

QUERCUS CERRIS L.: ANTIBACTERIAL AND ANTIBIOFILM POTENTIAL

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ABSTRACT

Invasion of pathogenic infections, antibiotic resistance, and formation of biofilms represent serious contemporary issues that threaten human health. *Quercus cerris* L. is a deciduous Mediterranean tree that has been used in folk medicine since the ancient times but without knowing its phytochemical profile. The aim of this study was to evaluate the antibacterial potential of aqueous and ethanolic leaf extracts against two Gram-positive bacteria (*Streptococcus intermedius* and *Enterococcus faecium*) and two Gram-negative bacteria (*Escherichia coli* and *Stenotrophomonas maltophilia*) using agar well diffusion and broth microdilution methods. The antibiofilm properties of *Quercus cerris* L. extracts were also tested against the listed bacteria. The results of this study showed that both extracts displayed antibacterial capacity. However, the test cultures were found to be more susceptible to the ethanolic extract than the aqueous one. The lowest minimum inhibitory concentration (MIC) (12 mg/ml) and minimum bactericidal concentration (MBC) (25 mg/ml) values of the ethanolic extract were registered against *Escherichia coli*, while the highest values were noted against *Streptococcus intermedius*. As for the aqueous extract, it showed only a bacteriostatic activity against all the tested bacteria with a MIC of 100 mg/ml. The results of the antibiofilm assays also showed that the ethanolic extract exhibited significant antibiofilm effects against the investigated microorganisms. In the light of the findings of this research study, the aqueous and ethanolic extracts of *Quercus cerris* L. may be very useful in the development of new plant-based antimicrobial agents.

Keywords: *Quercus cerris* L., extracts, antibacterial activity, antibiofilm activity

INTRODUCTION

Infectious diseases caused by pathogenic microorganisms represent one of the grave global issues. In addition to the financial costs in terms of losses to health services centers and human health, pathogenic microorganisms are responsible for approximately 50 % of deaths (Seshadri et al., 2020). *Streptococcus intermedius*, *Enterococcus faecium*, and *Escherichia coli* are part of the normal microbial flora of human body. However, due to poor hygiene practices, they can be transmitted to the environment, contaminating surfaces, foods, and water sources. These bacteria can also be found in sewage sludge. In some countries, untreated sewage is exploited in the agricultural sector as natural fertilizer or directly discharged into water bodies, leading to grave environmental issues and health complications. *S. intermedius* is a Gram-positive bacterium belonging to the group of *Streptococcus anginosus*. This bacterium has been involved in endocarditis, liver, and brain abscesses (Tran et al., 2008; Issa et al., 2020). *E. faecium* is a spherical Gram-positive bacteria involved in many human infections such as prostatitis, urinary tract infections (UTIs), endocarditis, and wound infections (Bush, 2022). *E. coli*, part of the normal bowel flora of humans and animals, has been associated with UTIs, respiratory pneumonia, hospital-acquired infections, and burn wound infections (Forson et al., 2017; Lee et al., 2018). As for the multidrug-resistant *Stenotrophomonas maltophilia*, it is a free-living Gram-negative bacterium commonly isolated from water sources, soil, food, plants, and animals. *S. maltophilia* has been found to cause multiple infections including bacteremia, endocarditis, UTI, pneumonia, and other nosocomial infections, especially in immunocompromised patients and thus resulting in an elevation in the morbidity and mortality rates among them (Chang et al., 2015; Subhani et al., 2016). In this context, antibiotics have been used by patients to cure microorganisms-induced diseases. However, synthetic drugs could be expensive and unobtainable in many countries, especially the developing ones, or could exhibit unwanted adverse effects that menace human health (Sohretoglu et al., 2007; Oseni & Issaf, 2012). Additionally, resistance of bacteria such as *E. faecium* and *S. maltophilia* to antibiotics and formation of biofilms constitute other problems of concern. Thus, researchers tend to develop more safe and efficient plant-derived drugs with antimicrobial potential as natural alternatives to synthetic ones. Medicinal plants synthesize bioactive secondary metabolites such as steroids, saponins, tannins, alkaloids, flavonoids, and phenolic acids (Atef, 2019; Sengun et al., 2021). These

phytochemicals are well known to possess anti-inflammatory, antimicrobial, antioxidant, and antitumor activities (Kl et al., 2018; Seshadri et al., 2020). As a result, the use of herbal medicine has gained a wide interest in the health community.

Quercus cerris L., a member of the *Fagaceae* family, is one of the deciduous species that are known to be used in traditional medicine by ancestors (Taib et al., 2020). *Quercus cerris* L. grows vigorously in the Mediterranean climate zone where it can develop up to 30-35 meters high (De Rigo et al., 2016). Across its distribution scope, it is remarkably present in Italy, France, England, Turkey, Albania, Syria, and Lebanon where it is dominant in the Ezer forest. *Quercus cerris* L. has been widely used by humans in folk healing practices to treat various ailments like wound infections, throat inflammations, psoriasis, diarrhea, intestinal infections, hemorrhoids, and gums infections problem (Stafasani & Toromani, 2015). Burlacu et al. (2020) stated that *Quercus* species barks can be soaked in water, boiled, and applied orally to treat gastrointestinal disorders. Oak barks can be also applied topically to cure burn wounds. Furthermore, several studies demonstrated the potent antimicrobial effect of leaf, stem, and bark *Quercus cerris* L. extracts on some pathogenic microorganisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus mutans*, *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Candida krusei* (Sohretoglu et al., 2007; Hobby et al., 2012; Smailagic et al., 2020). In Lebanon, the traditional medicinal uses of this tree remain unknown. *Q. cerris* L. is often seen as a source of wood and to the best of our knowledge, no studies have been carried out to explore the biomedical properties of the Lebanese *Quercus cerris*. Hence, the aim of this research study was to investigate the *in vitro* antibacterial efficacy of the aqueous and ethanolic extracts of *Q. cerris* L. leaves against four pathogenic bacteria *Streptococcus intermedius*, *Enterococcus faecium*, *Escherichia coli*, and *Stenotrophomonas maltophilia*. The potential antibiofilm activity of leaf extracts was also assessed.

MATERIAL AND METHODS

Sample collection and powders preparation

Q. cerris L. leaves were sampled in June 2021 from the Ezer forest, in the Akkar district of Lebanon, at 1300 meters of altitude. Full sun-exposed leaves were collected from oak trees about five feet high from ground level, placed in

polyethylene bags, and carried to the laboratories of Beirut Arab University. After that, *Q. cerris* L. leaves were appropriately washed with distilled water and then dried at room temperature for one week away from the sun to prevent their degradation. The air-dried leaves were ground into fine particles, preserved in plastic containers, and stored at room temperature away from light.

Aqueous and ethanolic extracts preparation

The aqueous extract was prepared by mixing 10 g of dried powdered leaves with 100 ml of distilled water and then boiling for 30 minutes at 100 °C. The extract was filtered using filter paper and the filtrate was kept in sterile plastic containers and stored at 4 °C till use in the experiments (Al-Manhel & Niamah, 2015).

As for the ethanolic extract, 10 g of powdered leaves were soaked in 100 ml of ethanol (95%) and left for 7 days at room temperature away from the light with periodic shaking. The extract was then filtered and the filtrate was stored at 4 °C in plastic containers (Abdulsalami et al., 2016).

Bacterial samples

Four bacteria (*Streptococcus intermedius*, *Enterococcus faecium*, *Escherichia coli*, and *Stenotrophomonas maltophilia*), isolated from sewage sludge of Southern Lebanese villages and identified by VITEK assay, were used to assess the antibacterial activity of *Quercus cerris* L. (Mezher et al., 2022). They were obtained from Beirut Arab University, Department of Biological Sciences, Microbiology Research Laboratory.

