Isolation, Biological Evaluation, and Molecular Docking Studies of Compounds from *Sophora mollis* (Royle) Graham Ex Baker

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**ABSTRACT:** The *Sophora mollis* is one of the best anti-inflammatory, antioxidant, and anticancerous plant; therefore, the isolated chemical constituents, that is, scopoletin (1), pinitol (2), 2-propenoic acid, 3-(3,4-dihydroxyphenyl)-octacosyl ester (3), betulin (4), and β-sitosterol glucoside (5) were tested for these folklores. The structures of the isolated compounds were confirmed by 1H NMR, 13C NMR, 2D-NMR, and mass spectral data. The anti-inflammatory, anticancer, antiglycation, and antioxidant activities of compounds 1–5 were evaluated using different assays. Compound 1 exhibited significant anti-inflammatory effect as it reduced edema of the paw (83.98%), which is more potent than the standard drug (ibuprofen) (which showed an inhibition percentage of 73.22% a), followed by compound 3. Furthermore, compound 3 showed significant free-radical scavenging activity using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free-radical assay. Percentage inhibition of DPPH recorded was 95.646 ± 0.003, 94.766 ± 0.014, and 94.516 ± 0.011% at concentrations of 400, 200, and 100 μg/mL, respectively. Evaluation of anticancer activity of isolated compounds reveals weak effect against HeLa and 3T3 cell lines. Docking studies of the most active compound into the binding sites of cyclooxygenase isoforms showed a better antagonistic potential against COX-1 than the COX-2 isofrom.

1. **INTRODUCTION**

Genus *Sophora*, belonging to the Fabaceae family, contains more than 52 species distributed in Australia, Southeast Europe, western South America, the United States, and Asia.1,2 Species of the genus *Sophora* play an important role in traditional medicine, where they have been extensively used for the treatment of various diseases.3,4 Genus *Sophora* is a rich source of bioactive metabolic compounds. According to recently published studies, more than 300 compounds have been isolated and identified from *Sophora* species and their therapeutic/pharmacological properties evaluated.2 *Sophora mollis* is a medicinal plant belonging to the genus *Sophora*. It grows in Afghanistan, Pakistan, Iran, India, Nepal, and China.5 Research findings revealed numerous metabolites such as flavonoids and alkaloids isolated from *S. mollis*.5–7 Moreover *S. mollis* exhibits significant analgesic, anti-inflammatory,8 anti-plasmodial,9 antibacterial,10 antioxidant, and cytotoxic activities.11 Because of the wide continued interest in the bioactivity profile of *S. mollis*, the aim of the present study was to isolate and characterize compounds from *S. mollis* and to evaluate the anti-inflammatory, anticancer, antiglycation, and antioxidant activities of these compounds.

2. **RESULTS AND DISCUSSION**

2.1. **Isolation and Characterization of Pure Compounds.** The chloroform (138 g) and ethyl acetate fractions (117 g) of the aerial parts of *S. mollis* extract were combined and then subjected to a phytochemical investigation, which led to the isolation of five compounds. The isolated compounds were identified by using mass and NMR (1H, 13C, and 2D) spectral data as: scopoletin (1), pinitol (2), 2-propenoic acid,
3-(3,4-dihydroxyphenyl)-octacosyl ester (3), betulin (4), and β-sitosterol glucoside (5). Structures of these compounds are shown in Figure 1. Chemical and physical properties of isolated compounds were in agreement with reported data.

Compound 1: Scopoletin (7-hydroxy-6-methoxy-2H-chromen-2-one) was isolated from the CHCl3/EtOAc subfractions as a yellowish crystalline solid. The compound showed blue fluorescence under UV light. The mass spectrum of this compound based on its electrospray ionization mass spectrometry (ESI-MS) displayed a molecular ion \( m/z \): 192 corresponding to the molecular formula C_{10}H_{8}O_{4}. The structure of this compound was determined with the aid of both one- and two-D NMR spectral data including \(^1\)H, \(^{13}\)C NMR, and heteronuclear multiple-bond correlation (HMBC)/COrrelated SpectroscopY (COSY)/heteronuclear single quantum coherence. The \(^1\)H NMR spectrum (CD\(_3\)OD, 500 MHz) showed signals due to the methyl and four methine protons, whereas the \(^{13}\)C NMR spectrum revealed the five quaternary carbon atoms in the structure. The three methyl protons appeared as singlets at 3.90 ppm, whereas each of the two aromatic protons appeared as a doublet at 6.21 and 7.86 ppm (H-3 and H-4), respectively. On the other hand, two aromatic protons appeared as singlets at 6.77 and 7.10 ppm (H-5 and H-8).

