IJPSR (2022), Volume 13, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 February 2021; received in revised form, 01 June 2021; accepted, 09 June 2021; published 01 January 2022

INFLUENCE OF DIFFERENT SOLVENTS ON ANTIBACTERIAL POTENTIAL OF THREE SPECIES OF HIMALAYAN OAKS

Suresh Chandra Sati, Amrendra Nath Tripathi * and Parikshit Kumar

Department of Botany, D.S.B. Campus, Kumaun University, Nainital - 263001, Uttarakhand, India.

Keywords:

Kumaun Himalaya, *Oaks* spp, Plant extract, Gentamycin, Antibacterial activity

Correspondence to Author:
Mr. Amrendra Nath Tripathi

U.G.C. S.R.F
Department of Botany,
DSB Campus, Kumaun University,
Nainital - 263001, Uttarakhand, India.

E-mail: amrendratripathi05@gmail.com

ABSTRACT: The antibacterial property of three Himalayan *Oaks* species (*Quercus floribunda*, *Q. leucotrichophora* and *Q. semecarpifolia*) in different solvents against eight gram-positive and gram-negative pathogenic bacteria employing disc diffusion method has been investigated. All the test microorganisms were inhibited significantly by *Oaks* species at 1000 µg/ml. The MIC and MBC values of each extract (where ZOI ≥ 15 mm) were also determined. *Q. semecarpifolia* exhibited significantly higher antibacterial activity compared to other species. Solvent systems showed species-specific response for extraction of total content (% yield) of dry extract. Maximum yield was found for ethanol extract (3.59-5.79%). Methanol extract of *Q. leucotrichophora* showed the highest inhibitory activity against all the tested bacteria (16-23 mm) ZOI, followed by acetone extract (17-23 mm), ethanol extract (15-20 mm). Furthermore, methanol and acetone extracts of *Q. leucotrichophora* showed significant activity against *A. tumefaciens* and *E. chrysanthemi*, with MIC values of 15.6-31.25 µg/ml and MBC values of 15.6-125 µg/ml. *Q. semecarpifolia* showed its highest antibacterial potential against *X. phaseoli* and *E. chrysanthemi* whereas extracts of *Q. floribunda* leaves were found with moderate activity against all the tested bacteria. The chloroform and ethanol extracts have the lowest inhibitory activity (ZOI, 22 mm each). All the tested bacterial strains were found resistant to aqueous extracts of *Oaks* species. The bacterial inhibitory potential of *Q. semecarpifolia* was found very close to gentamycin (30 mcg). The present work fully highlighted the utility of three *Oaks* spp. of Kumaun Himalaya for their antibacterial activities against pathogenic bacteria.

INTRODUCTION: Plants in various forms are being used in different traditional systems of medicine for the treatment of human ailments, particularly those caused by pathogenic microbes since time immemorial [1]. These are also valuable resources for the isolation of novel bioactive molecules to combat microbial diseases.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(1).197-05

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(1).197-05

Now a day's infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [2]. The discovery of novel active compounds against new targets is a matter of urgency. Almost every part of the plant possesses antimicrobial activity [3] it is due to the presence of phytoalexins formed in plants. The effect of plant extracts on bacteria has been studied by a large number of researchers in different parts of the world [3, 4, 5].

Quercus spp. (Family: Fagaceae) represents an important group of evergreen or semi-deciduous trees from temperate and tropical climatic areas. The genus *Quercus* is comprised of around 450

species worldwide, which often differ in their flowering and fruiting dynamics [6]. *Quercus* species are used in traditional medicine as astringent, antiseptic and haemostatic and for the treatment of acute diarrhea, hemorrhoid and oral, genital and anal mucosa inflammation [7]. The decoction of *Q. floribunda* plants can be used for burns and added to ointments for the healing of cuts [8]. Due to the multipurpose use of *Quercus* species, it has been the subject of intensive research.

Some recent reports revealed ethnobotanical uses of *Q. leucotrichophora* in the Himalayan region like, in North Pakistan, fruit and seed powder is taken for the treatment of urinary infection [9, 10] bark is used to cure toothache and piles leaves is used as an astringent and in the treatment of diarrhea while the gum resin paste is used as stomachache, to cure Gonorrhea, asthma, hemorrhages, diarrhea, dysentery [11, 12] and its dry resin with water has been used for stomach pain [13, 14]. Some authors reported that, *Q. floribunda* is also used as an astringent and antidiarrhoeal [15]. The seeds are used in the treatment of diarrhoea, menorrhagia, and gastrointestinal hypertrophy. It has been reported that different species of *Quercus* possess antibacterial activity [16, 17], antioxidant activity and *in vivo*- hepatoprotective activity [18, 19] and anti-inflammatory effect [20].

In the literature, many species of the genus *Quercus* have been studied for their biological activities *i.e.*, antioxidant, antibacterial, and antifungal. However, little is known on the antibacterial and antifungal properties of Himalayan *Quercus* species. The aim of this study was to investigate the antibacterial potential of various organic extracts of leaves of *Q. leucotrichophora, Q. semecarpifolia,* and *Q. floribunda*, occurring at high altitude in Kumaun Himalaya, India, against a wide range of pathogenic bacterial strains, which have not been evaluated earlier.