Table 1 Detailed description of the bacterial isolates after their identification by the VITEK assay.

Species	Probability (%)	Bio-number
<i>S. intermedius</i>	94	031510304711431
<i>E. faecium</i>	86	012047065763731
<i>E. coli</i>	99	0405610544406601
<i>S. maltophilia</i>	99	1002103003540020

Agar well diffusion assay

The preliminary antibacterial efficacy of *Q. cerris* L. extracts was evaluated using the agar well diffusion method (Atef et al., 2019). Briefly, 100 µl of bacterial suspension adjusted to 0.5 McFarland (10⁶ CFU/ml) was spread on Tryptone Soy agar (TSA) plate. Holes were then punched in the plates with a sterile cork-borer and filled with 100 µl of each extract at different concentrations ranging between 100 mg/ml and 6.25 mg/ml. Tetracycline (0.25 mg/ml) was used as standard antibiotic, while ethanol (95%) and distilled water were used as negative control. Petri dishes were incubated at 37 °C for 24 hours. Finally, the diameter of inhibition zone was measured. All experiments were performed in three replicates.

Broth microdilution assay

The MIC is defined as the lowest concentration of a drug that inhibits the visible growth of microorganisms. The MIC₅₀ of the two studied extracts against the four bacterial strains were determined using the microdilution method (Iseppi et al., 2020). First, 90 µl of Tryptone Soy broth (TSB) and 10 µl of standard inoculum (10⁶ CFU/ml) were pipetted into the wells of a sterile 96-well microplate. Then 100 µl of each extract ranging from 100 mg/ml to 6.25 mg/ml were added to the wells. Microplates were further incubated for 24 hrs at 37 °C and the optical density (O.D.) was measured using an ELISA microtiter plate reader at 595 nm. TSB inoculated with bacteria served as a negative control, while that with Tetracycline served as positive control.

The MBC, defined as the lowest concentration of a drug that kills microorganisms, was determined by transferring 10 µl of clear wells (resulting from the MIC test) to Tryptone Soy agar plates. The petri dishes were then allowed to incubate at 37 °C for 24 hrs. All experiments were performed in three replicates.

Time kill test

Time kill test was applied following the method of Iseppi et al. (2020) in order to determine the time needed by the investigated extracts to inhibit the growth of test cultures. First, 90 µl of Tryptone Soy broth (TSB) and 10 µl of bacterial pathogens (10⁶ CFU/ml) were pipetted into the wells of a sterile 96-well microplate. After that, 100 µl of each extract (at MIC value against each bacteria) was transferred to the wells. The microplates were then incubated for 24 hrs at 37 °C and the O.D. was read at the appropriate wavelength (595 nm) at specified time intervals (0, 1, 2, 3, 5, and 24 hrs).

Inhibition of biofilm formation

The biofilm inhibition assay was carried out to assess the capability of the extracts to prevent biofilm development (Famuyide et al., 2019). 100 µl of standard bacterial suspension were transferred to 96-well microtiter plates and incubated at 37 °C for 4 hrs. After that, 100 µl of *Q. cerris* L. extracts at different concentrations

ranging from 100 mg/ml to 6.25 mg/ml were pipetted into the wells. 100 µl Tetracycline diluted in 100 µl of standard culture served as positive control, while TSB inoculated with bacteria was used as negative control. The plates were then incubated at 37 °C for 24 and 48 hrs. After incubation, the plates were washed with distilled water several times, air-dried, and then oven-dried at 60 °C for 15 minutes. The wells were afterward stained with 100 µl of crystal violet (CV) and incubated for 15 minutes at room temperature, followed by washing with distilled water. Biofilms appeared as purple rings. Finally, 100 µl of 95% ethanol was added to de-stain the wells. Absorbance was read at 595 nm and the percentage of inhibition of biofilm formation was deduced from the following formula:

$$\% \text{ inhibition} = \frac{OD \text{ negative control} - OD \text{ experimental}}{OD \text{ negative control}} \times 100$$

Destruction of pre-formed biofilm

The potential of both extracts to destroy pre-formed biofilms was also examined. Concisely, 100 µl of each tested bacterium (10⁶ CFU/ml) were transferred to 96-well microtiter plates and incubated for 30 hrs at 37 °C to allow the formation of biofilms. 100 µl of *Q. cerris* L. extracts at different concentrations were then added into the wells, followed by incubation at 37 °C for 24 and 48 hrs. After incubation, the CV staining assay was performed to quantify biofilm biomass and the percentage inhibition was obtained from the formula used previously in the biofilm inhibition assay (Famuyide et al., 2019).

Data analysis

The tests were performed in three replicates and the results were given as mean ± standard error of the mean. All statistical tests were done in Microsoft Excel software (Version 2016). T-test was applied to assess the statistical significance of the samples. *P*-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Sensitivity of investigated bacteria against *Q. cerris* L. extracts

The in vitro antibacterial activity of *Q. cerris* L. extracts against the investigated Gram + and Gram – bacteria was assessed by the agar well diffusion method. The results of this study showed that the ethanolic leaf extract displayed a significant antibacterial activity against nearly all the tested bacteria except for *S. intermedius* where at 6.25 mg/ml it showed no activity (Fig 1). These results indicate that at the lowest concentration (6.25 mg/ml), the active components of the ethanolic extract were not able to exert their antibacterial properties against the Gram + bacteria *S. intermedius* that surrounds itself by a thick layer of peptidoglycan that constitutes a strong line of defense against antimicrobial agents. The different strains showed high susceptibility to the tested ethanolic extract with some variability depending on the concentration used. Ethanolic extract was found to exert a greater growth inhibition activity in a concentration-dependent manner. On the other hand, the tested microorganisms were found to be less susceptible to the aqueous extract. The leaf aqueous extract displayed a moderate inhibitory effect against Gram-negative strains and *E. faecium* bacteria with a diameter of inhibition zone of 7-7.33 mm when bacteria are treated with 100 mg/ml (Fig 2). However, it showed growth inhibition zones against *S. intermedius* at all doses used (*p*<0.05). These outcomes raise questions about the resistance mechanisms of *S. intermedius* and depict that the efficacy of the studied extracts against *S. intermedius* differs depending on their content of secondary metabolites. The aqueous extract but not the ethanolic extract might contain specific phytoconstituents responsible for such antibacterial effects against *S. intermedius* even at very low concentrations. Smailagic et al. (2020) reported that the type of the extract, its chemical composition, the concentration of bioactive compounds present in it, as well as the bacterial strain under investigation, will affect the bacterial growth. Therefore, phytochemical analysis of *Q. cerris* L. extracts is required to fully identify the active components responsible for these contradictory results of the extracts against *S. intermedius* at 6.25 mg/ml. Besides, the determination of their mechanisms of action and target potential is also a must. Previous studies have investigated the antibacterial potential of leaf aqueous extracts of different *Quercus* species against a wide range of Gram-positive and Gram-negative bacteria. Sánchez-Burgos et al. (2013) evaluated the antibacterial activity of aqueous extracts obtained from the leaves of four *Quercus* species (*Q. grisea*, *Q. laeta*, *Q. obtusata*, *Q. resinosa*) against a broad spectrum of microorganisms (*Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Proteus hauseri*, *Proteus mirabilis*, *Proteus vulgaris*). They reported that among the species studied, *Q. resinosa* and *Q. grisea* had enhanced inhibitory activity against all the test organisms. These researchers further indicated that *Klebsiella pneumoniae* was the most sensitive. Its growth was inhibited by all aqueous extracts. Additionally, the findings of the present study indicated that the ethanolic extract had a greater growth inhibition potential against the test cultures than the aqueous one. This could be attributed to the fact that the bioactive chemicals responsible for this antibacterial activity are more soluble in the ethanolic solvent than in water, as shown in the antibacterial capacities of both extracts (Oseni &

Issah, 2012). Sohretoglu et al. (2007) noted in their study that the *Quercus* genus is well distinguished by its richness in saponins, alkaloids, terpenoids, and phenolics, such as flavonoids and tannins. Burlacu et al. (2020) also reported that generally the leaves of this genus bear phenolic acids such as caffeic acid, ferulic acid, p-coumaric acid, ellagic acid, and gallic acid. These phytochemicals are well characterized by their biological activity and therefore help plants in their

antagonistic activity against microorganisms through binding to proteins and disrupting their functions, inactivating key enzymes and thus hampering metabolic pathways, inhibiting nucleic acid synthesis, and inducing microbial membrane disruption (Abdulsalami et al., 2016; Seshadri et al., 2020; Smailagic et al., 2020).

