons (75 MHz) found in the chemical structures of the semisynthetic compounds were reported by an instrument termed Bruker-AVANCEIII Spectrophotometer.

2.1. Synthetic Pathways of the Semisynthetic Compounds

The one-pot synthesis of the semisynthetic compounds RNs and GNs from their original precursors RN and GN, respectively, is displayed in figure 1.

2.1.1. Synthesis of the Semisynthetic Compound RNs

In a mortar, RN (0.554 g, 1.8 mmol) was mixed with anhydrous potassium carbonate (0.5 g, 3.6 mmol) under a solvent-free environment for 30 min. The resulted mixture was heated at 70°C for 60 min and subsequently diluted by anhydrous ethyl acetate to the reflux point for 3 h under anhydrous environment and then filtered. The gathered filtrate was treated with...
an equal amount of H$_2$O, and the ethyl acetate layer was vaporized under minimized pressure. The crude was translocated into a cube of powdered ice, filtered, washed with cold H$_2$O, and recrystallized from a mixture of diethyl ether: EtOH (2:1) mixture (22).

RN: Yellowish powder; Rf = 0.72, $\lambda_{\text{max}}$ (EtOH) = 318 nm; % yield = 71.89 (0.414 g); MP = 189-192°C; IR (cm$^{-1}$): 3094, 3054 (alkene C-H), 2892 (alkane C-H), 1733 (ester C=O), 1632, 1590 (Cis C=C), 1554 (aryl C=C), 1250, 1050 (alkyl-aryl ether C-O-C), 734 (C-Cl); $^1$H-NMR (DMSO-d$_6$, ppm): 8.08 (1H, d, $J$ = 9 Hz, H4), 6.70 (1H, s, H11), 6.22 (1H, d, $J$ = 9 Hz, H3), 4.35 (6H, s, H1', H2', H3'); $^{13}$C-NMR (DMSO-d$_6$, ppm): 160.8 (C2), 159.4 (C12), 146.7 (C5), 139.6 (C7), 137.6 (C9), 132.6 (C8), 115.5 (C3), 114.4 (C10), 112.9 (C6), 103.3 (C11), 64.2 (C13), 63.9 (C14), 62.6 (C2'), 30.9 (C1', C3').

2.1.2. Synthesis of the Semisynthetic Compound GNS

A conical flask protected from light by enveloping with an aluminum foil and containing a mixture of GN (0.944 g, 4 mmol) in 75 ml anhydrous ethyl acetate was settled in a salt-ice bath. As the temperature of the mixture dropped to 0°C, a cold solution of diiodomethane (0.16 ml, 2 mmol, CH$_2$I$_2$) in 6 ml anhydrous ethyl acetate was slowly added drop by drop. The reaction mixture was mixed at 90°C for 12 h, condensed, handled with H$_2$O (50 ml), and separated by CHCl$_3$ (3×25 ml). The gathered lipophilic layer was dehydrated and vaporized under minimized pressure. The titled compound was obtained by recrystallizing from CH$_2$I$_2$ (23).

GNS: White powder; Rf = 0.68, $\lambda_{\text{max}}$ (EtOH) = 279 nm; % yield = 48.02 (0.476 g); MP = 177-179°C; IR (cm$^{-1}$): 3061 (alkene C-H), 2904 (alkane C-H), 1726, 1703 (ester C=O), 1670 (Cis C=C), 1588 (aryl C=C), 1249, 1034 (aryl-alkyl ether C-O-C); $^1$H-NMR (DMSO-d$_6$, ppm): 7.76 (1H, d, $J$ = 9 Hz, H4), 7.53 ppm (1H, s, H5), 6.22 ppm (1H, d, $J$ = 9 Hz, H3), 5.95 (2H, s, H13), 4.20 (3H, s, H12); $^{13}$C-NMR (DMSO-d$_6$, ppm): 170.2 (C11), 160.9 (C2), 155.2 (C7), 145.4 (C9), 143.7 (C4), 137.5 (C8), 123.3 (C5), 115.4 (C3), 113.1 (C10), 110.1 (C6), 93.5 (C13), 53.5 (C12).