MATERIALS AND METHODS:

Collection of Plant Material: Green leaves of *Q. leucotrichophora* (QL), *Q. semecarpifolia* (QS), and *Q. floribunda* (QF) were collected in the month of April from Nainital, Kumaun Himalaya, India and authenticated by the plant taxonomist of the department of Botany, Kumaun University Nainital. Voucher specimens were deposited in the herbarium of the department for further references.

Extraction Procedure: Leaves of the plants were thoroughly washed with distilled water and dried at room temperature (20 ± 2°C). The dried material was powdered in an electric grinder. To prepare the stock solution 50 g of this powder was placed in a 500 ml conical flask mixed with 200 ml of solvents (w/v, 50g/ 200 ml). The mouth of flasks is tightly plugged with non-absorbent cotton and tightly wrapped with aluminum foil to prevent evaporation. Solvents used for extraction were methanol, ethanol, chloroform, ethyl acetate, acetone, hexane, and distilled water. All mixtures were shaken on a rotary incubator shaker at 190-220 rpm for 24 h at room temperature 37°C. The mixtures were filtered through Whatman filter paper no. 1 and filtrate collected separately in a clean beaker. The extracts were evaporated, using steam both to dryness at 30°C. The dry extracts were weighed and kept in sterile sample bottles and stored in the refrigerator at 4°C for further use.

Antibacterial Activity:

Microorganism Used: Five (Gram +ve and –ve) bacteria (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No. 40, *Agrobacterium tumefaciens* MTCC No. 609, procured from Institute of Microbial Technology Chandigarh, India, and *Ralstonia solanacearum* ITCC No. BH0007, *Xanthomonas campestris* ITCC No. BD0006, *Xanthomonas oryzae* ITCC No. PI0012 was obtained from Indian Type Culture Collection, IARI, New Delhi, India on the other hand, *Xanthomonas phaseoli* and *Erwinia chrysanthemi* obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India, were used in this investigation. Pathogens obtained from respective stock cultures were inoculated (1% v/v) into nutrient agar broth followed by incubation at 37°C (for 18 h) to activate cultures.

Screening of Antibacterial Activity: Antibacterial tests of selected microorganisms were carried out using the disc diffusion method [21]. Nutrient agar plates (90 mm size) were prepared and cool down at room temperature (20 ± 2°C). Test bacterial inoculums containing 10^6 CFU/ml of test bacteria were spread uniformly. A small sterile swab was dipped into 24 h old test culture of bacteria and was

inoculated by streaking the swab over the entire agar surface. The process was repeated by streaking the swab 2 or more times rotating the plates approximately 60° each time to ensure the even distribution of inoculums. Each bacterial culture was inoculated on three plates as replicates. Stock solutions of the tested samples were prepared into 10% v/v aqueous dimethyl sulfoxide solution. The sterile filter paper disc (5 mm) loaded with 20µl of extract were placed on the surface of the bacteria seeded agar plates at equidistance, and it was allowed to diffuse for 5 min then these plates were incubated at 37± 1°C for 24 h of incubation in the bacterial incubator. Gentamycin (30 mcg) was placed in to agar plates used as a positive control, and 10% v/v aqueous dimethylsulfoxide solution was also used as a negative control. After 24 h of incubation, the diameter was observed for the inhibition zone (measured in mm, including disc size). All tests were performed in triplicates, and observed values of ZOI are expressed as mean values with standard error of means (SEM).

Determination of Minimum Inhibitory Concentrations (MIC's): The most effective plant extracts which exhibiting a strong antibacterial activity with ZOI, 15 mm or more than 15 mm at 1000 µg/ml were tested to determine their MIC using disc diffusion method and evaluate their efficiency in controlling bacterial strains causing plant pathogenic diseases. MIC and MBC were performed at seven concentrations of extracts (500, 250, 125, 62.5, 31.25, 15.625, and 7.8 µg/ml) following two-fold serial dilution technique [22] and loaded their requisite amount over sterilized filter paper discs (6 mm in diameter). Nutrient agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the effective plant extract were placed on the top of the Nutrient agar plates. The plates were incubated at 35ºC for 24 h. The inhibition zones were measured and recorded against the concentrations of the effective plant extracts.

Determination of Minimum Bactericidal Concentrations (MBC's): Streaks were taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto sterile nutrient agar (NA) plates. The plates were incubated at 35 ± 2 ºC for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibit any bacterial growth on the freshly inoculated agar plates.

RESULTS AND DISCUSSION:

Plants Extract Yield: The results revealed that different solvent systems showed a variable percentage of yield. The percentage yield of leaves extracts of *Q. leucotrichophora* (QL), *Q. semecarpifolia* (QS) and *Q. floribunda* (QF) in methanol, acetone, ethanol, chloroform, hexane and aqueous solvents are presented in **Table 1**. The ethanol extracts of QS and QL were showed the highest yielding (5.79% and 5.57%, respectively), while the hexane extracts were observed the least yield (0.81% and 1.53 %, respectively). On the other hand, methanol extract of QF leave was showed its highest yield (4.42%), followed by ethanol extract (3.59%), aqueous extract (3.54%) but hexane extract gave the least yield (0.64%). The polarity of solvent used for extraction and the method of extraction play a vital role in the efficiency (yield) and efficacy (magnitude of bioactivity) of prepared extracts [23.] It is interesting to note that the hexane (non-polar) extracts of all selected plants were found lowest yield compare to others solvent extracts (polar solvent).