\(^{13}\)C NMR (CD\(_3\)OD, 125 MHz): \( \delta \) 164.1 (C-2), 56.8 (C-1′), 104.0 (C-8), 110.0 (C-5), 112.6 (C-3), 146.1 (C-6), 151.5 (C-9), 112.6 (C-10), 153.0 (C-7), 147.1 (C-4). The COSY spectrum showed the correlation of H-3 with H-4 (Figure 2). Similarly, the HMBC spectrum showed correlations of H-3 with C-3 and C-2, whereas H-4 showed correlation with C-2, C-9, and C-10; H-5 showed correlation with C-4, C-6, and C-7. H-8 showed correlation with C-7, C-6, and C-9, while H-1′ showed correlation with C-1′ and C-6 (Figure 2). In conclusion, these spectral data agree with those of scopoletin.

Compound 2: Pinitol was obtained from the CHCl3/EtOAc subfractions as white crystals. The molecular formula (C\(_{15}\)H\(_{10}\)O\(_7\)) was determined from its ESI-MS mass spectrum, which displayed a molecular ion of m/z: 194. The chemical structure of this compound was confirmed by means of NMR spectral data (\(^1\)H NMR, \(^{13}\)C NMR, and 2D NMR). The \(^1\)H NMR spectrum revealed methyl protons signal at 3.48 ppm (3H, s, H-1′) and six methine hydrogens. The H-1 and H-2 appeared as triplets at 3.22 (1H, t, \( J = 19.2 \) Hz, H-1) and 3.57 (1H, t, \( J = 19.2 \) Hz, H-2), respectively. On the other hand, each of H-3 and H-6 appears as a doublet at 3.65 (1H, dd, \( J_1 = \ldots \))
2.4 Hz, \( J_2 = 2.4 \) Hz) and 3.70 (1H, dd, \( J_1 = 2.8 \) Hz, \( J_2 = 2.0 \) Hz), respectively, whereas H-4 and H-5 resonated as doublets at 3.88 (1H, d, \( J = 4.0 \) Hz) and 3.90 (1H, d, \( J = 4.0 \) Hz), respectively.

\[ ^{13}C \text{NMR (D}_2\text{O, 125 MHz) and DEPT spectra of compound 2 showed seven signals corresponding to one methyl and six methine carbons. These signals appeared at } \delta 62.5 (C-1'), 85.5 (C-1), 74.5 (C-2), 73.0 (C-3), 74.0 (C-4), 74.0 (C-5), and 72.2 (C-6). The COSY spectrum confirmed the correlations of H-1 with H-2 and H-6, H-2 with H-1 and H-3, H-3 with H-4, and H-5 with H-6 (Figure 3A). Similarly, the HMBC spectrum confirmed the correlation of H-1 with C-2, C-6 and C-1', H-2 with C-1 and C-3, H-3 with C-2, H-6 with C-1, H-5 with C-1, and H-4 with C-5 (Figure 3B). These spectral data are in agreement with those reported for pinitol.\)

\[ ^{13}C \text{NMR (CDCl}_3, 100 MHz) and Distortionless Enhancement by Polarization Transfer (DEPT) spectra of compound 3 displayed 14 distinct signals at } \delta 114.0 (C-2), 144.6 (C-3), 146.5 (C-4), 122.5 (C-5), 115.5 (C-6), 145.0 (C-7), 114.6 (C-8), 168.0 (C-9), 64.6 (C-1'), 32.1 (C-2'), 31.2 (C-3'-26'), 23.0 (C-27'), and 14.5 (C-28'). The COSY spectrum showed correlations of H-5 with H-6, H-7 with C-6, C-5, and C-4, H-6 with C-6, C-5, and C-4, H-7 with C-8, C-6, C-5, and C-9, and H-8 with C-7 and C-9. COSY and HMBC correlations are depicted in Figure 4. These spectral data are similar to those reported for 2-propenoic acid, 3-(3,4-dihydroxyphenyl)-octacosyl ester.\)