2.2 Biological Assessment

2.2.1. Antiradical Potential

The capacity of natural products RN and GN and their derivatives RNs and GNS to quench the DPPH-(2,2-diphenyl-1-picyrylhydrazyl) and hydroxyl-free radicals was assessed using vitamin C (VC) as a reference. A total of six concentrations for every tested product or compound, including 200, 100, 50, 25, 12.5, and 6.25 μM, were retrieved from an original methanolic (1mM) solution by a double-minimizing method. The trapping percent (TP%) was equaled to ($A_A-A_0/A_0$)×100. The abbreviations $A_0$ and $A_A$ reflected the absorbances of VC and the tested agent, respectively. By plotting the log concentrations of the tested agent versus TP%, the non-linear regression was used to calculate the TP$_{50}$ for three independent tryouts.

To investigate the capacity of the tested agent for quenching the DPPH radicals, a 1.5 ml sample was combined with 0.5 ml DPPH (0.1 mM). The mixture was coated with aluminum foil to be protected from light and inoculated for 30 min at an ambient temperature. To calculate the TP%, the mixture was inspected at 517 nm via spectroscopy. The blank is composed of a mixture of 0.5 ml DPPH (0.1 mM) and 1.5 absolute MeOH (7).

For the hydroxyl radicals trapping assay, the test mixture was prepared by the following subsequent additions: 1.5 ml tested agent, 2.4 ml potassium phosphate-buffered solution (pH 7.8, 200 mM), 60 μl FeCl$_3$ (1 mM), 90 μl ortho-phenanthroline monohydrate (1 mM), and 150 μl H$_2$O$_2$ (170 mM). The test mixture was inoculated for 5 min at an ambient temperature, examined spectrophotometrically at 560 nm versus a negative control containing all the above additions but the tested agent (3).

2.2.2. Primary Antineoplastic Potential

The neoplastic cells of an elected tumorline were localized in a 96-well microplate at the density of 4×10$^4$ cell/well. The wells were occupied with a defined
incubational medium and treated separately by varying concentrations ranging between 6.25 and 200 μM of the test agent for 24 h. The cell viability assessment was performed after an incubation period of 72 h by striping the incubational medium, disseminating the tetrazolium-based dye (28 μl, 3.27 mM, MTT), and subsequently inoculating the handled wells for 90 min at 37±1°C. The antineoplastic percent symbolized as Ap% of each derivative was calculated via the formula: Ap%=(Hu−Ht)/Hu×100. The Hu and Ht represent the absorbances of the untreated and treated holes, respectively. The IC50 values of the investigated derivatives were determined for three separate experiments by plotting the Ap% versus log concentration and calculated by non-linear regression (24).

2.2.3. Antimicrobial Potential

In the antibacterial assay, the selected strain was incubated at 37°C in 5 ml nutrient broth for 16 h. The final inoculum of 1.5×10^8 CFU/ml was acquired by adjusting the turbidity of the incubated mixture to 0.5 McFarland standards utilizing normal saline. Discs (0.2 cm in diameter) prepared from Whatman Grade 3 filter papers were moistened with the dimethyl sulfoxide (DMSO) solution (10 μl, 20 mg/ml) of the investigated derivative. The incubated mixture (100μl) and molten agar (20 ml) were combined under aseptic conditions and flowed into cell-culture plates. The prepared discs were seeded on the surface of solidified agar using aseptic forceps. Upon incubation for one day at 37°C, the inhibition sector (I) of the individual derivative was detected in millimeters via a Mitutoyo digital vernier caliper series 500. The activity index (AI) of the investigated derivative was calculated by applying the mathematical law: AI=I0/Ib (25). The symbols I0 and Ib represent the inhibition sectors achieved by the investigated derivative and reference, respectively. In the antifungal assay, a similar technique was followed with only two adjustments incubating for two days at 30°C and using Potato dextrose agar as a culturing medium (26).

3. Results and Discussion

3.1. Chemical Modification

The isolation and structural characterization of the natural coumarins (RN and GN) have been described previously (27, 28). To evaluate the impact of the phenolic hydroxyl groups of these coumarins, two semisynthetic coumarins (RNs and GNs) were synthesized in such a way as to eliminate the ability of these functional groups to act as a hydrogen-bond donor. This structural modification may consequently influence the physicochemical properties, including hydrophilicity (29).

For RN, the nucleophilicity of its phenolic hydroxyl group was improved via the deprotonation achieved by potassium carbonate. The resulting phenoxide attacks the alkylating agent and DMS, affording the formation of the semisynthetic derivative RNs. As a result, the influence of the phenolic hydroxyl group was covered by etherification (22). Concerning GN, its catecholic hydroxyl groups were shielded by their incorporation into 1,3-dioxolane ring under the effect of CH2I2 (23).