TABLE 1: PERCENTAGE OF YIELD IN DIFFERENT EXTRACTS OF *QUERCUS* LEAVES

	Yield of extract (%)		
Plant extract	***Q. leucotrichophora***	***Q. floribunda***	***Q. semecarpifolia***
Methanol	4.15	4.42	4.43
Acetone	2.22	2.92	4.64
Ethanol	5.57	3.59	5.79
Chloroform	1.68	1.15	4.49
Hexane	1.53	0.64	0.81
Water	4.09	3.54	2.10

Antibacterial Activity of Plants Extract: Results obtained from disc diffusion method for antibacterial activity of the plant extracts of QL, QS, and QF in different solvents against eight pathogenic microorganisms are shown in **Table 2** and **3**. Relying upon the results obtained in the present investigation, it is clear that almost all leaves extracts of tested species were potentially effective in suppressing microbial growth of plant pathogenic bacteria with variable potency except aqueous extract. This might be due to the various

substances that show activity against bacteria are more soluble in organic solvents than water and might be absent in aqueous extract as suggested by Boer *et al.* [24] Now- a days a number of micro-organisms acquire several resistance mechanism; making them multi-drug resistance (MDR) and develop novel antimicrobial agents for combating resistant organism [25].

Methanol and acetone extract of all tested *Oaks* species were found most effective against all bacterial strains **Fig. 1, 2,** and **3**. All the plant extracts were found active against both Gram-positive and Gram-negative bacteria. This is contrary with the earlier reports, which suggest that plant extracts remain more active against Gram-positive bacteria as compared to Gram-negative bacteria [26, 27]. While comparing with standard drug (gentamycin), the plant extract demonstrated relatively lesser anti-microbial activity **Fig. 1** and **Table 2**.

These results show the potential utility of natural compounds over synthetic antibiotics considering the reported side effects of synthetic chemicals [28]. Also, synthetic drugs have high production costs; therefore, biologically active compounds derived from plants can be an alternative source to combat infectious diseases [29].

In a recent study, Semwal *et al.,* [30] reported that methanol extract of QL leaves has significant inhibitory activity against a panel of bacterial strains, and the result of the present investigation also supported the earlier findings. Out of six fractions tested of QL leaves, the methanol and acetone extracts were found most active against all the tested bacterial strains with a maximum zone of inhibition against *E. chrysanthemi* (ZOI, 23 mm each) except *X. compestris*, which was found sensitive to acetone extract of the species **Table 2**.

On the other hand, the second highest antibacterial activity (ZOI, 22 mm) was recorded at four instances *i.e.,* methanol and acetone extract of QL against *E. coli* and *B. subtilis* respectively. Quercetin and its 3-O -disaccharides were also isolated from the leaves of *Quercus sp.*[31`] and recently, some investigators reported the remarkable bacterial inhibitory potential of quercetin against a panel of bacteria [32, 33, 34]. Ethanol, methanol and acetone extracts of QS leaves showed significant activity against all studied bacterial strains **Fig. 2**.

The polarity of a solvent plays an important role on the composition of an extract and hence on its potential antibacterial activity. **Fig. 2** showed that chloroform extract of QS has the least inhibitory activity against maximum tested bacterial strains. But it was interesting to note that, the promising inhibitory activity of QS was recorded in chloroform and acetone extract against *X. phaseoli* and *E. chrysanthemi* (ZOI, 22 mm each) respectively **Table 2** and **Fig. 2**. The present study clearly demonstrated that chloroform extract of QS showed the comparable inhibition potential with used positive control (gentamycin) against *X. phaseoli* **Table 2** and **Fig. 2**. Hexane extracts of QS and QF were also showed promising antibacterial activity against all the studied bacterial strains.

On the other hand Jamil *et al.,* [35] studied the antibacterial activity of QF (Syn. *Q. dilatata*) and reported that polar fractions of the aerial part showed remarkable antibacterial activity with the highest against *Bordetella bronchiseptica*. Although, in the present study, polar extract (methanol and acetone) showed moderate inhibitory activity and ethanol has the least activity **Fig. 3**. This inhibitory effect might be influence by climatic conditions *i.e.,* CO_2 and temperature [36]. Recently, Top *et al.,* [37], studied the climatic influence on content and chemical composition of *Quercus rubra* tissues and reported that climatic factors directly influenced the biologically active polyphenolic and tannin content of *Q. rubra* tissues. As evident from **Table 2**, it was notable that all tested bacterial strains have resistant potential against aqueous extracts of all studied plants and chloroform extract of QL. Chloroform extract of QF was also found least active against *E. coli* and *X. compestris* with ZOI, 19 and 15 mm respectively, while it has no activity against other tested strains **Table 2** and **Fig. 3**. Also, hexane extracts of QL did inhibit no growth of *A. tumefaciens, E. coli* and *X. phaseoli.* Similar results were also reported by Berahou *et al.,* [38] that only ethyl acetate, butanol and aqueous phases of methanol extract of *Quercus ilex* bark showed antibacterial activity against all the bacterial strains, whereas the hexane and dichloromethane

phases were found almost inactive. This suggests that the quality of active compounds responsible for antimicrobial activity varies extracts to extracts **Table 2**.