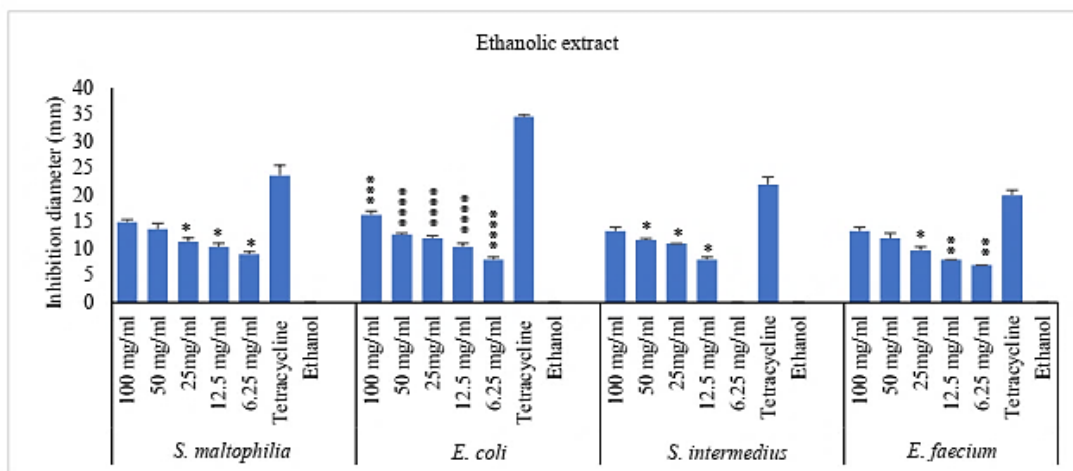


Figure 1 Zone of inhibition of ethanolic extract of *Q. cerris* L. against the investigated bacteria. Each value is a mean of three samples ± SEM. Significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

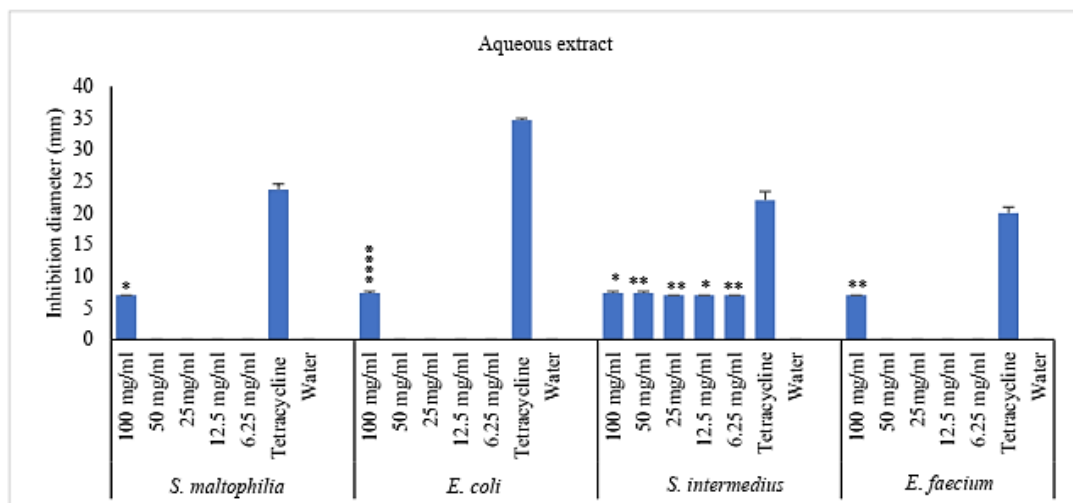


Figure 2 Zone of inhibition of aqueous extract of *Q. cerris* L. against the investigated bacteria. Each value is a mean of three samples ± SE. Significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

MIC and MBC of *Q. cerris* L. extracts against four tested bacteria

MIC broth microdilution assay and MBC test were performed to determine the minimum concentration at which the tested extracts inhibit or kill the test bacterial pathogens, respectively.

The results of the present study showed that the ethanolic extract exerted both bacteriostatic and bactericidal activities, while the aqueous extract had only a bacteriostatic capacity. The minimum inhibitory concentration of the ethanolic extract was 25 mg/ml against *S. maltophilia* and *E. faecium*, 12.5 mg/ml against *E. coli*, and 50 mg/ml against *S. intermedius* (Tab 2). The MBC values were 50 mg/ml against *S. maltophilia* and *E. faecium*, 25 mg/ml against *E. coli*, and 100 mg/ml against *S. intermedius* (Fig 3). The highest MIC and MBC values were recorded against *S. intermedius*. These observations are in line with the ones obtained in the agar well diffusion assay. In fact, *S. intermedius* was found to be resistant to the ethanolic extract applied at the lowest concentration 6.25 mg/ml. This indicates that besides having a thick protective peptidoglycan layer, *S. intermedius* might adapt definite survival strategies to resist the antimicrobials' toxic effects. However, the lowest values were noted against *E. coli*. Gram-negative bacteria are distinguished by a double-layered cell wall which consists of a thin peptidoglycan membrane surrounded by an outer polysaccharide layer. This second membrane is well known to provide resistance to the penetration of tiny antimicrobial agents. Additionally, the periplasmic space of Gram-negative bacteria is characterized by the presence of specific enzymes that scavenge and decompose the diffused molecules (Famuyide et al., 2019). The results of this study demonstrated that the bioactive compounds of ethanolic extract were able to pass these barriers and exert their antibacterial function. As for the water extract, it showed only a bacteriostatic

effect against all the studied bacteria with a MIC of 100 mg/ml (Tab 3, Fig3). Antimicrobial agents are considered as bactericidal when the MBC/MIC ratio is below 4 (Appiah et al., 2017). In the current study, The MBC/MIC ratios of the ethanolic extract against all tested microorganisms were below 4 which affirms that in addition to the bacteriostatic action, the ethanolic extract displayed a bactericidal activity. In the literature, few studies reported the antimicrobial properties of *Q. cerris* L. extracts. Sohretoglu et al. (2007) demonstrated that the leaf butanol, ethyl acetate, and aqueous extracts exhibited significant antifungal activities against *Candida albicans*, *Candida krusei*, and *Candida parapsilosis*, with the lowest MIC of the three extracts was recorded against *Candida krusei*. Smailagic et al. (2020) also tested and demonstrated the antibacterial activity of the wood ethanolic extract against *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus pyogenes*, Methicillin-resistant *Staphylococcus aureus*, and *Listeria monocytogenes*. The lowest MIC was noted for *Staphylococcus aureus*. Other research works carried out on different *Quercus* species have highlighted the antimicrobial potential of this genus on a wide variety of microorganisms known to be pathogenic. Tayel et al. (2018) noted that *Q. infectoria* ethanolic extract had enhanced bacteriostatic activity against *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Benyagoub et al. (2020) showed that *Q. robur* L. leaf extracts displayed significant inhibitory activity against *Enterococcus faecalis*, *Escherichia coli*, and *Staphylococcus aureus* with a MIC of 10 mg/ml and against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with a MIC of 30 mg/ml. In a study conducted by Zarei et al. (2022), the antibacterial activity of *Q. persica* was evidenced against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*.

