Isolation and Characterization of Two New Antimicrobial Acids from Quercus incana (Bluejack Oak)

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Received 24 August 2017; Accepted 27 November 2017; Published 28 January 2018

Abstract

Two new compounds [1-2] were purified from ethyl acetate fraction of Quercus incana. The structure of these compounds is mainly established by using advanced spectroscopic technique such as UV, IR, one-dimensional (1D) and two-dimensional (2D) NMR techniques, and El mass. The structural formula was deduced to be 4-hydroxydecanoic acid [1] and 4-hydroxy-3-(hydroxymethyl) pentanoic acid [2]. Both isolated compounds were tested for their antimicrobial potential and showed promising antifungal activity against Aspergillus niger and Aspergillus flavus.

1. Introduction

The family Fagaceae is large family comprising 8 genera and about 800–1100 species. Quercus is the largest genus of family Fagaceae having huge medicinal importance and mostly found in dry conditions [1]. The genus Quercus have long been considered among the clades of woody angiosperms in terms of species diversity, horticultural merit, ecological dominance, and industrial and economic values [2]. The Quercus robur is the only cultivated species while other 600 known species are found in temperate regions of the Northern Hemisphere, Southward through Central America to Colombia and through Turkey to Pakistan [3].

The wood is durable, is attractively grained, and is mostly utilized for timber purposes; it is particularly important in shipbuilding, construction for flooring, furniture, railroad ties, and veneers. The bark of Quercus spp. has been used for medicinal purposes and is an important source of phenolic compounds like tannins which are used for tanning leather and wine production [4]. The fruit (acorn) of Quercus has husk coating which is edible and highly nutritious and rich in carbohydrates and protein. Quercus (oak) species are utilized in conventional pharmaceutical, as astringent, antiseptic, and hemostatic and in addition to the treatment of acute diarrhea, hemorrhoid, and oral, genital, and anal mucosa inflammation. Moreover, the decoction plants from this genus can be used against burns and added to ointments for the healing of cuts [5]. Oak seeds are a major source of sugar, amino acids, lipids, and different sterols [6]. Quercus species have been utilized against problems of skin, wounds and gastrointestinal illnesses [7], astringent, mellow germ-free, small cuts [8], and mouth washes [9] all suggesting their antimicrobial potential.

Genus Quercus is characterized by six species found mostly in Northern areas of Pakistan. The most promising timber specie is Quercus incana Roxb. (Blue jack oak or cinnamon oak) locally called Ban shindar, Kharpata serci (Punjabi), Rein (Hindko), and Serie (Pushto) [10]. The Q. incana has huge medicinal usage; it may be used as astringent [11], diuretic, and antidiarrheal agent and for treatment of asthma. Bark and leaves of Q. incana may be used as antipyretic, antirheumatism, antidiabetic, and antiarthritic...
purposes [12]. These medical applications and therapeutic potential of Quercus incana prompted us to carry out the phytochemical investigation to explore biologically active compounds.

2. Material and Methods

2.1. Experimental Procedures. The ethyl acetate soluble fraction was selected for isolation of bioactive compounds using column chromatographic analysis having column silica and flash silica gel as an adsorbent material. The column was eluted by using n-hexane and ethyl acetate with increasing polarity, which yield two new compounds 4-hydroxy decanoic acid [1] and 4-hydroxy-3-(hydroxymethyl) pentanoic acid [2] by increasing polarity. The purity of compounds [1-2] was checked by using precoated TLC plates. The IR spectrum was recorded by using spectrophotometer JASCO-320A. The EI mass was recorded on double focusing Varian MAT-312 Spectrometer. 1H-NMR and 13C-NMR spectra were measured by using advance Bruker AMX-300 spectrometer machine. The chemical shifts in parts per million (δ) relative to tetramethylsilane as an internal standard and scalar (J) were described in Hz.

2.2. Plant Extraction and Fractionation. Extraction and fractionation of Quercus incana were reported in our previous study [13]. Ethyl acetate soluble fraction was subjected to repeated column chromatography which yielded two pure compounds [1-2].