The polar organic solvents exhibited greater antimicrobial activity **Fig. 1, 2,** and **3** and strengthening the fact that antimicrobial agents are polar in nature; therefore, can be extracted through polar organic solvents [7]. The lipophilic solutes extracted by hexane and chloroform extract (non-polar solvent) are, therefore, not much effective against Gram-negative bacteria. Generally, Gram-positive was found comparatively more susceptible than Gram-negative bacteria, which might be due to the presence of outer peptidoglycan layer, which is not an effective permeability barrier [39]. However, in this study, more sensitivity of Gram-negative bacterial strains might be due to other possible mechanisms of extracted constituents which are directly associated with membrane disruption [40].

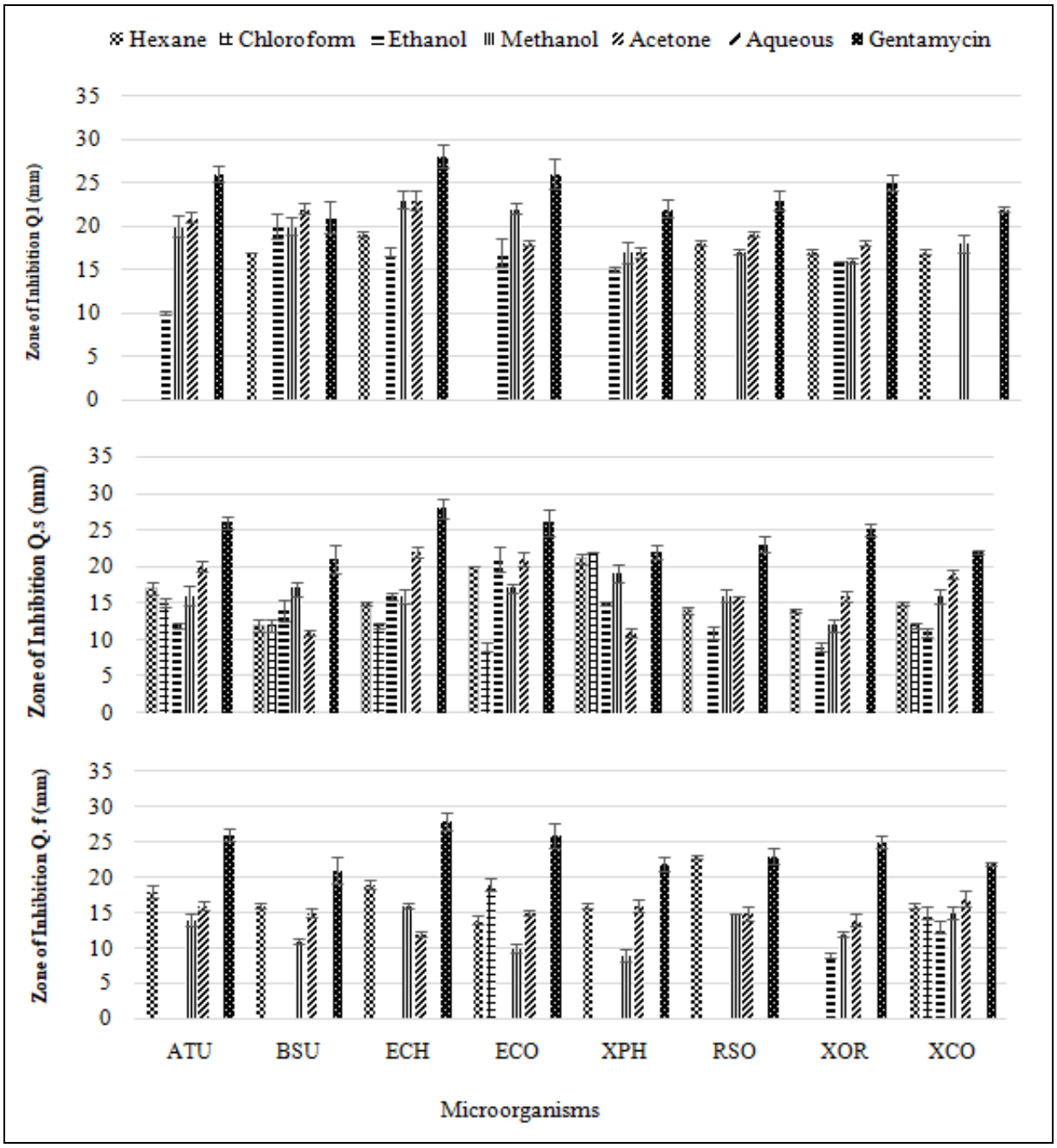

FIG. 1-3: ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF *Q. FLORIBUNDA* LEAVES AGAINST BACTERIAL STRAINS, ATU- *A. tumefaciens*; BSU- *B. subtilis*; ECH- *E. chrysanthemi*; ECO- *E. coli*; XPH- *X. phaseoli*; RSO- *R. solanacearum*; XOR- *X. oryzae*; XCO- *X. compestris*