Table 2 MIC and MBC values of ethanolic extract against test bacteria.

Concentrations of ethanolic extract (mg/ml) and O.D. (595nm) of bacterial growth										
Bacteria	100	50	25	12.5	6.25	Bacteria	Tet	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
<i>S. maltophilia</i>	0.35±0.00 ***	0.35±0.03 *	0.59±0.10 *	1.24±0.21 *	1.40±0.01 ****	0.66±0.00	0.08±0.00	25	50	2
<i>E. coli</i>	0.15±0.01 *	0.36±0.01 ***	0.61±0.02 ***	1.06±0.18 *	1.83±0.25 *	0.50±0.03	0.08±0.00	12.5	25	2
<i>S. intermedius</i>	0.11±0.03	0.30±0.05	0.72±0.02 ****	1.09±0.05 ***	1.87±0.14 **	0.53±0.02	0.07±0.00	50	100	2
<i>E. faecium</i>	0.16±0.03	0.24±0.04	0.77±0.06 **	0.95±0.16 *	2.15±0.19 **	0.54±0.06	0.09±0.00	25	50	2

Tet: Tetracycline.

TSB inoculated with bacteria without any treatment is negative control.

Each value is a mean of three samples ± SEM.

Significant at **p*<0.05, ***p*<0.01, ****p*<0.005, *****p*<0.001.

Table 3 MIC and MBC values of aqueous extract against test bacteria.

Concentrations of aqueous extract (mg/ml) and O.D. (595nm) of bacterial growth										
Bacteria	100	50	25	12.5	6.25	Bacteria	Tet	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
<i>S. maltophilia</i>	0.04±0.01	0.14±0.02	0.20±0.01 *	0.53±0.12	1.64±0.30 *	0.66±0.00	0.08±0.00	100	-	-
<i>E. coli</i>	0.20±0.01 ***	0.23±0.00 ****	0.37±0.01 ***	0.82±0.03 ***	1.84±0.07 ***	0.50±0.03	0.08±0.00	100	-	-
<i>S. intermedius</i>	0.08±0.01	0.11±0.02	0.18±0.03	0.41±0.10	1.54±0.17	0.53±0.02	0.07±0.00	100	-	-
<i>E. faecium</i>	0.18±0.04	0.21±0.04	0.29±0.04	0.54±0.08 *	1.49±0.18 *	0.54±0.06	0.09±0.00	100	-	-

Tet: Tetracycline.

TSB inoculated with bacteria without any treatment is negative control.

Each value is a mean of three samples ± SEM.

Significant at **p*<0.05, ***p*<0.01, ****p*<0.005, *****p*<0.001.

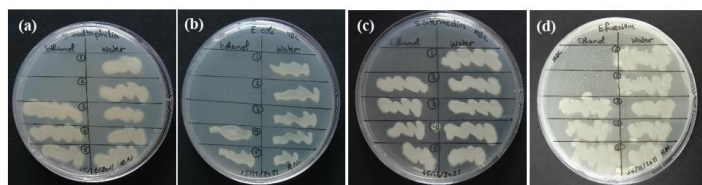


Figure 3 MBC results of ethanolic and aqueous extracts against test bacteria (a): *S. maltophilia*, (b): *E. coli*, (c): *S. intermedius*, and (d): *E. faecium*. 1, 2, 3, 4, and 5 represent the concentrations of *Q. cerris* L. extracts at 100, 50, 25, 12.5, and 6.25 mg/ml, respectively.

Time kill results of *Q. cerris* L. extracts against tested bacteria

Time kill test is a convenient tool that provides data on the dynamic interaction between the bioactive agent and microbial sample. It was done using the MIC values of the two tested extracts against all the studied bacteria to determine the required time for each extract to exhibit its antibacterial activity. As represented in Figure 4, the investigated microorganisms were sensitive to *Q. cerris* L. extracts between 2 and 3 hrs, indicating that the extracts were able to hinder the adaptation and duplication of bacterial pathogens. This substantial antibacterial activity of the extracts could be ascribed to the phytochemical profile of *Q. cerris* L. leaves in terms of their richness in biomolecules with bioactive properties. Previous investigations carried out on various *Quercus* species illustrated that the members of the *Quercus* genus are characterized by their important content of various secondary metabolites with potent antimicrobial capacity. Phenolic acids, flavonoids, and tannins are the most common antibacterial entities among all *Quercus* species with considerable variations in the amounts of bioactive phytochemicals between species due to the high phylogenetic diversity, geographical origin, tree developmental stage, harvesting time, and plant parts (Semwal et al., 2018).

Phenolic compounds, also referred to as phenols, are a class of secondary metabolites that are not directly involved in plant growth and fitness but rather take part in diverse ecological functions like defense, disease resistance, medicinal use, and flavoring. The members of this class consist of at least one aromatic ring that bears one or more hydroxyl groups, which together contribute to their biological activity. Phenols' antibacterial activity derives from their ability to interact with bacterial membrane structure, thus altering its permeability and resulting in the

leakage of intracellular materials out of the cell. The hydroxyl groups of phenolic compounds can also replace functional groups of proteins like sulfhydryl groups, thus inactivating them. Besides, phenols are also reported to inhibit peptidoglycan and nucleic acid synthesis (Slobodnikova et al., 2016).

Flavonoids, belonging to the family of polyphenols, are ubiquitous across plant species. These molecules, well characterized by the flavan nucleus, can be subdivided into six sub-classes defined by variations in heterocyclic rings and functional groups. Their antibacterial properties come from their capability to interact with the membrane proteins, porins in the cell membrane, and phospholipid bilayer if lipophilic enough, thus disrupting membrane integrity and increasing membrane fluidity. Flavonoids can also affect the synthesis and integrity of bacterial DNA and mRNA, hamper intracellular energy (ATP) production, and inhibit bacterial enzymes taking part in the virulence of microorganisms such as Sortase A (Kovac et al., 2023).

Tannins, a group of secondary polyphenolic metabolites, exert their antibacterial potential by inhibiting bacterial cell wall synthesis, interacting with cell membrane proteins, complexing with polysaccharides, and disrupting cell membranes. They can also inactivate enzymes, affect surface-exposed adhesins that facilitate the adherence of bacteria to the cells or surfaces, and suppress the expression of virulence factors (bacterial endotoxins, modulines, Sortase A...) (Kovac et al., 2023).

The effectiveness of the studied extracts could be attributed to a bioactive individual compound or more. The single action of the various components present in the extracts may conjointly play a part in the bioactivity of the extracts. This rapid antibacterial activity of the extracts may also be owing to the additive or antibacterial synergistic effects of the different bioactive components. Indeed, the chemical components may act synergistically to inactivate bacterial enzymes, impeding common metabolic pathways or facilitating the passage of other phytochemicals into the cell (Olajuyigbe & Afolayan, 2012; Sengun et al., 2021).