2.3. Antibacterial Activity of Compounds [1-2]. Antibacterial activity was performed by agar well diffusion method with some modifications [14]. Three Gram-positive (Staphylococcus aureus, Micrococcus luteus, and Bacillus subtilis) and Gram-negative (Escherichia coli, Pseudomonas pickettii, and Shigella flexneri) pathogens were used in study. 10 μg of each compounds [1-2] was dissolved in 1 mL DMSO. Standard drug and each sample (20 μL) were poured in 6 mm well. The assay plates were incubated at 37°C for 24 hrs. The zone of inhibition was dignified in mm and DMSO was used as a negative control in the experiment.

2.4. Antifungal Assay. Disc diffusion methods were used for determination of antifungal effects by using two selected fungal strains such as Aspergillus niger and Aspergillus flavus [15]. DMSO was used as a solvent; before applying compounds on petri plates DMSO was completely evaporated.

Characterization of Compound 1. Colorless oil; IR (KBr) νmax 3622 br (OH), 1714 (C=O) cm⁻¹, [α]D 25 + 34.80° (c = 0.78, CHCl₃). EI-MS m/z (rel. int.) 188 [M]+ (15), 176 (9), 157 (35), 128 (5), 115 (9). HR-EI-MS: m/z 188.4140 (calcd. for C₁₀H₁₀O₃). 1H-NMR (CDCl₃, 300 MHz): δ 3.62 (1H, m, H-10), 2.08 (2H, m, H-2), 1.49, 1.62 (2H, m, H-3), 1.45 (2H, m, H-5), 1.37 (2H, m, H-6), 1.30 (2H, m, H-7), 1.28 (2H, m, H-8), 1.29 (2H, m, H-9). 13C-NMR (CDCl₃, 75 MHz): δ 179.6 (C-1), 72.1 (C-4), 13.9 (C-10), 34.6 (C-2), 34.9 (C-3), 37.9 (C-5), 26.1 (C-6), 29.8 (C-7), 32.0 (C-8), 22.1 (C-9).

Characterization of Compound 2. Colorless oil: IR (KBr) νmax 3495 (OH), 1708 (C=O) cm⁻¹. [α]D 25 + 53.60° (c = 0.97, CHCl₃). EI-MS m/z (rel. int.) 131 [M-OH]+ (15), 131 (10), 115 (8), 86 (100), 71 (31). HR-EI-MS: m/z [M-OH]+ 131.0744 (calcd. 131.0736 for C₈H₇O₃). 1H-NMR (CDCl₃, 300 MHz): δ 2.35 (1H, m, H-2), 2.04 (1H, m, H-2), 2.58 (1H, m, H-3), 3.92 (1H, m, H-4), 1.23 (3H, d, J = 6.9 Hz, H-5), 4.31 (2H, m, H-1'). 13C-NMR (CDCl₃, 75 MHz): δ 177.5 (C-1), 273 (C-2), 45.9 (C-3), 68.1 (C-4), 20.9 (C-5), 65.5 (C-1').

3. Result and Discussion

Ethyl acetate soluble fraction was subjected to repeated column chromatography on silica gel using n-hexane and ethyl acetate as a solvent with gradual increasing in polarity up to 100% ethyl acetate, which resulted in four subfractions (Fractions A–D). The fractions obtained based on TLC profile were resubjected to pencil column chromatography and eluted with n-hexane:EtOAc, 25:75 and 10:5 to purify compound 1 (10.5mg) and compound 2 (9.8mg) (Figure 1).

Compound 1 was isolated as a colorless oil and has molecular formula of C₁₀H₁₀O₃ as suggested by molecular ion peak at m/z 188 [M]+ in HR-EIMS. The other fragment peaks were obtained at m/z 176, 157, 128, and 115. The HR-EIMS gave exact mass of compound 1 which was at m/z 188.1420 (calcd. m/z 188.1412). The IR spectrum displayed absorption bands for hydroxyl and carbonyl groups at 3622 and 1714 cm⁻¹, respectively. The 1H-NMR spectrum of compound 1 exhibited typical signal for aliphatic acid skeleton, which was strongly supported by DEPT experiment. The

Figure 1: Structure of compounds [1-2].
Table 1: $^1$H-NMR (CDCl$_3$, 300 MHz) data of compounds [1-2] in ppm, J in Hz.