\[ ^{13}C \text{NMR (CDCl}_3, 100 MHz) and Distortionless Enhancement by Polarization Transfer (DEPT) spectrum of compound 3 showed seven signals corresponding to one methyl and six methine carbons. These signals appeared at } \delta 62.5 (C-1'), 85.5 (C-1), 74.5 (C-2), 73.0 (C-3), 74.0 (C-4), 74.0 (C-5), and 72.2 (C-6). The COSY spectrum confirmed the correlations of H-1 with H-2 and H-6, H-2 with H-1 and H-3, H-3 with H-4, and H-5 with H-6 (Figure 3A). Similarly, the HMBC spectrum confirmed the correlation of H-1 with C-2, C-6 and C-1', H-2 with C-1 and C-3, H-3 with C-2, H-6 with C-1, H-5 with C-1, and H-4 with C-5 (Figure 3B). These spectral data are in agreement with those reported for pinitol.\)

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8.0 Hz, H-5), 1.62 (2H, t, $J = 13.0$ Hz, H-6), 1.91 (2H, d, $J = 11.0$ Hz, H-7), 1.58 (2H, t, $J = 8.5$, H-11), 1.45 (2H, m, H-13), 1.82 (2H, d, $J = 15.0$ Hz, H-12), 1.31 (2H, m, H-15), 1.29 (2H, d, $J = 6.0$ Hz, H-16), 1.38 (2H, d, $J = 7.0$ Hz, H-17), 1.83 (2H, m, H-17), 1.85 (2H, m, H-18), 1.45 (2H, d, $J = 8.0$ Hz, H-19), 0.78 (3H, s, H-20), 0.71 (3H, d, $J = 10.0$ Hz, H-21), 1.45 (1H, d, $J = 7.0$ Hz, H-22), 1.38 (1H, d, H-23), 1.15 (1H, d, $J = 7.0$ Hz, H-24), 1.10 (1H, d, $J = 10.0$ Hz, H-25). These spectral data agree well with those reported for betulin.15,16

Compound 5: β-Sitosterol glucoside was obtained from the CHCl3/EtOAc subfractions as a white crystalline solid. On the basis of its mass spectrum as evidenced by its ESI-MS $m/z$: 576, its molecular formula was C15H10O7. The structure of this compound was elucidated with the aid of 1H, 13C, and 2D-NMR spectroscopy. 1H NMR (CDCl3/CD3OD, 500 MHz), displayed signals of the different protons in the compound:

| Compound | 1st | 2nd | 3rd | 4th | 5th |
|----------|-----|-----|-----|-----|-----|
| 1        | 79.09 ± 0.90 | 83.98 ± 0.12 | 83.76 ± 0.95 | 81.87 ± 0.98 | 78.04 ± 0.77 |
| 2        | 1.74 ± 0.99   | 51.5 ± 0.86   | 7.66 ± 0.00   | 5.83 ± 0.98   | 5.83 ± 0.98   |
| 3        | 50.76 ± 0.66  | 51.7 ± 0.86   | 51.02 ± 0.98  | 50.98 ± 0.76  | 50.07 ± 0.90  |
| 4        | 73.14 ± 0.56  | 73.22 ± 0.09  | 73.22 ± 0.44  | 72.09 ± 0.54  | 71.08 ± 0.08  |
| 5        | 4.661, 4.50 (2H, s, H-29a, H-29b), 1.66 (3H, s, H-30). |

Figure 5. Inflammatory effect of compounds 1–5 isolated from the aerial parts of S. mollis.

Table 1. Anti-Inflammatory Activity of Isolated Compounds from Aerial Parts of S. mollis

| compound (0.5 mg/kg) | 1st | 2nd | 3rd | 4th | 5th |
|---------------------|-----|-----|-----|-----|-----|
| 1                   | 79.09 ± 0.90 | 83.98 ± 0.12 | 83.76 ± 0.95 | 81.87 ± 0.98 | 78.04 ± 0.77 |
| 2                   | 1.74 ± 0.99   | 51.5 ± 0.86   | 7.66 ± 0.00   | 5.83 ± 0.98   | 5.83 ± 0.98   |
| 3                   | 50.76 ± 0.66  | 51.7 ± 0.86   | 51.02 ± 0.98  | 50.98 ± 0.76  | 50.07 ± 0.90  |
| 4                   | 73.14 ± 0.56  | 73.22 ± 0.09  | 73.22 ± 0.44  | 72.09 ± 0.54  | 71.08 ± 0.08  |
| 5                   | 4.661, 4.50 (2H, s, H-29a, H-29b), 1.66 (3H, s, H-30). |

2.2. Biological Activity. Pure compounds isolated from the aerial parts of S. mollis were evaluated for anti-inflammatory, anticancer, antiglycation, and antioxidant activities.