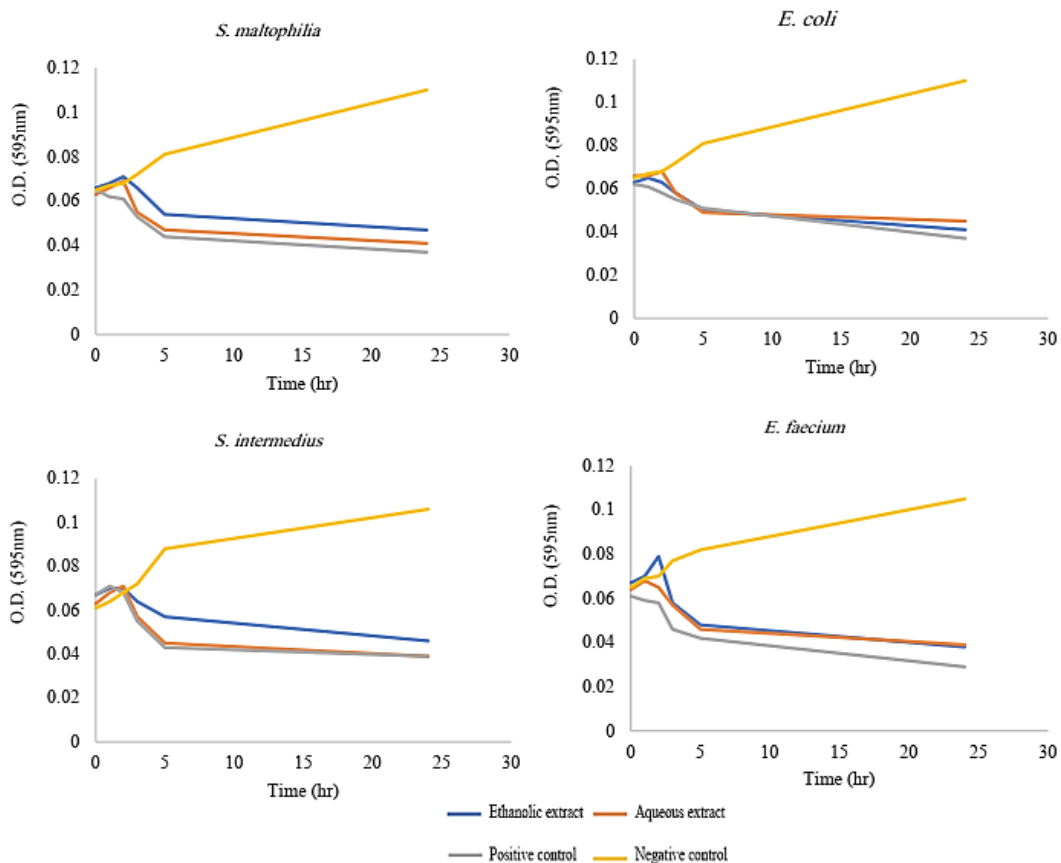
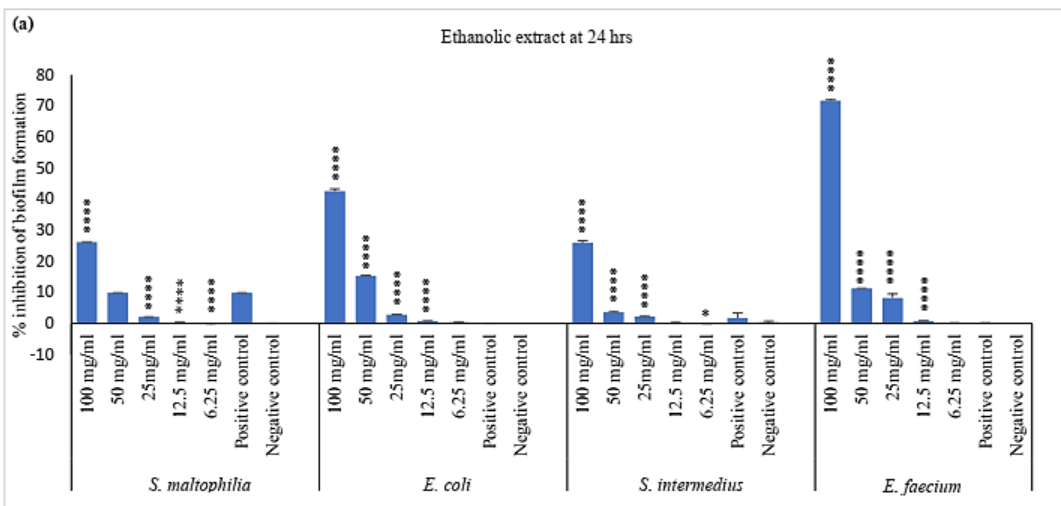


Figure 4 Time kill curve plots of *Q. cerris* L. extracts against (a): *S. maltophilia*, (b): *E. coli*, (c): *S. intermedius*, (d): *E. faecium*. TSB inoculated with bacteria and Tetracycline is a positive control. Bacteria without any treatment is negative control.

Antibiofilm activity of *Quercus cerris* L. extracts

The antibiofilm formation assay was performed to test the capability of *Q. cerris* L. extracts to prevent the formation of biofilms. The results are presented in Figures 5 and 6. The percentage inhibition which is less than 0% indicates the enhancement of biofilm formation, while percentage inhibition above 10% is deemed effective in the prevention of biofilm formation (Famuyide et al., 2019). Serwecinska (2020) reported that at subinhibitory concentrations, antimicrobials can act as signaling molecules promoting biofilm establishment through triggering different physiological changes, increasing levels of second messengers involved in the regulation of biofilm formation and virulence, upregulating drug resistance genes, activating quorum sensing system, along with facilitating horizontal gene exchange between bacteria. Biofilm represents an interacting community of bacteria that adhere to a surface and secrete a slimy extracellular matrix of polysaccharides, proteins, and nucleic acids. This extracellular matrix combines the bacteria together, provides support and protection from external factors, and

confers resistance to antimicrobial agents (Verderosa et al., 2019). In the current study, water extract did not exhibit any antibiofilm effect. In contrast, the ethanolic extract was found to exhibit a significant biofilm inhibitory activity at 24 hrs against *S. maltophilia* (26.23±0.03 % inhibition) and *S. intermedius* (25.98±0.68 % inhibition) at a concentration of 100 mg/ml and against *E. coli* (15.30±0.17 and 42.57±0.72 % inhibition) and *E. faecium* (11.30±0.00 and 71.73±0.27 % inhibition) at two test concentrations 50 and 100 mg/ml. It is worthy of note that the % inhibition of biofilm formation was higher at 100 mg/ml than 50 mg/ml. The results indicate that the antibiofilm activity of ethanolic extract is dose-dependent. Indeed, the percentage inhibition of biofilm formation increased as the concentration of ethanolic extract increased. These results are consistent with the findings of Hobby et al. (2012). They demonstrated that the inhibitory activity of the ethanolic extract of *Q. cerris* L. leaves against *Staphylococcus aureus* biofilm formation was dose-dependent.



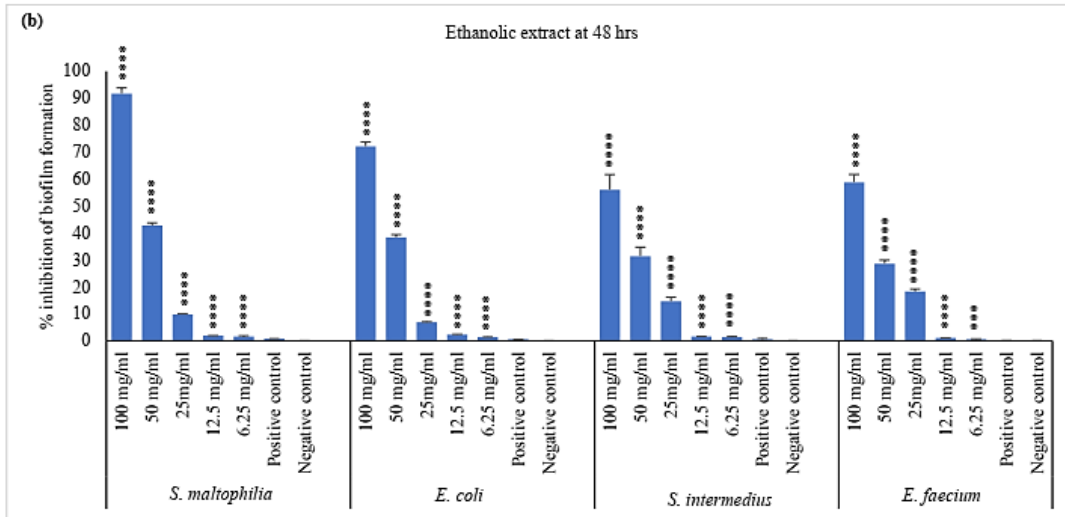


Figure 5 Antibiofilm activity of *Q. cerris* L. ethanolic extract at (a): 24 hrs and (b): 48 hrs. Tetracycline diluted in standard culture is a positive control. Bacteria without any treatment is negative control. Significant at *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

