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Screening of phytoconstituents and antibacterial activity of leaves and bark of *Quercus leucotrichophora* A. Camus from Uttarakhand Himalaya

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Abstract

Background: *Quercus leucotrichophora* A. Camus (QL) belongs to the family Fagaceae, commonly known as Banj oak in the Garhwal region of Himalaya, where it is the principal source of fuel, fodder, and medicine.

Methods: In the present study, GC-MS analysis has been performed for profiling the chemical composition of methanolic extracts of leaves and bark of QL. The antibacterial activity was evaluated by using the disk diffusion method against five bacterial strains.

Results: Total 23 components in bark and 62 components in leaves extracts of QL were identified. The major components identified in the bark extracts were Linoleic acid (19.77%), Lupeol (17.91%), Epi-psi-Taraxastanonol (14.20), and cis-Vaccenic acid (13.10%), while others were present in relatively small amounts. For the leaves extract, the major components were Linoleic acid (17.09%), Simiarene (15.29%), Flavone 4′-oh, 5-oh,7-di-o-glucoside (15.26%), and D-Quinic acid (9.29%), respectively. As far as antibacterial assays are concerned, it was observed that both the extracts are active against most of the tested bacterial strains with the zone of inhibition ranging between 8.53 ± 0.50 to 19.07 ± 0.31 mm, respectively.

Conclusion: The GC-MS results revealed the presence of several phytochemical compounds in leaves and bark of QL extract and are recommended as a plant of pharmaceutical importance. The antibacterial analysis showed that both the extracts (leaves and bark) of QL have antibacterial activity against all gram positive (*S. aureus, B. subtilis* and *S. pyogenes*) and gram negative (*E. coli*, *P. aeruginosa*) bacterial strains.

Keywords: Antibacterial activity, Chemical composition, Himalaya, *Quercus leucotrichophora*

Background

Use of plants and plant extracts as a source of medicine has been inherited and is an important component of the health care system in the world. India is the largest producer of medicinal herbs and is known as the botanical garden of the world [1]. The Himalayan region is well known for its huge diversity of flora with more than 10,000 natural plant species, especially medicinal plants. Banj oak (*Quercus leucotrichophora* A. Camus) belonging to the family Fagaceae is an evergreen tree of approximately 40 m height and commonly found throughout the Himalayan region with a latitudinal range from 800 to 2300 m [2]. Several species of Quercus genus possess immense medicinal properties and therapeutic applications [3–6]. Banj oak is the principal source of fuel supply as well as the main fodder tree in the Himalayan region [7]. The leaves, seeds and bark of QL are used in human health care system as well as for livestock health care [8, 9]. Gum of the tree is traditionally used for the treatment of gonorrhoeal and digestive disorders, especially in children [10, 11]. The seeds act as astringent and diuretic agents and are also used in the treatment of indigestion, diarrhoea and asthma in humans [12]. Previously, active compounds like, quercetin and kaempferol were

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isolated from the ethanolic stem bark extract of QL, whereas the antimicrobial activity of the extract showed highest activity against *E. coli* followed by *S. aureus*, *P. auroginosa* and *B. subtilis*, respectively [13]. Further, the presence of twenty-three phytoconstituents (major phytocomponent: monoterpenoids) in the volatile extract of bark of QL were analyzed by GC-MS analysis [14]. The fruit extract of QL revealed the presence of higher amount of saturated fatty acid compared to unsaturated fatty acid. The bark and fruit extract of QL possess antimicrobial activity [14, 15]. The QL is used in traditional system of medicine, but still there are not many scientific reports to confirm its phytochemical activity and medicinal properties [16]. Thus, the present study was aimed to investigate the chemical composition and antibacterial activity of methanolic leaves and bark extracts of QL.

Methods

Plant collection and preparation of crude extracts

Leaves and bark of QL were collected from the Uttarakhand Himalaya (Tehri district), India and voucher specimens (BSI/NRC-115222) have been kept in the herbarium of Botanical Survey of India (BSI/NRC--Dehradun), Uttarakhand, India. Plant samples (leaves and bark) of QL were cleansed, shade dried and coarsely powdered. Crude powdered material (500 g) was extracted with methanol (80%) using a Soxhlet extractor. The extracts obtained were filtered and concentrated using a rotary vacuum evaporator (Strike-12, Steroglass, Italy) and used for further analysis (GC-MS and antibacterial analysis).

