CHEMICAL INVESTIGATION AND SCREENING OF ANTIMICROBIAL ACTIVITY OF STEM BARK OF QUERCUS LEUCOTRICHOPHORA

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ABSTRACT

7-Methoxy kaempferol and 3-O-[(α-L-rhamnopyranosyl-(1"→4"))-(α-L-rhamnopyranosyl-(1"→6"))]-β-D-glucopyranosyl quercetin were isolated from the ethanolic extract of stem bark of Quercus leucotrichophora along with β-sitosterol. The ethanolic extract exhibited a potent antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli.

Keywords: Quercus leucotrichophora and antimicrobial activity.

INTRODUCTION

Various plant products have been used worldwide since time immemorial for medicinal purposes. According to a WHO study based on publications on pharmacopoeias and medical plants in 91 countries, the number of medicinal plants is nearly 20,0001. Though the therapeutic use of medicinal plant products has been supported by world health organization2, there is an indisputable need to generate corroborative evidence substantiating their proposed medical application. Furthermore, in view of growing antibiotic resistance among bacteria there exists an emergent need to explore novel plant products for possible antibacterial action. Though innumerable such preparations are used regularly by traditional communities on an empiric basis, laboratory data substantiating their antibacterial effect is relatively rare3,4.

The Himalayan region is home to a rich treasure of more than 10,000 natural plant species, many of which have medicinal importance. Quercus leucotrichophora, Banj belonging to family Fagaceae is an evergreen tree of approximately 40 m height and is commonly found throughout the Himalayan region at altitudes ranging from 800-2000 m5. Gum of the tree is traditionally used for gonorrhoeal and digestive disorders6. The seeds are astringent and diuretic and are used in the treatment of gonorrhoea, indigestion, diarrhoea and asthma7. The leaves, seeds and bark are also used in traditional medicine. The Himalayan region is home to a rich treasure of more than 10,000 natural plant species, many of which have medicinal importance. Quercus leucotrichophora vern. Banj belonging to family Fagaceae is an evergreen tree of approximately 40 m height and is commonly found throughout the Himalayan region at altitudes ranging from 800-2000 m. Gum of the tree is traditionally used for gonorrhoeal and digestive disorders. The seeds are astringent and diuretic and are used in the treatment of gonorrhoea, indigestion, diarrhoea and asthma. The leaves, seeds and bark are also used in traditional medicine.

MATERIAL AND METHODS

Collection of plant material

The stem barks of Q. leucotrichophora were collected in January 2008 from Nag Nathi Pokhari, District Chamoli Garhwal, Uttarakhand. The plant was properly identified from Taxonomy Laboratory, Department of Botany, H. N. B. Garhwal University, Srinagar Garhwal, Uttarakhand and the voucher specimen (GUH8835) was kept in the Departmental herbarium.

Extraction and isolation

The air-dried and chopped stem bark was defatted with petroleum spirit using soxhlet. The defatted bark material was extracted exhaustively with 85% EtOH at 30-50°C for 15 h, 3 times on a heating mantle and concentrated under reduced pressure. The extract was then fractionated through column chromatography using Chloroform: Methanol as eluting solvent. The polarity of the solvent was gradually increased by addition of methanol. The repeated column chromatography afforded Compound 1 and 2 together with β-Sitosterol (Direct comparison with authentic sample).

RESULTS AND DISCUSSION

Characterization of Compound 1:

M.P. - 226-228°C
Molecular Formula - C16H12O6
Molecular Weight - 284.28 amu
UV (λmax nm) nm - 270, 275, 280, 373, 390
IR (γMax KBr) cm⁻¹ - 3480, 3260, 1650, 1600, 1500, 1420, 1350, 1340, 1280, 1220.