2.2.1. Anti-Inflammatory Activity. Pure compounds (1–5) obtained from the aerial parts of S. mollis were evaluated for anti-inflammatory activity, and the results are shown in Table 1.
Among the compounds examined, compound 1 exhibited significant attenuation in induced edema with an inhibition percentage of 83.98%. This compound was more potent as an anti-inflammatory agent than the standard drug (ibuprofen), which showed percent inhibition as 73.22%. On the other hand, compound 3 also exhibited mild anti-inflammatory activity. Compounds 2, 4, and 5 were inactive, as shown in (Figure 5).

Scopoletin (1) is a coumarin that is widespread in numerous plants and documented for its antifungal and antibacterial activity.18 In this investigation, compound 1 exhibited considerable anti-inflammatory effect. Our findings are in agreement with results reported by Ding and his co-workers.19 Meanwhile, compound 3 displayed good anti-inflammatory effect, which may be attributed to the presence of an OH group at the para position that is usually described as having biological properties.20

2.2.2. Anticancer Activity (HeLa and 3T3 Cells). Anticancer effect of the pure compounds (1–5) isolated from S. mollis was evaluated against the human cervical cancer cells (HeLa) and 3T3 cell lines; the results are summarized in Table 2.

Table 2. Anticancer Activity of Isolated Compounds Isolated from Aerial Parts of S. mollis against HeLa and 3T3 Cell Lines

| compound | HeLa cell line | 3T3 cell line |
|----------|----------------|---------------|
|          | % inhibition   | IC₅₀ ± SD      | % inhibition | IC₅₀ ± SD      |
| 1        | 28             | ND            | 19          | ND            |
| 3        | 24             | ND            | 1           | ND            |
| 4        | 41             | ND            | 30          | ND            |
| 5        | 48             | ND            | 39          | ND            |
| doxorubicin (standard) | 71 | | |
| cycloheximide | 71 | 0.8 ± 0.2 |

“IC₅₀ = 50% inhibitory concentration, ND = not detected, SD = standard deviation.

The results revealed that compound 5 exhibits a moderate anticancer effect against the HeLa cell line with a percentage inhibition of 48% as compared with the standard drug (doxorubicin, 71%), followed by compound 4, which showed a percentage inhibition of 41%. Our findings also indicated that the order of potency against the HeLa cell line follows the order 5 > 4 > 1 > 3. On the other hand, compounds 5 and 4 exerted a weak effect against the 3T3 cell line with a percentage inhibition of 39 and 30%, respectively, as compared with the standard anticancer drug (cycloheximide), which exhibited 71% inhibition. The anticancer effect of tested compounds against the 3T3 cell line follows the order 5 > 4 > 1 > 3. Compound 2 was inactive against both cell lines.

2.2.3. Antiglycation Activity. Antiglycation activity of isolated compounds from S. mollis including pinitol, 2-propenoic acid, 3-(3,4-dihydroxyphenyl)-octacosyl ester, and β-sitosterol glucoside was evaluated. All compounds tested failed to show antiglycation activity (Table 3).

2.2.4. Antioxidant Activity. Isolated compounds from S. mollis were evaluated for their radical scavenging potency using the DPPT assay. The results revealed that compound 3 exhibits significant radical scavenging activity, as shown in Table 4.

The highest radical scavenging activity found was 95.646 ± 0.003% at a concentration of 400 µg/mL. On the other hand, scavenging activities of 94.766 ± 0.014% and 94.516 ± 0.011% at the concentrations of 200 and 100 µg/mL, respectively, were obtained. The lowest radical scavenging activity of compound 3 was 30.956 ± 0.025 recorded at the concentration of 10 µg/mL. The remaining compounds exhibited weak antioxidant activity as compared with the positive control (ascorbic acid) (Figure 6).