GC-MS analysis

GC-MS analysis was performed at University Science Instrumentation Centre, Jawaharlal Nehru University (JNU), Delhi (India). The analyses of the methanolic extracts were carried out on a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan). The system was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), a mass selective detector with an ion source (220 °C) and an interface (260 °C). Rtx-5 MS capillary column (Restek Company, Bellefonte, USA) having 30 m (length) × 0.25 mm (diameter) × 0.25 μm (film thickness) was used for GC-MS analyses. The mass range of 40–650 m/z with 1000 ev of threshold was used. The injector was set in the split injection mode having 250 °C of temperature. The starting temperature was adjusted to 80 °C (3 min), which afterwards increased to 280 °C with a ramp rate of 10 °C/min. Helium (> 99.99%) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow according to stranded methods [17, 18]. The bark and leaves extract components were identified on the basis of retention time (RT) by gas chromatography and interpretation of mass spectrum was performed by comparing spectral fragmentation obtained, to the database provided by NIST11.LIB and Wiley8.LIB [17, 18].

Antibacterial activity

Five pathogenic bacterial strains were used in this study for assessing the antibacterial activity of QL, including the Gram-negative and Gram-positive strains namely; *Escherichia coli* (MTCC-582); *Pseudomonas aeruginosa* (MTCC-2295); *Staphylococcus aureus* (MTCC-3160); *Bacillus subtilis* (MTCC-441); and *Streptococcus pyogenes* (MTCC-1924). The reference bacterial strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh (India) and were maintained at 4 °C on slants of nutrient agar (NA) (Merck, Germany). The antibacterial activity of plant extracts was carried out using the disk diffusion method [19]. The methanolic bark and leaves extracts were dissolved in 10% of dimethyl sulfoxide (DMSO). The concentration and

Table 1 GC-MS analysis of *Quercus leucotrichophora* (Bark) extract

SN	Retention time	Area per cent	Name of compounds
1	10.086	0.19	Beta-Himachalene
2	11.992	0.14	.Alpha.-Eudesmol
3	12.152	0.62	Myristyl acrylate
4	13.239	0.08	1-Octadecene
5	14.150	0.10	Phthalychloride
6	14.643	0.14	Methyl Palmitate
7	15.067	4.53	Pentadecanoic acid
8	16.030	0.11	Heptadecanoic acid
9	16.318	0.24	Linoleic acid methyl ester
10	16.772	19.77	Linoleic acid
11	16.803	13.10	cis-Vaccenic acid
12	16.860	4.13	Ambrettolide
13	16.974	2.92	Octadecanoic acid
14	17.566	0.67	10,12-Hexadecadien-1-ol
15	20.814	0.41	Lignoceric alcohol
16	24.147	0.28	Nonadecyl pentafluoropropionate
17	26.641	0.31	2,3-Oxidosqualene
18	36.274	2.59	Taraxerone
19	36.631	1.45	Clionasterol
20	37.952	6.21	Simiarene
21	38.517	14.20	Epi-psi-Taraxastanonol
22	39.357	17.91	Lupeol
23	41.461	1.81	Sitostenone
Total		91.91	
Unidentified		0.34	

Table 2 GC-MS analysis of *Quercus leucotrichophora* (Leaves) extract

SN	Retention time	Area percent	Name of compounds
1	5.184	0.23	2,3-Dihydro-3,5-dihydroxy-6-methyl-4 h-pyran-4-one
2	8.397	0.26	4-Propylphenol
3	10.625	0.07	Lauric acid
4	11.354	0.36	.beta.-Methylglucoside
5	11.750	9.29	D-Quinic acid
6	11.924	0.14	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl
7	12.111	0.87	Tetradecyl acrylate
8	12.367	0.12	Methoxyeugenol
9	12.533	0.08	2-Hydroxy-5-isopropyl-2,4,6-cycloheptatrienone
10	12.733	0.25	Methyl-(4-hydroxy-3-methoxyphenyl) acetat
11	12.856	1.44	Coniferol
12	13.200	0.07	Cyclopentadecane
13	13.283	0.26	(–)-Loliolide
14	13.453	0.84	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-
15	13.711	0.67	Oleic acid
16	13.970	0.15	Neophytadiene
17	14.093	0.05	Caprylone
18	14.164	0.25	E-2-Tetradecen-1-ol
19	14.458	0.20	3,5-Dimethoxy-4-hydroxyphenethylamine
20	14.596	0.27	Methyl palmitate
21	15.020	5.04	Pentadecanoic acid
22	15.254	0.18	5,9-Dimethyl-2-(1-methylethyl)cyclodecane-1,4-dione
23	15.405	0.11	2,4,4-Trimethyl-3-(3-oxobutyl)cyclohex-2-enone
24	15.490	0.20	Benzenepropanoic acid, 2,5-dimethoxy-
25	15.996	0.37	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester
26	16.270	0.17	Methyl linoleate
27	16.317	0.14	Methyl oleate
28	16.456	1.21	Phytol
29	16.726	17.09	Linoleic acid
30	16.919	1.43	Octadecanoic acid
31	18.527	0.42	Methyl hexadecadienoate
32	18.718	0.11	11-Eicosenoic acid
33	18.978	0.62	Arachidic acid
34	19.279	0.12	cis-9-Hexadecenal
35	20.133	0.13	Methyl tetrahydroionol
36	21.618	0.50	cis-Vaccenic acid
37	23.680	0.11	Ethyl linoleate (JAN)
38	23.807	0.09	1,1′-Biphenyl, 2-formyl-4′,5′,6′-trimethoxy-
39	24.061	2.93	1-Heptacosanol
40	25.631	0.10	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol
41	25.889	0.56	Octadecanal
42	26.567	0.41	2,8-Dimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol
43	26.915	0.44	Butanedioic acid, di-9-dodecyn-1-yl ester
44	27.372	0.49	2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene

Table 2 GC-MS analysis of *Quercus leucotrichophora* (Leaves) extract *(Continued)*

SN	Retention time	Area percent	Name of compounds
45	27.673	0.45	Tocopherol
46	28.406	0.56	3-Hydroxycholest-4-en-6-one
47	29.332	0.47	.beta.-Tocopherol
48	29.583	0.24	.gamma.-Tocopherol
49	30.461	1.17	Baccharane
50	31.294	2.78	Vitamin E
51	32.319	1.25	(+)-γ-Tocopherol, O-methyl-
52	33.809	0.47	1H-Indole
53	36.231	15.29	Simiarene
54	36.396	0.74	Clionasterol
55	36.793	0.82	Verticiol
56	37.508	0.49	.beta.-Amyrin
57	37.714	0.45	Methyl ursolate
58	39.351	0.67	D:C-Friedo-B′:A′-neogammacer-9(11)-en-3-one
59	40.473	0.62	9,19-Cyclolanost-23-en-3-ol, 25-methoxy-, acetate, (3.beta.,23e)-
60	40.807	0.16	-Heptadecyloxirane
61	41.130	3.81	Stigmast-4-en-3-one
62	44.451	15.26	Flavone 4′-OH,5-OH,7-di-o-glucoside
Total		94.54	
Unidentified		1.10	

volume of the extracts used for the analysis of antibacterial activity were 5 mg/ml and 20 μl (extract soaked by each disc), respectively. The antibacterial activity was assessed by measuring the zone of inhibition surrounding the disks and each experiment was carried out in triplicate. In the present study, DMSO (10%) and ampicillin (1 mg/ml) were used as negative and positive controls, respectively.

Results and discussion

This study focused on the chemical composition and antibacterial screening of QL extracts. The yield of bark and leaves extracts were found to be 9.7% and 13.6%, respectively. A range of volatile phytoconstituents have been identified by GC-MS in different Quercus species other than QL [20, 21]. In the present study, the percentages (area per cent) and the retention time (RT) of the components are listed in Tables 1 and 2. In leaves extract of QL, 62 components were identified, representing 94.54% of the total plant extract, in which Linoleic acid (17.09%), Simiarene (15.29%), and Flavone 4′-OH,5-OH,7-di-O-glucoside (15.26%) were the major components, however, in bark extract of QL, 23 components were identified, representing 91.91% of the total plant extract, in which Linoleic acid (19.77%), Lupeol (17.91%), Epi-psi-Taraxastanonol (14.20%), and cis-Vaccenic acid (13.00%) were the major compounds. Linoleic acid is an omega-6-fatty acid and is enormously used in cosmetic industries, whereas the conjugated linoleic acid was accounted to have anticarcinogenic, fat reducing, antiatherogenic and immune enhancing activity [22]. Lupeol is a triterpenoid which possess

Table 3 Antibacterial profile of QL extracts

Bacterial strains	QLB (ZOI)		QLL (ZOI)		DMSO (ZOI)
	QLB (Ave ± SD) mm	Ampi (Ave ± SD) mm	QLL (Ave ± SD) mm	Ampi (Ave ± SD) mm	
E. coli	9.37 ± 0.65	22.7 ± 0.65	8.53 ± 0.50	21.5 ± 0.62	0.00
P. aeruginosa	13.97 ± 0.42	23.2 ± 0.74	13.27 ± 0.25	23.3 ± 0.70	0.00
S. aureus	16.97 ± 0.25	22.6 ± 0.62	13.80 ± 1.51	20.7 ± 0.56	0.00
S. pyogenes	15.97 ± 0.65	21.2 ± 0.46	15.83 ± 0.29	22.4 ± 0.52	0.00
B. subtilis	19.07 ± 0.31	22.2 ± 0.51	17.03 ± 0.55	20.6 ± 0.57	0.00