1H-NMR (CD3OD, 6 ppm)

3.83 (3H, s, OMe), 6.21 (1H, d, J=2.1 Hz, H-6), 6.39 (1H, d, J=2.1 Hz, H-8), 8.04 (2H, d, J=8.9 Hz, H-3, 5'), 6.93 (2H, d, J=8.8 Hz, H-2, 6')

13C-NMR (CD3OD, 6 ppm)

148.3 (C-2), 137.4 (C-3), 175.8 (C-4), 105.4 (C-4a), 161.1 (C-5), 98.5 (C-6), 167.2 (C-7), 92.8 (C-8), 158.2 (C-9a), 123.7 (C-1'), 130.0 (C-2', 6'), 116.0 (C-3', 5'), 1602 (C-4'), 56.4 (OMe)

Compound 1 was crystallized as pale yellow needle shaped from methanol. It gave green colour with FeCl₃ and positive test with Mg/HCl thereby indicating the flavonoid nature of compound11. The IR spectrum of compound furnished two absorption bands at 3480 cm⁻¹ and 3260 cm⁻¹ for chelated and non-chelated OH functions, the other IR absorption bands were observed at 1650 cm⁻¹ and 1600 cm⁻¹ for α, β unsaturated carbonyl and 1500 cm⁻¹ for ether functional group. The 1H NMR spectrum of compound 1 indicated six aromatic protons, two doublets at δ 6.21 (d, J=2.1Hz) and 6.39 (d, J=2.1Hz) assigned to H-6 and H-8, respectively, and other two doublets at δ 6.93 (d, J=8.9Hz) and 8.04 (d, J=8.8Hz) due to a pair of protons (H-2', H-6' and H-3'; H-5') having an AB system, which is characteristic of a p-substituted aromatic ring. It was further supported by its 13C-NMR data appeared at δ 130.7 (C-3', 5') and δ 116.3 (C-3', 5') which were corresponded with hydrogen bearing carbon of p-cresol11. The 1H NMR spectrum of compound 1 also signified one singlet for 3H at δ 3.83 ppm assigned to methoxy proton. The 13C NMR revealed 16 peaks of carbon resonance characteristic for aromatic nucleus and the downfield value at δ 175.8 (C-4) which were corresponded with carbonyl functional group and a up field value at δ 56.4 indicated the presence of methoxy group. The 1H-NMR spectrum showed other peaks a benzylic carbon at δ 148.3 (C-2) and an oxygen bounded ethylenic carbon atom at δ 137.4 (C-3).
On the basis of above observation compound 1 was identified as 7-Methoxy kaempferol. This was further supported by reported data of rhamnocitrin. On the basis of above observation compound 1 was identified as 7-rhamnocitrin 13.

**Characterization of Compound 2**

- **M.P:** - 232-234°C
- **Molecular Weight:** - 756amu
- **FAB-MS** (m/z): 146[(M-H) - (2x146)] - 301[(M-H) - (2x146+162)]
- **IR** (γMaxKBr) Cm−1: 3410, 3220, 1650, 1600, 1577, 1509, 1464, 1458, 1430, 1400, 1377, 1250, 1120, 1040, 890, 840, 755, 609
- **Molecular Weight:** - 756amu
- **M.P:** - 232-234°C

**1H-NMR (CD3OD, δppm)**

1.27 (s H-6" and H-6""'), 4.4(1H, d, J=3.4Hz, H-1'''), 4.6(1H, d, J=3.4Hz, H-1''''), 5.3(1H, d, J=6.5Hz, H-1'''), 7.8 (d, J=2.5 Hz, H-2''), 6.20 (d, J=2.5 Hz, H-6), 7.7 (d, J=2.5 Hz, H-8), 6.80 (d, J=2.5 Hz, H-5'), and 7.15 (d, J=6.0 Hz, H-6'), 5.3(1H, d, J=6.5Hz, anomeric of glucose)

**13C-NMR (CD3OD, δppm) (Aglycone)**

160.7(C-2'), 149.6(C-3), 177.4(C-4), 160.9(C-5'), 146.1(C-7), 92.3(C-8'), 157.7(C-8a), 104.6 (C-4a), 133.6(C-2'), 150.0(C-3'), 145.8(C-4'), 156.3(C-5'), 97.3(C-6').