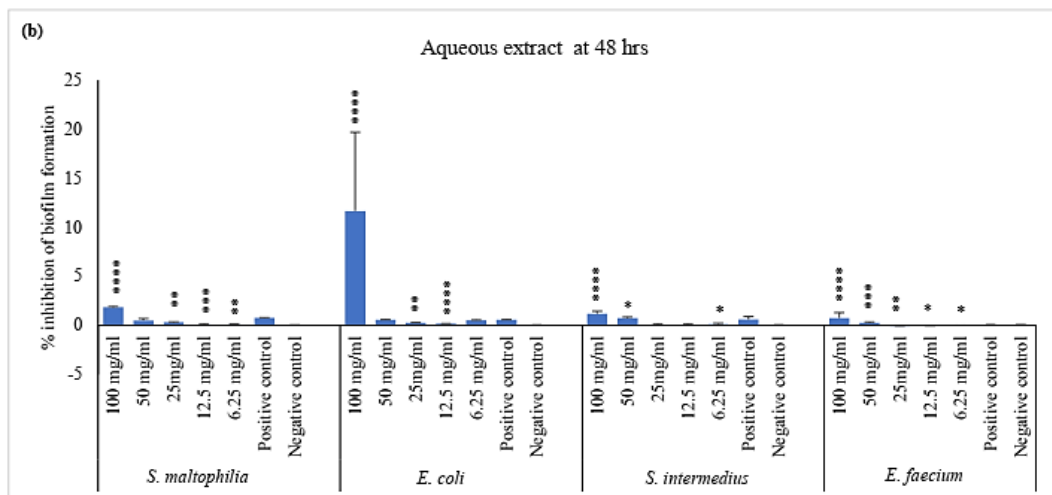
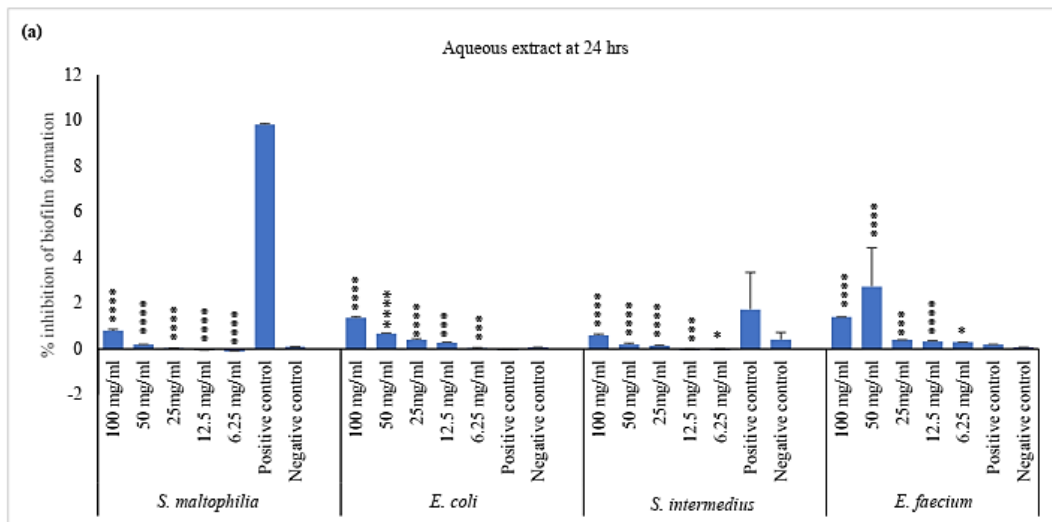


Figure 6 Antibiofilm activity of *Q. cerris* L. aqueous extract at (a): 24 hrs and (b): 48 hrs. Tetracycline diluted in standard culture is a positive control. Bacteria without any treatment is negative control. Significant at *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

On the other hand, the ethanolic extract investigated in the present study was also found to exert an antibiofilm effect against *S. maltophilia* at 50 mg/ml and against *S. intermedius* and *E. faecium* bacteria at two tested doses 50 and 25 mg/ml but after 48 hrs of incubation. These results depict that the antibiofilm formation activity of the ethanolic extract is also time-dependent. The activity of ethanolic extract at 48 hrs was found to be greater than after 24 hrs of incubation. This better antibiofilm activity at 48 hrs could be attributed to the fact that the constituents of

ethanolic extract penetrate the bacterial cells of mature biofilms at diverse intervals of time and that some reactions require a longer time than others (Aires et al., 2021). In literature, the antibiofilm formation activity of various *Quercus* species has been described by several researchers. Chusri et al. (2012) demonstrated that *Quercus infectoria* extracts prevented methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* biofilms formation by affecting the cell surface hydrophobicity and cell wall of staphylococcal strains.

Mohammadi-Sichani et al. (2015) also showed that *Quercus infectoria* extracts were effective on *Streptococcus mutans* biofilm formation at doses higher than 19.5 µg/ml. Bahar et al. (2017) determined in their study that *Quercus persica* had a substantial biofilm inhibitory effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermis*. Regarding *Q. cerris* L., this is the first such investigation carried on it to examine its antibacterial potential against the chosen microorganisms, which substantiates the potent antibacterial properties of *Quercus* genus generally and *Q. cerris* L. specifically and provides new results to the literature.

Pre-formed biofilm destruction potential of *Quercus cerris* L. extracts

As for the pre-formed biofilm destruction capacity, the results are shown in Figures 7 and 8. The results indicated that the leaf ethanolic extract was considerably effective ($p < 0.05$) against *S. maltophilia* (44.2±0.33 % inhibition) and *E. faecium* biofilms (10.23±0.18 % inhibition) after 48 hrs and against *S. intermedius* biofilm (12.77±0.84%) after 24 hrs at a concentration of 100 mg/ml. The ethanolic extract

was also found to be effective against *E. coli* pre-formed biofilm after 48 hrs at all concentrations including 6.25 mg/ml. These results depict that the *Q. cerris* L. leaves contain bioactive chemicals with promising antibiofilm potential. In literature, the antibiofilm potential of phenolic compounds has been described thoroughly. Phenolics such as flavonoids, tannins, and phenolic acids are well recognized to inhibit the primary stages of biofilm development by hindering quorum sensing (QS) activity, modifying expression of certain genes concerned in adhesion to solid surfaces, or by binding to bacterial proteins and prevention of attachment (Slobodníková et al., 2016; Burlacu et al., 2020; Smailagic et al., 2020). These compounds can also intervene with forces that enhance the deposition and attachment of bacterial cells to surfaces (Famuyide et al., 2019). Additionally, the above-mentioned phenolic compounds are acknowledged to display a potent activity against certain pre-formed biofilms by impeding lipid metabolism, affecting the bacterial cytoplasmic membrane stability, or by binding to the exopolysaccharides matrix to inhibit their attachment (Rozalski et al., 2013; Slobodníková et al., 2016).