Interestingly, compound 3 was isolated for the first time from the genus Sophora. This compound is phenolic, having two OH groups attached to a benzene ring. Our findings from this investigation indicated that compound 3 exhibits potent antioxidant activity due to its significant 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging potential as compared to the standard agent (ascorbic acid). Therefore, the potential of radical scavenging capacity could be attributed to the presence of these two OH groups or may be due to the alkyl ester group, which increases the hydrophobicity of the compound and thus improves its antioxidant activity.22,23

2.2.5. Docking Studies. In an anti-inflammatory assay, compound 1 displayed remarkable inhibition potential with an IC₅₀ value of 1.56 ± 0.34 µg. We performed docking studies on compound 1 against cyclooxygenases (COX-1 and COX-2) as molecular targets. In this study, the three-dimensional (3D) structures of both isoforms were obtained from the Protein Data Bank (PDB) with accession codes 1EQG and 1CX2, respectively. The 3D binding orientation of 1 superposes on ibuprofen into the binding site of COX-1 isoform, as shown in Figure 7a. In addition, the 3D interaction plot of 1 depicted in Figure 7b showed two hydrogen bond interactions with Tyr355 and Ser350, whereas Ile523 and Leu352 form the π-alkyl type of interaction to stabilize the ligand–enzyme complex. On the other hand, the standard drug ibuprofen showed bifurcated hydrogen bond interaction with Arg120 and Tyr355 (Figure 7c). Tyr355 also establishes the π-alkyl type of interaction. Other residues that form the π-alkyl type of interaction are: Tyr385, Trp385, Phe318, and Ala527 (Figure 7c).

The binding energy value of 1 into the binding site of COX-1 is −6.6990 kcal/mol. In a similar fashion, the 3-D interaction plot of 1 into the binding site of COX-2 isoform is shown in Figure 8.

Results show that 1 forms hydrogen bond interaction with Tyr385 and Arg120 residues, and forms π-alkyl type with Val349, Leu352, and Ala527. The computed binding energy value of compound 1 into the binding site of 1CX2 is −5.5464 kcal/mol. These results suggest that 1 exhibits excellent inhibition potential against COX-1 more than the COX-2 isoform.

3. EXPERIMENTAL SECTION

3.1. Collecting Plant. Aerial parts of S. mollis were collected from Chitral region (latitude: 35.50° N and the
longitude: 71.47° E), Khyber Pakhtunkhwa, Pakistan, in May 2015. Mr. Naveed, a taxonomist at the Department of Botany, University of Peshawar, Pakistan, identified and authenticated the plant. A voucher specimen (JA-08-C) was deposited at the herbarium located at the Department of Botany, University of Peshawar, Pakistan.

3.2. Extraction and Fractionations. The shade dried aerial parts of *S. mollis* were extracted with commercial grade methanol using a Soxhlet extractor. The methanol extract was concentrated and dried by means of rotary evaporation. Extracts were then fractionated with solvents of increasing polarity, followed by solvent evaporation by means of a rotary evaporator for further concentration.

3.3. Isolation and Purification of Compounds. The chloroform (138 g) and ethyl acetate fractions (117 g) of aerial parts of *S. mollis* were combined and then subjected to column chromatography (CC) using silica gel (E. Merck’s) [(0.063−0.200 mm, 5 × 60 cm)] as the stationary phase and a solvent system consisting of mixtures of n-hexane:ethyl acetate (100→0−100). This process led to collection of 15 fractions (A–O); on the basis of their thin-layer chromatography profiles, fraction (K) afforded a white-yellowish powder (300 mg) using the mobile phase system n-hexane/ethyl acetate (45:55) (compound 1), and fraction (F) was subjected to CC and eluted with n-hexane/ethyl acetate (75:25) as a solvent system and mobile phases; this results in the isolation of compound 2 obtained as a white powder (31 mg). On the other hand, fractions (H) and (I) were combined and subjected to CC, which led to the isolation of compound 3 as a yellowish crystalline solid (63 mg) using dichloromethane/ethyl acetate (80:20) as a mobile-phase system. Similarly, fraction (M) afforded compound 4 as colorless crystals colorless using the ethyl acetate/methanol (90:10) solvent system. Finally, fraction (G) was subjected to CC, resulting in the isolation of compound 5 obtained as a white