Note: *QLB Quercus leucotrichophora* bark, *QLL Quercus leucotrichophora* Leaves, *ZOI* Zone of inhibition, *Ampi* Ampicillin, *DMSO* Dimethyl sulfoxide

anticancer and anti-inflammatory activities [23]. Flavone 4'-OH,5-OH,7-di-O-glucoside is a isoflavonoid and possess antioxidant activity [24]. Cis-vaccenic acid is a omega-7 fatty acid is known for its antibacterial activity and hypolipidemic effect in rats [24]. Epi-psi-Taraxastanonol is a terpenoid and is known for its therapeutic activity against cardiovascular diseases [25]. A total of seven components were found to be the common for both extracts of QL. Previous studies on Quercus genus suggested that the species are rich in monounsaturated fatty acids, mostly oleic acid and also essential fatty acids such as linoleic (ω-6) and linolenic (ω-3) fatty acids, sesquiterpenes, terpenoids, flavonoids and phenolic acid [20, 21, 26] and in the present study same pattern of phytoconstituents were observed in the leaves and bark extracts of QL. Differences in quantity and quality of chemical components of any plant extract are highly influenced by several genetic and environmental factors, such as the genetic and seasonal variation, geographical origin, and the part of the plant used for the study, even agronomic conditions, developmental stage, time of collection, extraction method and solvent system [27].

The quantification of antibacterial activity for methanolic extracts of QL has been evaluated against five bacterial species by means of the agar disk diffusion method. The results of antibacterial activity of QL extracts are expressed as the diameter of the inhibition zone in millimetre (shown in Table 3). QLB and QLL extracts showed zone of inhibition (ZOI) from a range of 9.37 ± 0.65 to 19.07 ± 0.31 mm and 8.53 ± 0.50 to 17.03 ± 0.55 mm, respectively. Both the extracts showed the maximum and minimum zone of inhibition (ZOI) against *B. subtilis* and *E. coli*, respectively. Ampicillin showed ZOI from a range of 21.2 ± 0.46 to 23.3 ± 0.70 mm for all the bacterial strains, and DMSO was used as a negative control, which showed no zone of inhibition. Previously, the antimicrobial profile of the volatile extract of QLB was recorded against three microbial cultures, namely; *Streptococcus pyogenes, Streptococcus aureus*, and *Escherichia coli.* The volatile extract of QLB exhibited a potential antimicrobial activity against *Streptococcus pyogenes,* compared to *Streptococcus aureus*, and *Escherichia coli* [14]. The antibacterial activity of the fatty acid methyl ester (FAME) extract of QL fruits was recorded against four bacterial stains namely; *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* from a range of 7.8 to 15.9 mm [15]. The extract of FAME showed dissimilar activity against different bacterial strains due to the chemical nature, antimicrobial agents, and their mode of action on different microorganism [28]. In the present study, both the extracts of QL demonstrated better antibacterial activity compared to previous studies.

Conclusion

The GC-MS analysis of methanolic extract of bark and leaves of QL revealed the presence of highly composite profiles of medicinally important bioactive components. This study also revealed the antibacterial activity of QLB and QLL against pathogenic microbes. Therefore, it can be concluded that the methanolic leaf and bark extracts of QL have shown the presence of active compounds having pharmacological and industrial importance.

Abbreviations

Ampi: Ampicillin; *B. subtilis*: *Bacillus subtilis*; DMSO: Dimethyl sulfoxide; *E. coli*: *Escherichia coli*; GC-MS: Gas Chromatography- Mass spectrometry; *P.aeruginosa*: *Pseudomonas aeruginosa*; QL: *Quercus leucotrichophora*; QLB: *Quercus leucotrichophora* bark; QLL: *Quercus leucotrichophora* leaves; RT: Retention time; *S. aureus*: *Staphylococcus aureus*; *S. pyogenes*: *Streptococcus pyogenes*; ZOI: Zone of inhibition

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Availability of data and materials

All data generated or analysed during this study are included in this article.

Authors' contributions

PS, SP and HB reviewed the literature, collected the samples, performed all the experiments, and drafting the manuscript with RKB. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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