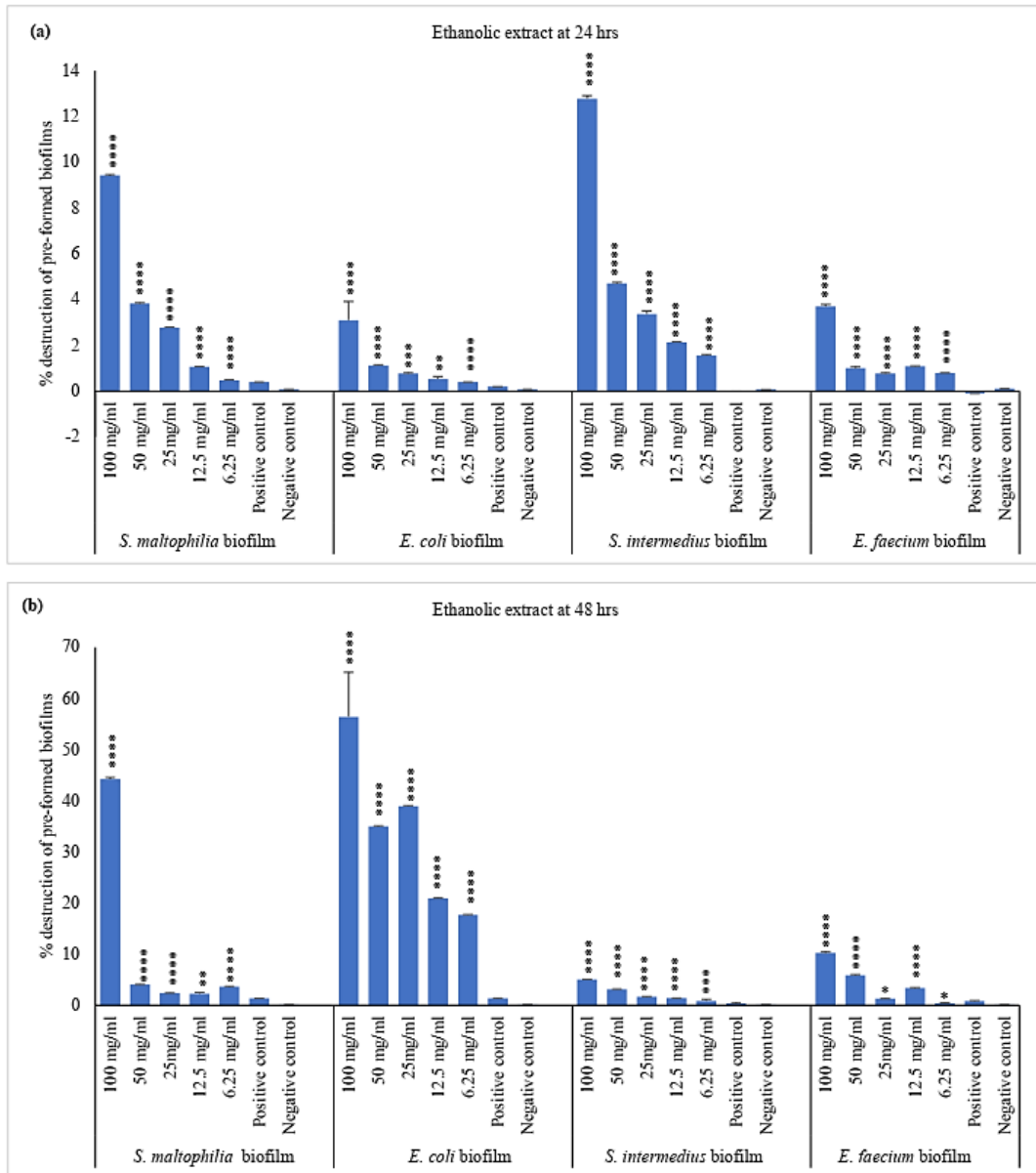


Figure 7 Pre-formed biofilm destruction activity of *Q. cerris* L. ethanolic extract at (a): 24 hrs and (b): 48 hrs. Tetracycline diluted in standard culture is a positive control. Bacteria without any treatment is negative control. Significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

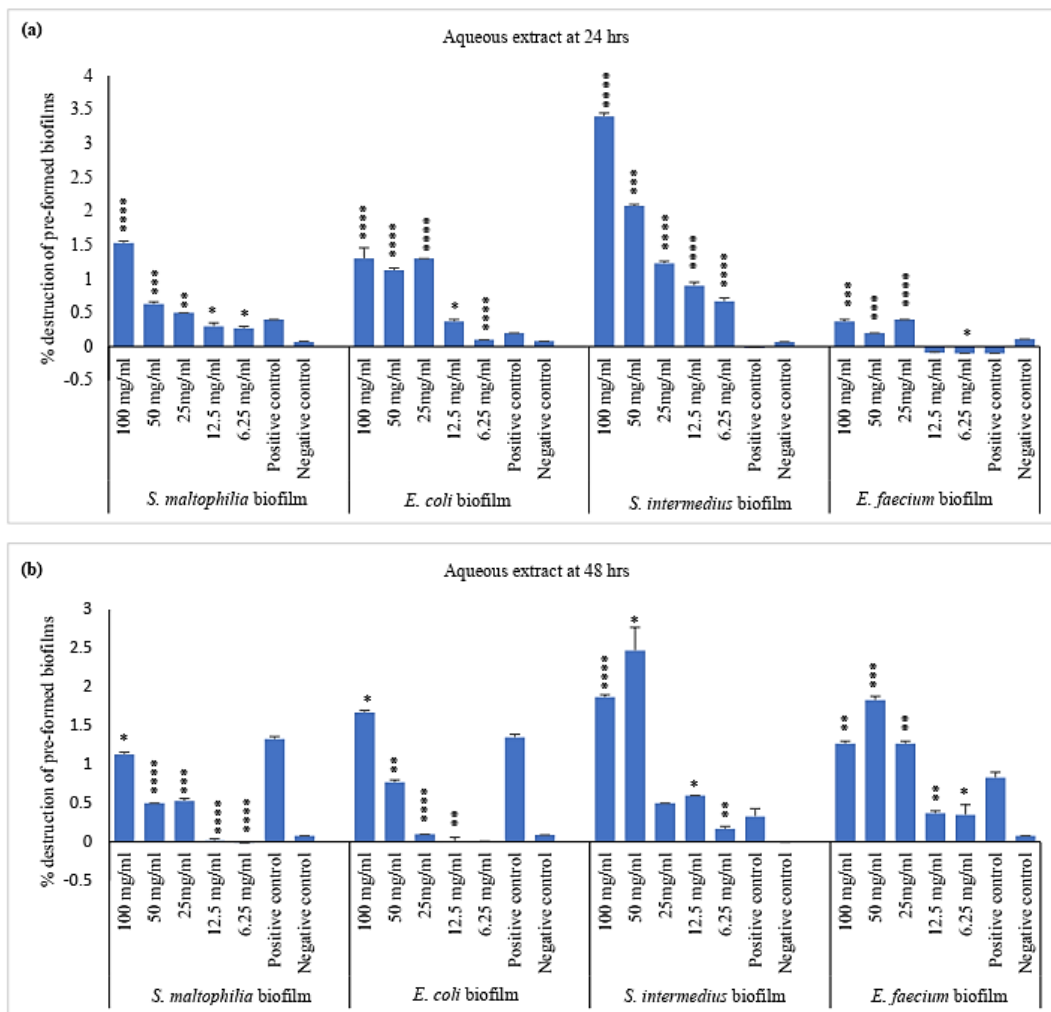


Figure 8 Pre-formed biofilm destruction activity of *Q. cerris* L. aqueous extract at (a): 24 hrs and (b): 48 hrs. Tetracycline diluted in standard culture is a positive control. Bacteria without any treatment is negative control. Significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