TABLE 2: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *Q. LEUCOTRICHOPHORA Q. SEMECARPIFOLIA* AND *Q. FLORIBUNDA* LEAVES

Plant extracts	Diameter of Inhibition Zone (mm)*							
Quercus leucotrichophora	**ATU**	**BSU**	**ECH**	**ECO**	**XPH**	**RSO**	**XOR**	**XCO**
Hexane extract	Na	17±0.0	19±0.3	Na	Na	18±0.3	17±0.3	17±0.3
Chloroform extract	Na	Na	Na	Na	Na	Na	Na	Na
Ethanol extract	20±0.3	20±1.4	17±0.5	17±1.6	15±0.3	Na	16±0.0	Na
Methanol extract	20±1.3	20±1.0	23±1.0	22±0.6	17±1.2	17±0.3	16±0.3	18±1.0
Acetone extract	21±0.6	22±0.6	23±1.1	18±0.3	17±0.6	19±0.3	18±0.3	Na
Aqueous extract	Na	Na	Na	Na	Na	Na	Na	Na
Q. semecarpifolia								
Hexane extract	17±0.8	12±0.8	15±0.3	20±0.0	21±0.8	14±0.5	14±0.3	15±0.3
Chloroform extract	15±0.6	12±0.8	12±0.3	9±0.6	22±0.0	Na	Na	12±0.3
Ethanol extract	12±0.3	14±1.4	16±0.5	21±1.6	15±0.3	11±0.8	9±0.6	11±0.6
Methanol extract	16±1.3	17±1.0	16±1.0	17±0.6	19±1.2	16±0.8	12±0.9	16±0.9
Acetone extract	20±0.7	11±0.3	22±0.8	21±1.0	11±0.6	16±0.0	16±0.7	19±0.6
Aqueous extract	Na	Na	Na	Na	Na	Na	Na	Na
Q. floribunda								
Hexane extract	18±0.9	16±0.3	19±0.7	14±0.7	16±0.3	23±0.3	Na	16±0.3
Chloroform extract	Na	Na	Na	19±0.8	Na	Na	Na	15±0.9
Ethanol extract	Na	Na	Na	Na	Na	Na	9±0.3	13±0.8
Methanol extract	14±0.9	11±0.3	16±0.3	10±0.6	9±1.0	15±0.0	12±0.3	15±0.9
Acetone extract	16±0.7	15±0.6	12±0.3	15±0.3	16±0.9	15±1.0	14±0.9	17±1.2
Aqueous extract	Na	Na	Na	Na	Na	Na	Na	Na
Gentamycin (Positive Control)	26±0.9	21±1.9	28±1.3	26±1.8	22±1.0	23±1.1	25±0.9	22±0.3

ATU- *A. tumefaciens*; BSU- *B. subtilis*; ECH- *E. chrysanthemi*; ECO- *E. coli*; XPH- *X. phaseoli*; RSO- *R. solanacearum*; XOR- *X. oryzae*; XCO- *X. compestris*; na- not active

Minimum Inhibitory Concentrations (MIC's) and Minimum Bactericidal Concentrations (MBC's): The MIC and MBC results are summarized in **Table 3**. Organic extracts from the leaf part of QL appeared to be more potent than that of QS and QF. In many instances, MIC and MBC values of QL and QS extracts were lesser than QF extracts, it showed that higher MIC and MBC values represents the least bioactivity. Among all the extracts tested lowest MBC values demonstrated by methanol and acetone extracts of QL against *E. chrysanthemi* (15.6 and 31.25 µg/ml, respectively). Methanol extract of QL also had significant MBC value against *E. coli* (31.25 µg/ml). Lower limit of MIC values (15.6 µg/ml) was also demonstrated by chloroform, ethanol and acetone extracts of QS against *X. phaseoli, E. coli* and *E. chrysanthemi*, respectively **Table 3**.

The hexane extract of QF had a significant MIC value against *R. solanacearum* (15.6 µg/ml). It is evident from **Table 3** that *X. oryzae* had more resistant potential for different tested organic extracts (polar and non-polar) of QS and QF as compare to QL. Only acetone extract of QS had significant MIC and MBC to inhibit the *X. compestris* growth (62.5 and 125 µg/ml respectively) while other tested plant extracts were more sensitive for *X. compestris* different concentrations **Table 3**.