**Glucose**

- Glucose: 104.96(C-1"'), 73.58(C-2"'), 71.15 (C-3"'), 78.0(C-4"'), 73.05(C-5"'), 65.65(C-6'').
- **Rhamnose**
  - Doublet at δ 4.4(J=3.4Hz), 4.6(J=3.4Hz) and 5.3(J=6.5Hz) showed the position of anomeric protons of rhamnose and glucose. The 13C NMR of compound 2 displayed a downfield signal at δ 177.4 (C-4) was attributed due to carbonyl functional group. Three peaks at δ 104.96 (C-1''), 99.2 (C-1'') and 97.2 (C-1'') were assigned for anomic carbon of sugar whereas other downfield signals displayed at δ 160.9, 165.1, 150.0 and 145.8 was attributed to four oxygenated carbon atoms. Acidic hydrogen of the compound gave an aglycone identified as quercetin (by direct comparison with authentic sample) and a mixture of mono saccharide identified as glucose and rhamnose (PC with sugar samples). On per methylation compound 2 afforded 2,3-di-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose. The types of linkage at the glycosidic points were found to be D-glucose-β and L-rhamnose-α by 1H-NMR and 13C-NMR data. From above spectral studies compound 2 was identified as and 3-O-[(α-L-rhamnopyranosyl) (1→4)](α-L-rhamnopyranosyl- (1→6))-β-D-glucopyranosyl quercetin.

**Antimicrobial activity**

The antimicrobial study of ethanolic extract of Quercus leucotrichophora was carried out against Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli. These microorganisms were isolated from different culture media and studied for inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC) using Erythromycin and Ampicillin as a positive control for test microorganisms (Table 1). The agar diffusion method was adopted for the inhibition zone diameter (IZD) study14. MICs of the extracts were determined by tube dilution method (turbimetric method). The microbial cultures were grown in nutrient broth for 24 hrs before being used. The cultures were diluted in broth at a density adjusted to a 0.5 McFarland turbidity standard [1-2×10^5 CFUs/ml].

The bacterial suspensions were diluted 1:10 in broth and 100 μl of it were used for the study. 2 ml of the sterilized nutrient broth was introduced in each of the 5 test tubes. The extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) (not toxic to germs at this percentage) and serially diluted to give a concentration of 250, 125, 62.5, 31.2 and 15.6μg/ml. In all the test tubes 0.1 ml of suspension of bacteria in saline was added and incubated at 37°C/24hr. Post-incubation the plates were observed for turbidity. The MIC was determined as the least concentration of ethanolic extract of Quercus leucotrichophora inhibiting the growth of the test organisms15.
### Table 1: Antimicrobial activity of QB against tested microorganisms compared with reference compounds

<table>
<thead>
<tr>
<th>S No.</th>
<th>Test microorganism</th>
<th>IZD (cm)</th>
<th>E (1mg/ml)</th>
<th>A (1mg/ml)</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. aureus</td>
<td>23.0 ± 1.0</td>
<td>31.2 ± 0.25</td>
<td>28.2 ± 0.34</td>
<td>250</td>
</tr>
<tr>
<td>2</td>
<td>B. Subtilis</td>
<td>15.0 ± 0.00</td>
<td>32.1 ± 0.98</td>
<td>26.4 ± 0.48</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>P. auropinosa</td>
<td>21.0 ± 1.0</td>
<td>27.5 ± 1.14</td>
<td>20.6 ± 0.52</td>
<td>125</td>
</tr>
<tr>
<td>4</td>
<td>E. coli</td>
<td>26.3 ± 1.53</td>
<td>24.6 ± 0.75</td>
<td>19.3 ± 0.22</td>
<td>125</td>
</tr>
</tbody>
</table>

E=standard Erythromycin; A=standard Ampicillin; QB=Ethanolic extract of Quercus leucotrichophora bark.

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**REFERENCES:**