| conc (µg/mL) | compound 1 | compound 2 | compound 3 | compound 4 | compound 5 | ascorbic acid (ST) |
|-------------|------------|------------|------------|------------|------------|--------------------|
| 10          | 10.73 ± 0.03 | 32.83 ± 0.019 | 30.956 ± 0.025 | 16.40 ± 0.16 | 18.26 ± 0.20 | 30.5 ± 0.113 |
| 50          | 13.03 ± 0.08 | 37.80 ± 0.19 | 87.657 ± 0.145 | 18.59 ± 0.24 | 19.27 ± 1.41 | 95.58 ± 0.57 |
| 100         | 13.69 ± 0.03 | 42.66 ± 0.17 | 94.516 ± 0.011 | 25.62 ± 0.05 | 21.39 ± 0.05 | 96.37 ± 0.01 |
| 200         | 14.01 ± 0.06 | 44.12 ± 0.07 | 94.766 ± 0.014 | 25.89 ± 0.77 | 24.86 ± 1.09 | 96.41 ± 0.01 |
| 400         | 16.64 ± 0.10 | 46.83 ± 0.03 | 95.646 ± 0.003 | 26.78 ± 0.09 | 29.09 ± 0.09 | 96.73 ± 0.33 |

“SD = standard deviation.

Figure 6. Antioxidant activity of compounds 1–5 isolated from aerial parts of *S. mollis*.

Figure 7. (a) Ribbon model of the superimposed binding pose of scopoletin into the binding site of human COX-1 (1EQG). (b,c) 3D binding interaction pattern of scopoletin and ibuprofen in the binding site of COX-1. Hydrogen bond interactions are shown in green dotted lines, while the π-alkyl type of interactions are shown in light pink.

Figure 8. 3D binding interaction pattern of scopoletin in the binding site of COX-2 (1CX2).
powder (30 mg), using the solvent system n-hexane/ethyl acetate (75:25).

### 3.4. Biological Study

#### 3.4.1. Anti-Inflammatory Activity

Anti-inflammatory activity of isolated compounds was evaluated in vivo using male Wistar rats (120–140 g) according to a published procedure. In brief, the Wistar rats were starved 12 h prior to the experiment. Animals were then randomly divided into five groups (n = 8) of five rats each. One group was orally fed with ibuprofen (standard drug) (5 mg/kg) in 0.5% carboxymethyl cellulose (CMC), whereas the control given vehicle (0.5 mL of 0.5% CMC). While tested groups were treated with isolated compounds (0.5 mg/kg, PO). Approximately 1 h after drug administration, rats were lightly anesthetized using diethyl ether. This was followed by injection of carrageenan (0.1 mL of 1%) subcutaneously into the subplanter of paw edema of each rat to induce inflammation. Inflammation was evaluated hourly for 5 h by means of a water plethysmometer after injection of carrageenan by measuring the volume of edema before and after injection. The percent inhibition of edema was calculated for all groups including the standard drug-treated group and test sample-treated groups by using the formula

\[
\text{% inhibition} = \frac{A - B}{A} \times 100
\]

where \(A\) = edema volume of control and \(B\) = paw edema of the tested group.

#### 3.4.2. Anticancer Activity (HeLa and 3T3) Cell Lines

Cytotoxic activity of compounds 1–5 was evaluated in 96-well flat-bottomed microplates by conducting cell viability using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to published procedures. In Dulbecco’s modified Eagle medium, 3T3 and HeLa cell lines were cultured, treated with streptomycin (100 μg/mL), penicillin (100 IU/mL), and 4% of fetal bovine serum (FBS) and placed in an incubator (37 °C) with (5% CO₂). The growing cells were rapidly collected and counted by using a haemocytometer and then diluted with a medium (5% FBS). Cell cultures (with a concentration of \(1 \times 10^4\) cells/mL) were prepared in (85 μL/well) into 96-well plates, and incubated overnight. Cells were then treated with various concentrations (5–500 μM) of the tested compounds. In all assays, tested compounds were dissolved in dimethyl sulfoxide immediately before the addition to cell cultures, and equal amounts of the solvent were added to control cells. After 48 h, 0.4 mg/mL (250 μL) of MTT was added to every well, followed by incubation for 4 h. MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microreader (Spectra Max plus, CA, Molecular Devices, USA). The cytotoxicity was considered as the concentration causing 50% growth inhibition (IC₅₀). The percent inhibition was calculated using the following formula

\[
\text{inhibition (\%)} = 100 - \frac{M_0 - M_i}{M_0 - M_p} \times 100
\]

where \(M_0\) = mean of the OD of the test sample, \(M_i\) = mean of the OD of the negative control, and \(M_p\) = mean of the OD of the positive control; Max Pro software was used to process results (inhibition %).

