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Research Article

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 auroginosa*, *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 20,000[1]. Though the therapeutic use of medicinal plant products has been supported by world health organization[2], 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 rare [3-5].

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 800-2000m[6]. Gum of the tree is traditionally used for gonorrhoeal and digestive disorders[7]. The seeds are astringent and diuretic and are used in the treatment of gonorrhoea, indigestion, diarrhoea and asthma[8]. The leaves, seeds and bark are also used in livestock healthcare[9]. Previously, only quercetin and its 3-O-disacchride were isolated from the leaves of the plant[10]. This article deals with the chemical investigation and antimicrobial activity of ethanolic extract of *Q. leucotrichophora* stem bark.

MATERIAL AND METHODS

Collection of plant material

The stem bark of *Q. leucotrichophora* were collected in January 2008 from Nagnath 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 sprit using soxhlet. The defatted bark material 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 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 Compound1:

M.P. - 226-228°C

Molecular Formula - $C_{16}H_{12}O_6$

Molecular Weight - 300 amu

UV (λ_{Max} ^{MeOH}) nm - 270, 275, 280, 373, 390

IR (γ_{Max}^{KBr}) Cm^{-1} - 3480, 3260, 1650, 1600, 1500, 1420, 1350,1340,1280, 1220.

^{1}H-NMR (CD_3OD, δ ppm)

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

^{13}C-NMR (CD_3OD, δ ppm)

148.3 (C-2), 137.4 (C-3), 175.8 (C-4), 105.4 (C-4a), 162.1 (C-5), 98.5 (C-6), 167.2 (C-7), 92.8 (C-8), 158.2 (C-8a), 123.7 (C-1′), 130.0 (C-2′, 6′), 116.0 (C-3′, 5′), 160.2 (C-4′), 56.4 (OMe)

Compound 1 was crystallized as pale yellow needle shaped from methanol. It gave green colour with $FeCl_3$ and positive test with Mg/HCl thereby indicating the flavonoid nature of compound[11]. The IR spectrum of compound furnished two absorption bands at 3480cm^{-1} and 3260cm^{-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 1500cm^{-1}, 1420 cm^{-1} for ether functional group. The ^{1}H NMR spectrum of compound 1 indicated six aromatic protons, two doublets at δ 6.21 (d,1H, J = 2.1Hz)and 6.39 (d,1H, J = 2.1Hz) assigned to H-6 and H-8, respectively, and other two doublets at δ 6.93 (d,2H, J = 8.8Hz) and 8.04 (d,2H, J = 8.9Hz) due to a pair of protons (H-2′, H-6′ and H-3′, H-5′) having an A_2B_2 system, which is the characteristic of a p-substituted aromatic ring. It was further supported by its ^{13}C–NMR data appeared at δ 130.7 (C-2′, 6′) and δ 116.3 (C-3′, 5′) which were corresponded with hydrogen bearing carbon of p-cresol [12]. The ^{1}H NMR spectrum of compound 1 also signified one singlet for 3H at δ 3.83 ppm assigned to methoxy proton. The ^{13}C NMR revealed 16 peaks of carbon resonance characteristic for aromatic nucleus and the downfield value at δ 175.8 (C-4) indicated the presence of carbonyl functional group and a up field value at δ 56.4 indicated the presence of methoxy group. The ^{13}C-NMR spectrum showed other peaks a benzylic carbon at δ 148.3 (C-2) and an oxygen bounded ethylenic carbon atom at δ137.4 (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 [13].

Characterization of Compound 2

M.P	-	232-234°C
Molecular Weight	-	756amu
FAB-MS (m/z)$^-$	-	755[M-H]$^-$, 609[(M-H)-146]$^-$, 463[(M-H) - (2x146)]$^-$ 301[(M-H) - (2x146+162)]$^-$
IR (γ_{Max}^{KBr}) Cm^{-1}	-	3410, 3220, 1650, 1600, 1525, 1430

^{1}H-NMR (CD_3OD, 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)

^{13}C-NMR (CD_3OD, δppm) (Aglycone)

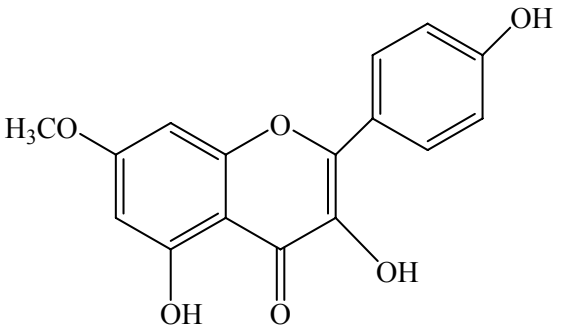
160.7(C-2), 149.6(C-3), 177.4(C-4), 160.9(C-5), 149.2(C-6), 165.1(C-7), 92.3(C-8), 157.7(C-8a), 104.6 (C-4a), 133.6(C-2'), 150 (C-3'), 145.8 (C-4'), 156.3(C-5'), 97.3(C-6').