<table>
<thead>
<tr>
<th>Position</th>
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<th>2</th>
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<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2.08, m</td>
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<tr>
<td>3</td>
<td>1.62, m</td>
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</tr>
<tr>
<td>4</td>
<td>3.62, m</td>
<td>2.58, m</td>
</tr>
<tr>
<td>5</td>
<td>1.45, m</td>
<td>1.23 (H, d, J = 6.9 Hz)</td>
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<td>6</td>
<td>1.47, m</td>
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<tr>
<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>1.28, m</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>1.29, m</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.88 (t, J = 7.7)</td>
<td>-</td>
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</table>

$^{13}$C-NMR spectrum revealed the presence of one methyl, one methine, seven methylene, and one quaternary carbon signals. The methine signal appearing at $\delta_{C}$ 3.62 (1H, m) was assigned to H-4 while methyl group resonated at $\delta_{H}$ 0.88 (3H, t, J = 7.7 Hz). Typical methylene signals were resonated as multiplet for seven methylene carbons at $\delta_{C}$ 2.08 (2H, m), $\delta_{H}$ 1.49 (1H, m), $\delta_{C}$ 1.45 (2H, m), $\delta_{H}$ 1.33 (1H, m), $\delta_{C}$ 1.47 (1H, m), $\delta_{H}$ 1.30 (2H, m), $\delta_{C}$ 1.28 (2H, m), and $\delta_{H}$ 1.29 (2H, m) assigned to H-2, H-3, H-5, H-6, H-7, H-8, and H-9, respectively (Table 1). The $^{13}$C-NMR spectrum (BB and DEPT) corroborated the presence of seven methylene carbons, one methine carbon, one terminal methyl carbon, and one quaternary carbon. The carbonyl carbon showed signals at $\delta_{C}$ 179.6 whereas methine signal centered at $\delta_{C}$ 72.1. The $^{13}$C-NMR chemical shift of CH$_3$-ClO was observed at $\delta_{C}$ 13.9 and seven methylene carbons appeared at $\delta_{C}$ 34.6, 34.9, 37.9, 26.1, 29.8, 32.0, and 22.1 for C-2, C-3, C-5, C-6, C-7, C-8, and C-9, respectively (Table 2). The HMBC and COSY spectra were quite helpful for accurate placement of various substituents in the molecule. The HMBC spectrum showed strong correlation of methine proton at $\delta_{H}$ 3.62 (H-4) with C-2, C-3, C-5, and C-6 [13]. The methyl proton at $\delta_{H}$ 0.88 showed strong HMBC correlation with C-9 ($\delta_{C}$ 22.1), C-8 ($\delta_{C}$ 32.0) which was quite supportive in the establishment of structure. Finally all spectral data confirmed, compound 1 as an aliphatic acid having straight chain of -(CH$_2$)$_2$-(CH$_3$)-moiety [16] and was proposed to be 4-hydroxy decanoic acid.

Compound 2 was isolated as colorless oil. Its structure was mainly established by $^1$H-NMR and high resolution mass spectroscopy and supported by $^{13}$C-NMR spectrum. Its molecular formula C$_8$H$_{12}$O$_4$ was concluded from the accurate mass measurement of peak at $m/z$ [M-OH]$^+$ 131, corresponding to molecular composition C$_8$H$_{12}$O$_3$-OH. In addition to its molecular ion peak, it showed some characteristic fragments at $m/z$ 115, 86, and 71. The HR-EI-MS gave exact mass of compound 2 at $m/z$ 131.0744 (calcld. 131.0736 for C$_8$H$_{12}$O$_3$-OH). The IR spectrum showed absorption bands at 3495 cm$^{-1}$ and 1708 cm$^{-1}$ indicating presence of the hydroxyl group and carbonyl carbon, respectively. Similarly, broad absorption centered at 2935 cm$^{-1}$ suggested the presence of carboxylic acid. The $^1$H-NMR showed a signal for a secondary methyl group at $\delta_{H}$ 1.23 (3H, d, J = 6.9 Hz, H-5), connected to a methine group resonated at $\delta_{H}$ 3.92 (1H, m, H-4) possessing hydroxyl group, while the other methine signal appeared at $\delta_{H}$ 2.58 (1H, m, H-3). The downfield methylene bearing hydroxyl group appeared at $\delta_{C}$ 4.31 (2H, m, H-1’) and the other methylene centered at $\delta_{H}$ 2.04 (1H, m, H-2) and $\delta_{H}$ 2.35 (1H, m, H-2) was directly connected to carboxylic acid (Table 1). The $^{13}$C-NMR spectrum confirmed the presence of one methyl carbon, two methylene groups, two methine carbons, and one quaternary carbon in the structure.