3.2. Biological Evaluation

3.2.1. Antiradical Effect

The trapping capacity of the natural and semisynthetic derivatives was tested versus DPPH and hydroxyl radicals. Many research papers reported the effects of various substituents on the antiradical efficiency of many natural and synthetic coumarins (30-32). This efficiency has been correlated to the number of phenolic hydroxyl groups linked to the aromatic component of the coumarin backbone (32) and the capability of the substituent ortho to the hydroxyl group to grant electrons (31, 33). This correlation is matched with the outcomes reported in table 1 and figure 1. In comparison with natural coumarins, the antiradical activity of their parallel semisynthetic derivatives is significantly declined. This may indicate the important role of the phenolic hydroxyl group (s) in the antiradical activity of the natural coumarins.
3.2.2. Primary Antineoplastic Effect

The investigated derivatives were screened for their primary antineoplastic activity utilizing MTT dye and six different concentrations. This investigation also incorporated 5-fluorouracil (5-FU) as a standard antineoplastic drug and DMSO as a solvent. The cancer cell lines involved in this preliminary test included HeLa (Epitheloid cervix carcinoma, 93021013), SK-OV-3 (Caucasian ovary adenocarcinoma, 91091004), AR42J (Rat exocrine pancreatic tumor, 93100618), MCF-7 (Caucasian breast adenocarcinoma, 86012803), AB12 (Mouse malignant mesothelioma, 10092306), KYSE-30 (Human Asian esophageal squamous cell carcinoma, 94072011), LC540 (Rat Fischer Leydig cell testicular tumor, 89031604), and AMN3 (murine mammary adenocarcinoma).

The outcomes manifested in table 2 and figure 2 report three main imports. Firstly, the investigated derivatives show higher IC50 values in comparison with that of 5-fluorouracil. Secondly, the antineoplastic activity of the natural derivatives versus the test cell lines is superior to that of their matching semisynthetic products. Finally, the decline observed in the antineoplastic activity of the semisynthetic derivatives is parallel to the lowering in their antiradical activity. In the literature, many studies have assigned the antitumor activity of diverse natural and synthetic coumarins with their antiradical activity (34-36).

### Table 1. Results of the antiradical activity of natural and semisynthetic coumarins

<table>
<thead>
<tr>
<th>Derivative symbol</th>
<th>Scavenger activity versus DPPH free radicals (µM±SD, n=3)</th>
<th>Scavenger activity versus hydroxyl free radicals (µM±SD, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC</td>
<td>46.29±0.67</td>
<td>50.33±0.91</td>
</tr>
<tr>
<td>RN</td>
<td>64.18±0.90</td>
<td>68.48±0.95</td>
</tr>
<tr>
<td>GN</td>
<td>48.20±0.86</td>
<td>52.84±0.76</td>
</tr>
<tr>
<td>RNs</td>
<td>89.31±1.05</td>
<td>101.06±0.90</td>
</tr>
<tr>
<td>GNs</td>
<td>114.05±0.81</td>
<td>107.14±0.72</td>
</tr>
</tbody>
</table>

3.2.3. Antimicrobial Effect

The natural and semisynthetic derivatives were scanned for their antimicrobial activity utilizing a well-defined agar disc dissemination method (23). This method involved the employment of DMSO as a negative control and a standard antimicrobial agent as a positive control, which was either ciprofloxacin (10 µg/disc, CP) for the antibacterial activity or nystatin (100 units/disc, NY) for the antifungal activity.

The test pathogens involved six standard bacterial and three standard fungal sorts. The experimental bacteria were Escherichia coli (ATCC 25922, Ec), Salmonella typhi (ATCC 6539, St), Klebsiella pneumonia (ATCC 700603, Kp), Haemophilus influenzae (ATCC 49247, Hi), Shigella dysenteriae (ATCC 13313, Sd), and Pseudomonas aeruginosa (ATCC 27853, Pa). The fungal sorts encompassed Candida albicans (ATCC 10231, Ca), Aspergillus flavus (ATCC 9643, Af), and Aspergillus niger (ATCC 16888, An).

The data recorded in tables 3-6 and their graphical representations displayed in figures 3-7 reveal four main points. The first is that the antimicrobial activity of the investigated derivatives was lower than that of the standard. The second issue is that the semisynthetic derivatives showed a towering antimicrobial effect in comparison with their corresponding natural products. The third one is that the semisynthetic derivative RNS had a more inhibitory effect on the growth of the tested bacteria than those of the GNS and natural products.
derivatives. The last issue is that the semisynthetic derivative GNs had a more inhibitory effect on the growth of the tested fungi than those of the RNS and the natural derivatives.