It was noteworthy that different organic extracts of QF leaves had moderate MIC values range (125-500 µg/ml) but hexane extract showed significant MIC (62.5-250 µg/ml) and MBC (62.5-500 µg/ml) values against tested bacterial strains except *E. coli* and *X. oryzae* **Table 3**. Jamil *et al.*, [35] reported that methanol extract of QF had effective MIC (range 200-500 µg/ml) against tested pathogenic bacterial strains and they reported that bacterium *E. coli* most sensitive with lowest MIC value (200 µg/ml) for methanol extract. In the present investigation acetone extract also had close proximity of MIC (250 µg/ml) against *E. coli* **Table 3**. This proximity of inhibitory potential in acetone and methanol extracts might be possible due to the propinquity of polarity in observed solvent system.

It was also notable that, in some instances studied plant extracts showed significant MIC against tested bacteria but do not have MBC against the same bacteria *i.e.*, ethanol extract of QL and QS against *X. phaseoli*, acetone extract of QS against *R. solanacearum*, hexane extract of QF against *B.*

subtilis, methanol extract of QS and chloroform extract of QF against *X. compestris* **Table 3**. MIC and MBC observations clearly showed that the studied plant extracts had less bactericidal potential as compare to bacteriostatic potential against gram-negative bacteria. The antibacterial activity of selected *Quercus* species had never been checked before against plant pathogenic bacteria. Thus, this is the first report on antibacterial activity of leaves extracts of Kumaun Himalayan *Quercus* species *i.e.*, QL, QS and QF against a panel of plant pathogenic bacteria. The results of the present investigation are largely in agreement with the earlier studies on antimicrobial activity of other *Quercus* species [7, 16, 17, 35, 38, 41] and provide a lead towards searching for new source of antibacterial substances. The results of this study indicate valuable findings for promoting the use of such species as a potential supplement in drug development. On the basis of available literature, it is observed that there is no previous record on the sensitivity of these plants' pathogenic bacterial strains *E. chrysanthemi*, *A. tumefaciens*, and *X. phaseoli* which are responsible for various plant disease.

TABLE 3: MIC AND MBC VALUES OF DIFFERENT EXTRACTS OF *Q. LEUCOTRICHOPHORA*, *Q. SEMECARPIFOLIA* AND *Q. FLORIBUNDA* LEAVES

Plant extracts	MIC and MBC values (µg/ml)															
	ATU		BSU		ECH		ECO		XPH		RSO		XOR		XCO	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Q.leucotrichophora																
Hexane extract	Nt	nt	250	500	62.5	250	nt	Nt	nt	nt	62.5	250	125	500	125	500
Chloroform extract	Nt	Nt	Nt	Nt	Nt	nt	nt	Nt	nt	nt	Nt	Nt	Nt	nt	nt	Nt
Ethanol extract	31.25	62.5	15.6	62.5	125	500	125	500	250	na	Nt	Nt	250	500	nt	Nt
Methanol extract	15.6	62.5	31.25	125	15.6	15.6	15.6	31.25	125	500	250	500	250	na	125	500
Acetone extract	15.6	125	15.6	62.5	15.6	31.25	62.5	250	125	500	62.5	250	62.5	250	nt	Nt
Aqueous extract	Nt	Nt	Nt	Nt	Nt	nt	nt	Nt	nt	nt	Nt	Nt	Nt	nt	nt	Nt
Q. semecarpifolia																
Hexane extract	125	500	Nt	Nt	500	na	31.25	125	31.25	62.5	Nt	Nt	Nt	nt	250	Na
Chloroform extract	250	500	Nt	Nt	Nt	nt	nt	Nt	15.6	62.5	Nt	Nt	Nt	nt	nt	Nt
Ethanol extract	Nt	nt	Nt	Nt	250	500	15.6	62.5	250	na	Nt	Nt	Nt	nt	nt	Nt
Methanol extract	250	500	250	500	125	500	125	250	31.25	62.5	125	500	Nt	nt	250	Na
Acetone extract	31.25	62.5	Nt	Nt	15.6	62.5	31.25	62.5	nt	nt	250	Na	500	na	62.5	125
Aqueous extract	Nt	Nt	Nt	Nt	nt	nt	nt	Nt	nt	nt	Nt	Nt	Nt	nt	nt	nt
Q. floribunda																
Hexane extract	62.5	250	250	Na	62.5	62.5	nt	Nt	250	500	15.6	62.5	Nt	nt	250	500
Chloroform extract	Nt	nt	Nt	Nt	nt	nt	62.5	125	nt	nt	Nt	Nt	Nt	nt	250	Na
Ethanol extract	Nt	nt	Nt	Nt	nt	nt	nt	Nt	nt	nt	Nt	Nt	Nt	nt	nt	Nt
Methanol extract	Nt	nt	Nt	Nt	250	500	nt	Nt	nt	nt	500	Na	Nt	nt	250	500
Acetone extract	250	500	250	500	nt	nt	250	500	250	500	500	Na	Nt	nt	125	250
Aqueous extract	Nt	Nt	Nt	Nt	nt	nt	nt	Nt	nt	nt	Nt	Nt	Nt	nt	nt	Nt
Gentamycin (Positive Control)	0.48	3.9	1.9	3.9	0.48	0.48	1.9	1.9	3.9	7.8	1.9	7.8	1.9	3.9	3.9	7.8