In $^{13}$C-NMR spectrum, signal for secondary methyl appeared at $\delta_{C}$ 20.9 whereas the methine signal bearing hydroxyl group was observed at $\delta_{C}$ 68.1 for C-4. The signal for another methine appeared at $\delta_{C}$ 45.9 for C-3. The side chain methylene having free hydroxyl group resonated at $\delta_{C}$ 65.5, while the second methylene group at position C-2 appeared at $\delta_{C}$ 273. Similarly, the quaternary carbon in the form of carboxylic acid showed signal at $\delta_{C}$ 177.5 (Table 2). Based on the HMBC and H-H COSY correlation (Figure 2), the connectivity of the C-1 to C-5 chain was found in agreement with literature [17]. The HMBC spectrum showed H-C correlation of CH$_2$-5 with that of C-4 and C-3. Similarly the position of hydroxyl group at C-1’ was confirmed by strong HMBC correlation of CH$_2$-1’ with C-3, C-2, and C-4 and weak interaction with C-1. The structure of compound 2 was mainly established by $^1$H-NMR, high resolution mass spectrometry and supported by $^{13}$C-NMR spectrum. From all spectral data it was evident that compound 2 was 4-hydroxy-3-(hydroxymethyl) pentanoic acid.

3.1. Antibacterial Activity. The antibacterial activity of isolated compounds [1-2] was determined by agar well diffusion method (Table 3). Compound 1 was significantly active against Bacillus subtilis, Staphylococcus aureus, and Micrococcus luteus (Gram-positive). Both compounds [1-2] showed promising antibacterial activity against Staphylococcus aureus with 16 mm and 13 mm zone of inhibition. Compound 2 was
Important HMBC correlation of 1

Important HMBC correlation of 2

**Figure 2:** HMBC correlation of compounds [1-2].

**Table 3:** Antibacterial activity of isolated compounds [1-2].

<table>
<thead>
<tr>
<th>S. number</th>
<th>Culture</th>
<th>Zone of inhibition (mm)</th>
<th>Ciprofloxacin</th>
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<tbody>
<tr>
<td>1</td>
<td><em>Bacillus subtilis</em></td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em></td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td><em>Micrococcus luteus</em></td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas pickettii</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td><em>Escherichia coli</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td><em>Shigella flexneri</em></td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

**Table 4:** Antifungal activity of isolated compounds [1-2].

<table>
<thead>
<tr>
<th>Extract</th>
<th>Pathogenic fungi</th>
<th>Zone of inhibition (mm)</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aspergillus flavus</em></td>
<td>Aspergillus niger</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12 mm ± 0.50</td>
<td>15 mm ± 0.70</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17 mm ± 0.28</td>
<td>22 mm ± 0.57</td>
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</tr>
<tr>
<td>Nystatin (standard)</td>
<td>16 mm ± 0.92</td>
<td>21 mm ± 0.28</td>
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</tbody>
</table>

Note: Each value in the table was obtained by calculating the average of three experiments.

moderately active against *Bacillus subtilis* and *Micrococcus luteus* with 5 mm and 9 mm zone of inhibition. Both compounds were inactive against *Escherichia coli* and *Shigella flexneri*.

3.2. Antifungal Activity. Antifungal activity of both compounds [1-2] was done against *Aspergillus flavus* and *Aspergillus niger*. Both compounds [1-2] showed immense activity against *Aspergillus niger* with 15 mm ± 0.70 and 22 mm ± 0.57 zone of inhibition (Table 4). Moderate activity was observed by compound 1 against *Aspergillus flavus* having 12 mm ± 0.50 zone of inhibition.

**4. Conclusion**

The current study describes the isolation, characterization, and antimicrobial activity of isolated compounds from ethyl acetate fraction of *Quercus incana*. Both compounds displayed promising antimicrobial activity against human bacterial and fungal strains. Therefore, these isolated compounds may be considered as the lead compounds as an antimicrobial agents.

**Conflicts of Interest**

There are no conflicts of interest regarding this paper.

**Acknowledgments**

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for its funding this prolific research group no. RGP-007.

**References**