#### 3.4.3. Antiglycation Activity

The antiglycation activity of isolated compounds was performed according to a previously published protocol. According to the published protocol, fatty acid-free bovine serum albumin (1 mg/mL) was incubated with D-glucose (200 mM) and 0.5 mg of each tested compound in 45 mM pH 7.4 phosphate buffer and 0.2 g/L of sodium azide (NaN₃). The mixture was incubated for 7 days at 37 °C, and rutin was used as a standard. Emission at 440 nm and excitation at 370 nm were measured with the aid of a spectrophotometer, and the fluorescence intensity was calculated using the following equation

\[
\text{% inhibition} = \frac{F_0 - F_i}{F_0} \times 100
\]

where \(F_0\) = fluorescence intensity of control and \(F_i\) = fluorescence intensity of control; IC₅₀ values were calculated by plotting % inhibition against the compound’s concentration.

#### 3.4.4. DPPH Radical Scavenging Activity Assay

Radical scavenging activity of isolated compounds was evaluated using the DPPH assay according to published procedures. In brief, stock solutions were prepared by dissolving 2 mg of each tested compound in analytical-grade methanol (50 mL). Solutions of various concentrations including 10, 50, 100, 200, and 400 μg/mL were then prepared from the stock solution, followed by addition of DPPH [1 mL solution prepared by adding DPPH (4 mg) to 50 mL methanol]. The control was also prepared by adding 1 mL of DPPH solution (1 mL) to pure methanol. All solutions were incubated for 30 min at room temperature in the dark. The absorption was then determined for each mixture at 517 nm by means of a UV/visible spectrophotometer (OPTIMA TOKYO, JAPAN). The radical scavenging (antioxidant) capacity was then calculated by using the equation

\[
\text{DPPH} = \frac{A_0 - A_i}{A_0} \times 100
\]

where \(A_0\) = absorption of the control and \(A_i\) = absorption of the sample.

#### 3.4.5. Docking Studies

We performed docking studies on compound 1 against COX-1 and COX-2 as molecular targets by using Molecular Operating Environment (MOE 2016) software. The 3D structures of both isoforms were obtained from the PDB with the accession codes 1EQG and 1CX2, respectively. The docking procedure was validated by redocking of the native ligands.

Preparation of ligands, downloaded enzyme (3D) protonation, energy minimization, and determination of binding sites were carried out according to our previously reported method. All ligand structures were drawn using the Builder option in MOE. The database of compounds was built as ligand.mdb. The compounds were then energy minimized up to 0.0001 gradient using MMFF94X force field, and the enzyme structure was opened in the MOE window. Water molecules (if present) were removed, and 3D protonation was done for all atoms in an implicit solvated environment at pH = 7, 300 K, and salt concentration of 0.1 M. The complete structure was energy minimized using MMFF94X force field. Finally, all compounds were docked into the binding sites of the prepared enzymes. Default docking parameters were set, and 10 different conformations were generated for each compound. The lowest binding energy ligand enzyme complexes were analyzed by the MOE ligand interaction module, whereas Studio Visualizer was used for 3D interaction plots (Biovia 2017).
4. CONCLUSIONS

In summary, findings from this investigation showed that the following five compounds were isolated from the aerial parts of S. mollis: β-sitosterol glucoside, betulin, pinitol, scopoletin, and 2-propenoic acid, 3-(3,4-dihydroxyphenyl)-octacosyl ester. These compounds were characterized by means of NMR and mass spectral data. Biological evaluation of isolated compounds revealed that scopoletin exhibits remarkable anti-inflammatory activity, whereas 2-propenoic acid, 3-(3,4-dihydroxyphenyl)-octacosyl ester shows significant free-radical scavenging capacity against DPPH. In addition, the isolated compounds displayed weak anticancer activity against HeLa and 3T3 cells lines. Binding orientation and energy data computed via docking simulations showed that scopoletin exhibits good inhibition potential against COX-1 higher than the COX-2 isoform. These findings may explain the medicinal use of S. mollis in folk medicine. However, more detailed studies are required to establish the safety and efficacy of these compounds as potential drugs.

ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c01532.

Spectroscopic data of compounds 1−5 (PDF)

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Notes

The authors declare no competing financial interest.

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