ATU- *A. tumefaciens*; BSU- *B. subtilis*; ECH- *E. chrysanthemi*; ECO- *E. coli*; XPH- *X. phaseoli*; RSO- *R. solanacearum*; XOR- *X. oryzae*; XCO- *X. compestris*; na- not active; nt- not test

CONCLUSION: The antimicrobial activity of different organic extracts of *Q. leucotrichophora* (QL), *Q. semecarpifolia* (QS*)* and *Q. floribunda* (QF) leaves were studied and indicated that the tested *Quercus* species have good antibacterial potentiality against all tested bacterial strains, especially against gram-negative bacteria. Furthermore, methanol and acetone extracts of *Q. leucotrichophora* showed more significant activity against *A. tumefaciens* and *E. chrysanthemi*, with MIC values of 15.6-31.25 µg/ml and MBC values of 15.6-125 µg/ml. The efficacy of each species differs depending on the chemical profile of the plants. The results suggest that these plant extracts should be further analyzed, as it might provide a new compound that is effective against multi-resistant infections.

ACKNOWLEDGEMENT: The authors wish to thank the Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India), and Microbial Type Culture Collection (MTCC), Chandigarh (India) for providing bacterial strains. One of us (ANT) is also thankful to University Grant Commission (UGC), New Delhi, for financial support under UGC-JRF Fellowship.

DECLARATION OF CONFLICT OF INTEREST: No potential conflict of interest was reported by the authors.

REFERENCES:

1. Samuelsson G: Drugs of natural origin: a textbook of pharmacognosy, 5th Swedish Pharmaceutical press, Stockholm 2004.
2. WHO- World Health Organization: Quality control methods for medicinal plant materials, Geneva 1992.
3. Sati, SC, Khulbe K and Joshi S: Antibacterial evaluation of the Himalayan medicinal plant *Valeriana wallichii* DC. (Valerianaceae). Research Journal of Microbiology 2011; 6: 289-96.
4. Sati SC and Joshi S: Antimicrobial potential of leaf extracts of *Juniperus communis* L. from Kumaun Himalaya, African Journal of Microbiology Research 2010; 4: 1291-94.
5. Sati SC and Joshi S: Antimicrobial activities of Ginkgo biloba L. Leaf extracts, The Science World Journal 2011; 11: 2241-46.
6. Tejerina D, Garcia TS, Vaca MC, Vasquez FM and Cava R: Acorns *Quercus rotundifolia* Lam) and grass as natural sources of antioxidants and fatty acids in the "montanera" feeding of Iberian pig: intra- and inter-annual variations. Food Chemistry 2011; 124: 997-1004.
7. Sohertoglu D, Ekizoglu M, Kilic E and Sakar MK: Antibacterial and antifungal activities of some quercus species growing in Turkey. FABAD Journal of Pharmaceutical Science 2007; 32: 127-30.
8. Baytop T, Turkiye de, Bitkilerle and Tedavi: 2nd ed. Istambul: Nobel Tip Kitabevi 1999.
9. Akhtar N, Rashid A, Murad W and Bergmeier E: Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan. Journal of Ethnobiology 2013; 25(9): 1-13.
10. Rawat VS: Medicinal plants and sustainable livelihood in Pauri district of Garhwal Himalaya, Uttarakhand, India. International Journal of Bioassays 2016; 5: 4589-92.
11. Gairola S, Sharma J and Bedi YS: A cross-cultural analysis of Jammu & Kashmir and Ladakh India medicinal plant use. Journal Ethnopharmacology 2014; 55: 925-86.
12. Chauhan PS, Bisht S and Ahmad S: Traditional and ethnobotanical uses of medicinal trees in district Tehri Garhwal Western Himalayas. Annals of Geriatric Education and Medical Sciences 2016; 1(1): 14-19.
13. Uniyal B and Shiva V: Traditional knowledge on medicinal plants among rural women of the Garhwal Himalaya, Uttaranchal. Indian Journal of Traditional Knowledge 2005; 4(3): 259-66.
14. Joshi AK and Juyal D: Traditional and ethnobotanical uses of *Quercus leucotrichophora* A. Camus (*Quercus oblongata* D. Don) in Kumaun and Garhwal regions of Uttarakhand, India: A review. International Journal of Herbal Medicine 2017; 5(5): 6-8.
15. Gorunovic MS and Lukic PB: University of Belgrade-Faculty of Pharmacy. Pharmacognosy 2001; Belgrade.
16. Gulluce M, Adiguzel A, Ogutcu H, Sengul M, Karaman I and Sahin F: Antimicrobial effects of *Quercus ilex* L. extract. Phytotherapy Research 2004; 18: 208-11.
17. Sanchez- Burgos JA, Ramirez- Mases MV and Larrosa MM: Antioxidant, antimicrobial, antitopoisomerase and gastroprotective effect of herbal infusions from four *Quercus* species. Industrial Crops and Products 2013; 42: 57-62.
18. Al- Mustafa, AH and Al-Thunibat OY: Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes. Pakistan Journal of Biological Sciences 2008; 11(3): 351-58.
19. Medina- Torres A and Bisht M: Evaluation of *in- vitro* antioxidant potential and in- vivo hepatoprotective activity of root extracts of *Quercus oblongata* D. Don. Journal of Drug Delivery and Therapeutics 2018; 8 (5): 152-61.
20. Haung J, Wang Y, Li c, Wang X and He X: Triterpenes isolated from acorns of *Quercus serrata*. Journal of Functional Foods 2018; 50: 18-25.
21. Bauer AW, Kirby WM, Sherris, JC and Truck M: Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 1966; 45(4): 493-96.
22. Zaidi SFH, Kazuki Y, Makoto K, Khan U and Toshiro S: Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against Helicobacter pylori. Journal Ethnopharmacology 2009; 121: 286-91.
23. Gupta A, Naraniwal M and Kothari V: Modern extraction method for preparation of bioactive plant extracts. International Journal of Applied and Natural Sciences 2012; 1(1): 8-26.
24. Boer HJde, Kool A, Broberg A and Levenfors J: Antifungal and antibacterial activity of some herbal remedies from Tanjania. Journal Ethnopharmacology 2005; 96(3): 461-9.
25. Kenneth VIR: New antimicrobial agents for treatment of bacterial infections in cancer patients. Hematology Oncology 2009; 27: 107-14.
26. Vlietinck AJ, Van- Hoof L, Totte J, Lasure A, Vanden-Berghe D, Rwangabo PC and Mvukiyumwami J: Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. Journal Ethnopharmacology 1995; 46: 31-47.
27. Vansey S, Vashney P, Dash SK, Gupta MK, Kumar A, Sing B and Sharma A: Antibacterial activity of fruits of *Terminalia chebula* and *Terminelia belerica* against mastitie fields isolates. Medici Plants 2012; 4(3): 167-69.
28. Karaman FS, Gulluce M, Ogutcu H, Sengul M and Adiguzel A: Antimicrobial activity of aqueous and methanol extracts of *Juniperous oxycedrus* L. Journal Ethnopharmacology 2003; 85: 213-35.
29. Essawi T and Srour M: Screening of some Palestinian medicinal plants for antibacterial activity. Journal Ethnopharmacology 2000; 70: 343-49.
30. Semwal P, Painuli S, Badoni H and Bacheti RK: Screening of phytoconstituents and antibacterial activity of leaves and bark of *Quercus leucotrichophora* A. Camus from Uttarakhand Himalaya, clinical. Phytoscience 2018; 4(3): https:doi.org/10.1186-018-0090-y.
31. Kalra VK, Kukla AS and Seshadri TR: A Chemical Examination of *Quercus robur* and *Quercus Incana*. Current Science 1996; 35: 204-05.
32. Jaisinghani RN: Antibacterial properties of quercetin, Microbiology Research 2017; 8: 13-14.
33. Wang S, Yao, J, Zhou, B, Yang J, Chaudry MT, Wang M, Xiao F, Li Y and Yin W: Bacteriostatic effect of Quercetin as an antibiotic alternative *in-vivo* and its antibacterial mechanism *in-vitro*. J of Food Protect 2018; 81(1): 68-78.
34. Qin XRMJ, Zhang XN, Gao Y, Lin MA, Li HE and Si-Yi: Study on the antibacterial activity of quercetin. Chemical Bioengineering Journal 2009; 26: 55-57.
35. Jamil M, Haq IU, Mirza B and Qayyum M: Isolation of antibacterial compounds from *Quercus dilatata* L. through bioassay guided fractionation, Annals of Clinical Microbiology, Antimicrobiology 2012; 11: 11.