Table 2. Results of the primary antineoplastic activity of the investigated derivatives

<table>
<thead>
<tr>
<th>Cancer cell line</th>
<th>Derivative symbol</th>
<th>5-FU</th>
<th>RN</th>
<th>GN</th>
<th>RNs</th>
<th>GNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td></td>
<td>13.11±0.80</td>
<td>20.18±1.00</td>
<td>25.11±0.90</td>
<td>57.63±1.10</td>
<td>55.54±1.05</td>
</tr>
<tr>
<td>SK-OV-3</td>
<td></td>
<td>22.16±1.05</td>
<td>29.58±0.90</td>
<td>31.58±1.00</td>
<td>62.91±0.95</td>
<td>73.36±0.95</td>
</tr>
<tr>
<td>AR42J</td>
<td></td>
<td>19.86±0.95</td>
<td>28.09±1.10</td>
<td>30.32±1.15</td>
<td>44.67±0.80</td>
<td>62.48±0.95</td>
</tr>
<tr>
<td>MCF-7</td>
<td></td>
<td>12.46±1.10</td>
<td>22.81±1.10</td>
<td>24.17±0.85</td>
<td>47.82±1.20</td>
<td>54.56±0.90</td>
</tr>
<tr>
<td>AB12</td>
<td></td>
<td>18.93±1.25</td>
<td>28.90±1.35</td>
<td>28.69±0.80</td>
<td>61.94±1.05</td>
<td>59.18±1.00</td>
</tr>
<tr>
<td>KYSE-30</td>
<td></td>
<td>29.38±1.05</td>
<td>40.12±1.05</td>
<td>33.88±0.95</td>
<td>60.87±1.45</td>
<td>67.55±1.15</td>
</tr>
<tr>
<td>LC540</td>
<td></td>
<td>23.67±0.85</td>
<td>52.47±1.10</td>
<td>47.17±1.05</td>
<td>83.04±1.20</td>
<td>76.48±1.05</td>
</tr>
<tr>
<td>AMN3</td>
<td></td>
<td>24.64±1.20</td>
<td>37.63±1.10</td>
<td>42.11±1.15</td>
<td>49.37±1.00</td>
<td>59.32±1.05</td>
</tr>
</tbody>
</table>

The outcomes are represented as IC50±SD. The IC50 value was computed in μM, while the standard deviation (SD) was calculated for three separate experiments.

Table 3. Results of the antibacterial activity of the natural and semisynthetic derivatives

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>CP</th>
<th>RN</th>
<th>GN</th>
<th>RNs</th>
<th>GNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec</td>
<td>32.63±0.90</td>
<td>10.54±1.15</td>
<td>12.98±1.05</td>
<td>22.16±1.30</td>
<td>19.16±1.25</td>
</tr>
<tr>
<td>St</td>
<td>26.12±1.05</td>
<td>9.84±0.95</td>
<td>10.02±1.15</td>
<td>19.50±1.00</td>
<td>14.05±1.20</td>
</tr>
<tr>
<td>Kp</td>
<td>31.47±1.00</td>
<td>12.47±1.05</td>
<td>11.59±0.95</td>
<td>20.81±0.95</td>
<td>20.57±1.00</td>
</tr>
<tr>
<td>Hi</td>
<td>27.46±1.25</td>
<td>10.46±1.00</td>
<td>12.11±1.05</td>
<td>20.67±1.00</td>
<td>18.82±1.15</td>
</tr>
<tr>
<td>Sd</td>
<td>24.56±1.00</td>
<td>8.22±1.00</td>
<td>13.28±1.35</td>
<td>21.04±1.20</td>
<td>21.24±1.05</td>
</tr>
<tr>
<td>Pa</td>
<td>35.32±1.05</td>
<td>6.22±0.95</td>
<td>11.67±1.15</td>
<td>18.24±1.05</td>
<td>23.59±0.95</td>
</tr>
</tbody>
</table>

The outcomes represent the means of the inhibition sectors expressed in mean±SD, which was detected for three separate experiments.