Glycon

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'').

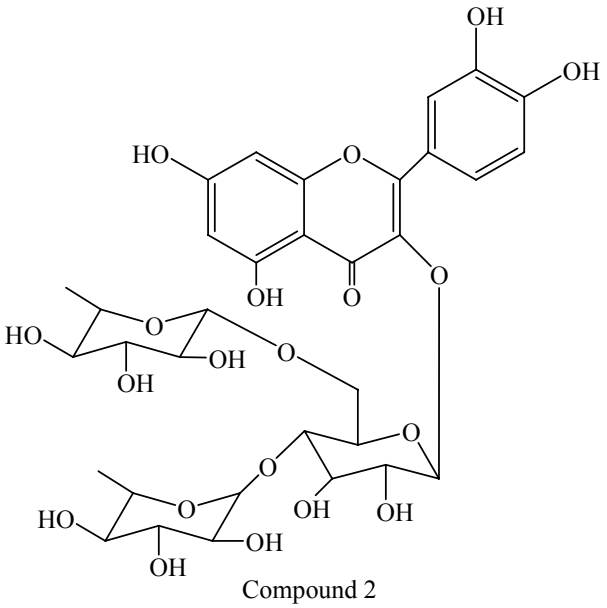
Rhamnose: 102.28 (C-1'''), 70.90 (C-2'''), 72.18 (C-3'''), 70.50 (C-4'''), 70.40 (C-5'''), 17.77 (C-6''').

Rhamnose: 100.21 (C-1''''), 70.00 (C-2''''), 68.57 (C-3''''), 68.24 (C-4''''), 68.15 (C-5''''), 17.60 (C-6'''').



Compound 1

Compound 2 was crystallized as pale yellow crystals from ethanol. It gave positive test (green colour) with alcoholic $FeCl_3$ and also with Mg/HCl showing flavonoidal nature of compound. It also gave positive Molish's reagent test indicating the glycosidic nature of compound[11]. The molecular ion peak observed at m/z 755 [M-H] in its FAB-MS, which conclude the molecular weight of compound is 756 amu. Other fragmentation peaks appeared at m/z 609 [(M-H)-146]$^-$, 463[(M-H) - (2x146)]$^-$, 301[(M-H) - (2x146+162)]$^-$ corresponding the sequential loss of two deoxy hexosyl and one hexose unit from molecular ion peak. The IR spectra of Compound 2 showed characteristic absorption bands at 3500 (OH) and 1600 (C=O). The ^{1}H NMR of Compound 2 displayed doublet at δ 7.8 (*J*=2.5 Hz), 6.20 (*J*=2.5 Hz), 7.7 (*J*=2.5 Hz), 6.80 (*J*=2.5 Hz) and 7.15 (d, *J*=6.0 Hz) for H-2', H-6, H-8, H-5' and H-6' respectively. A singlet at δ 1.27 ppm indicated the presence of rhamnose methyl proton. Three 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 ^{13}C 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 anomeric 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 hydrolysis 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 ^{1}H-NMR and ^{13}C-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.



Compound 2

Antimicrobial activity

The antimicrobial study of ethanolic extract of *Quercus leucotrichophora* was carried out against *Staphylococcus aureus*, *Pseudomonas auroginosa*, *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) study[14]. 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 turbity standard [1-2 x10^8 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 organisms[15].

Table 1: Antimicrobial activity of QB against tested microorganisms compared with reference compounds

S No.	Test microorganism	IZD (cm)			MIC (µg/ml)
		QB (10mg/ml)	E (1mg/ml)	A (1mg/ml)	QB (ethanolic extract)
1	*S. aureus*	23.0 ± 1.0	31.2 ± 0.25	28.2 ± 0.34	250
2	*B. Subtilis*	15.0 ± 0.00	32.1 ± 0.98	26.4 ± 0.48	250
3	*P. auroginosa*	21.0 ± 1.0	27.5 ± 1.14	20.6 ± 0.52	125
4	*E. coli*	26.3 ± 1.53	24.6 ± .75	19.3 ± 0.22	125

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

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