36. Kuokkanen K, Julkunen-Tiitto R, Keinänen M, Niemelä P and Tahvanainen J: The effect of elevated CO2 and temperature on the secondary chemistry of *Betula pendula* seedlings. Trees 2001; 15: 378-84.
37. Top SM, Preston CM, Dukes JS and Tharayil N: Climate Influences the Content and Chemical Composition of Foliar Tannins in Green and Senesced Tissues of *Quercus rubra*. Plant Science 2017; 8: 423.
38. Berahou A, Auhmani A, Fdil N, Benharref A, Jana M and Gadhi CA: Antibacterial activity of *Quercus ilex* bark's extracts. Journal Ethnopharmacology 2007; 112: 426-29.
39. Nostro A, Germano MP, Angelo VD, Marino A and Cannnatelli MA: Extraction method and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology 2000; 30: 379-84.
40. Cowan MM: Plant product as antimicrobial agent. Clinical Microbiology Review 1999; 12: 564-82.
41. Andrensek S, Simonovska B, Vovk I, Fyhrquist P, Vuorela H and Vuorela P: Antimicrobial and antioxidative enrichment of oak (*Quercus robur*) bark by rotation planar extraction using Extra Chrom. International Journal of Food Microbiology 2004; 92: 181-87.

How to cite this article:
Sati SC, Tripathi AN and Kumar P: Influence of different solvents on antibacterial potential of three species of Himalayan oaks. Int J Pharm Sci & Res 2022; 13(1): 197-05. doi: 10.13040/IJPSR.0975-8232.13(1).197-05.