Table 4. Outcomes assumed from examining the antifungal activity of the natural and semisynthetic derivatives

<table>
<thead>
<tr>
<th>Fungus</th>
<th>NY</th>
<th>RN</th>
<th>GN</th>
<th>RNs</th>
<th>GNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>19.08±0.90</td>
<td>7.18±1.15</td>
<td>4.44±1.05</td>
<td>11.45±1.10</td>
<td>14.05±0.85</td>
</tr>
<tr>
<td>Af</td>
<td>13.67±1.05</td>
<td>6.89±1.00</td>
<td>5.37±0.85</td>
<td>9.11±1.25</td>
<td>11.36±1.05</td>
</tr>
<tr>
<td>An</td>
<td>12.22±0.95</td>
<td>6.93±0.90</td>
<td>4.28±0.85</td>
<td>8.14±1.20</td>
<td>9.22±1.10</td>
</tr>
</tbody>
</table>

The outcomes represent the means of the inhibition sectors expressed in mm±SD, which was detected for three separate experiments.

Table 5. Values of A50 for the natural and semisynthetic derivatives versus the experimental bacteria

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>RN</th>
<th>GN</th>
<th>RNs</th>
<th>GNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec</td>
<td>0.32</td>
<td>0.40</td>
<td>0.70</td>
<td>0.59</td>
</tr>
<tr>
<td>St</td>
<td>0.38</td>
<td>0.38</td>
<td>0.75</td>
<td>0.54</td>
</tr>
<tr>
<td>Kp</td>
<td>0.40</td>
<td>0.37</td>
<td>0.66</td>
<td>0.65</td>
</tr>
<tr>
<td>Hi</td>
<td>0.38</td>
<td>0.44</td>
<td>0.75</td>
<td>0.69</td>
</tr>
<tr>
<td>Sd</td>
<td>0.33</td>
<td>0.54</td>
<td>0.86</td>
<td>0.87</td>
</tr>
<tr>
<td>Pa</td>
<td>0.18</td>
<td>0.33</td>
<td>0.52</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Table 6. Values of A50 for the natural and semisynthetic derivatives versus the experimental fungi

<table>
<thead>
<tr>
<th>Fungus</th>
<th>RN</th>
<th>GN</th>
<th>RNs</th>
<th>GNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.38</td>
<td>0.23</td>
<td>0.60</td>
<td>0.73</td>
</tr>
<tr>
<td>Af</td>
<td>0.50</td>
<td>0.39</td>
<td>0.67</td>
<td>0.83</td>
</tr>
<tr>
<td>An</td>
<td>0.57</td>
<td>0.35</td>
<td>0.66</td>
<td>0.75</td>
</tr>
</tbody>
</table>
The towering antimicrobial activity of the semisynthetic derivatives may be assigned to the replacement of the hydroxyl group found in their corresponding natural derivatives with less hydrophilic moiety. This replacement may increase the total lipophilicity of the semisynthetic derivatives resulting in the enhancement of their permeation into the microorganisms (37, 38). In addition, it is believed that the presence of two aryl-alkyl ether groups in the ortho or para position to each other could enhance the antimicrobial activity of various natural and semisynthetic coumarins (39).

Figure 3. Graphical representation of the data collected from assaying the antineoplastic activity of the investigated derivatives and positive control

Figure 4. Graphical representation of the data collected from examining the antibacterial activity of the investigated derivatives and positive control

Figure 5. Graphical representation of the data collected from examining the antifungal activity of the investigated derivatives and positive control

Figure 6. Graphical representation of the A1 values for the investigated derivatives as antibacterial agents

Figure 7. Graphical representation of the A1 values for the investigated derivatives as antifungal agents

The towering antimicrobial activity of the semisynthetic derivatives may be assigned to the replacement of the hydroxyl group found in their corresponding natural derivatives with less hydrophilic moiety. This replacement may increase the total lipophilicity of the semisynthetic derivatives resulting in the enhancement of their permeation into the microorganisms (37, 38). In addition, it is believed that the presence of two aryl-alkyl ether groups in the ortho or para position to each other could enhance the antimicrobial activity of various natural and semisynthetic coumarins (39).
This study reported the chemical modification of two natural coumarins to evaluate the role of their hydroxyl groups in biological activity. It can be concluded from the results of this study that the phenolic hydroxyl groups are important for the antiradical and antitumor activities, while they may contribute to lower antimicrobial activity.

**Authors’ Contribution**

Study concept and design: Y. F. M.
Acquisition of data: R. R. K.
Analysis and interpretation of data: E. T. M.
Drafting of the manuscript: M. K. B.
Critical revision of the manuscript for important intellectual content: M. K. O.
Statistical analysis: Y. F. M.
Administrative, technical, and material support: R. R. K.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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**References**

28. Mohammed ET, Mustafa YF. Coumarins from red delicious apple seeds: extraction, phytochemical analysis, and evaluation as antimicrobial agents. Syst Rev Pharm. 2020;11.