CONCLUSION

Despite the progress made in the pharmaceutical industry, the investigation of medical plants in laboratories takes a large section of interest, being an inexhaustible source rich in bioactive metabolites. Through assessment of biological properties of medicinal plants, the leaf aqueous and ethanolic extracts of *Q. cerris* L. were investigated for their antibacterial and antibiofilm activities against two Gram-positive and two Gram-negative bacteria. This research seems to be the first study to investigate the antibacterial activity of *Q. cerris* L. extracts against the selected bacteria in their planktonic and biofilm states. The findings of this study revealed that both extracts inhibited the growth of the test bacterial pathogens with various susceptibility. In addition to the bacteriostatic activity, the leaf ethanolic extract also displayed a potent bactericidal activity and a significant antibiofilm effect against all test bacteria. On the basis of the results obtained, the phytochemicals responsible for this antimicrobial potential should be clarified and mechanisms of activity should be determined. Additional studies must be also done to check other actions like the toxicity and antioxidant effects as this tree could be exploited in the formulation of new promising antimicrobial agent

Abbreviations: *Enterococcus faecium*: *E. faecium*; *Escherichia coli*: *E. coli*; *Stenotrophomonas maltophilia*: *S. maltophilia*; *Streptococcus intermedius*: *S. intermedius*; *Quercus cerris* L.: *Q. cerris* L.

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SUPPLEMENTARY INFORMATION

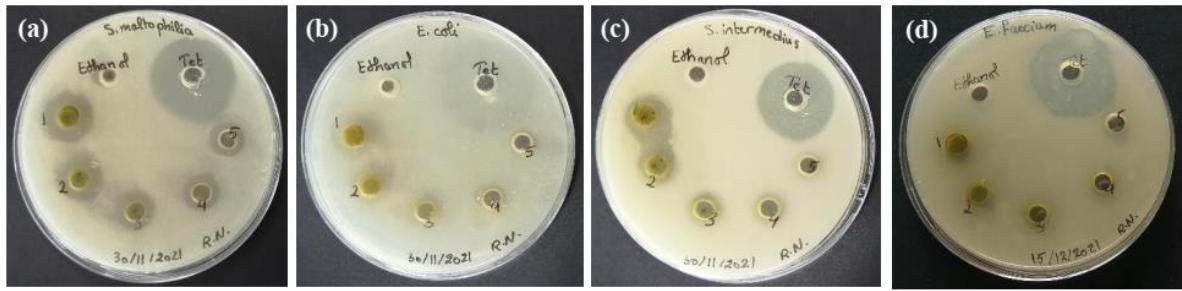


Figure S1 Susceptibility of test microorganisms (a): *S. maltophilia*, (b): *E. coli*, (c): *S. intermedius*, and (d): *E. faecium* to ethanolic extract of *Quercus cerris* L. leaves. Tetracycline is used as a standard antibiotic, while ethanol is negative control.

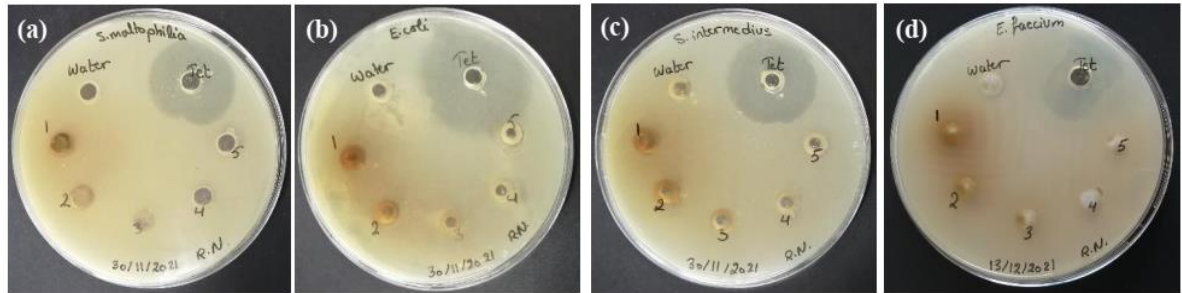


Figure S2 Susceptibility of test microorganisms (a): *S. maltophilia*, (b): *E. coli*, (c): *S. intermedius*, and (d): *E. faecium* to aqueous extract of *Quercus cerris* L. leaves. Tetracycline is used as a standard antibiotic, while distilled water is a negative control.

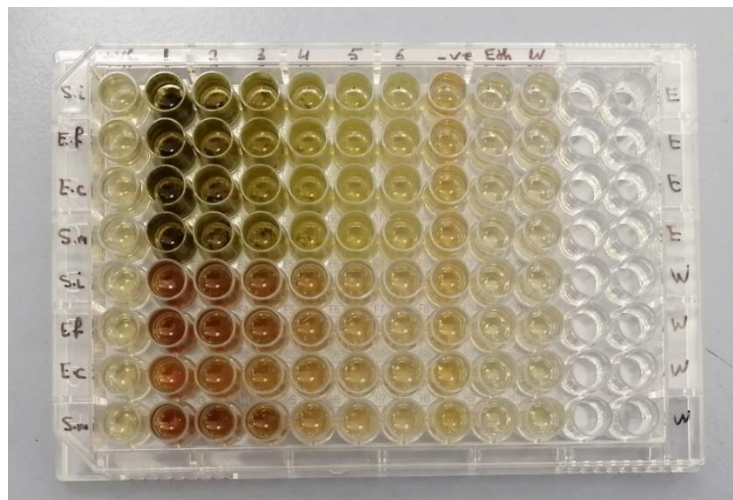


Figure S3 MICs of the extracts on the different bacteria.

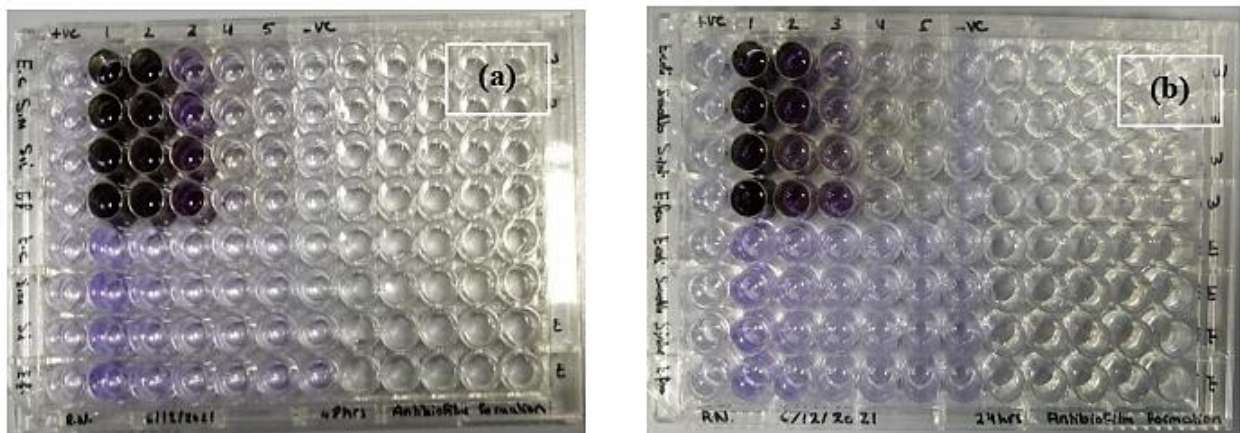


Figure S4 The inhibitory activity of *Quercus cerris* L. leaf extracts on the formation of bacterial biofilms at (a): 24 hrs and (b): 48 hrs.

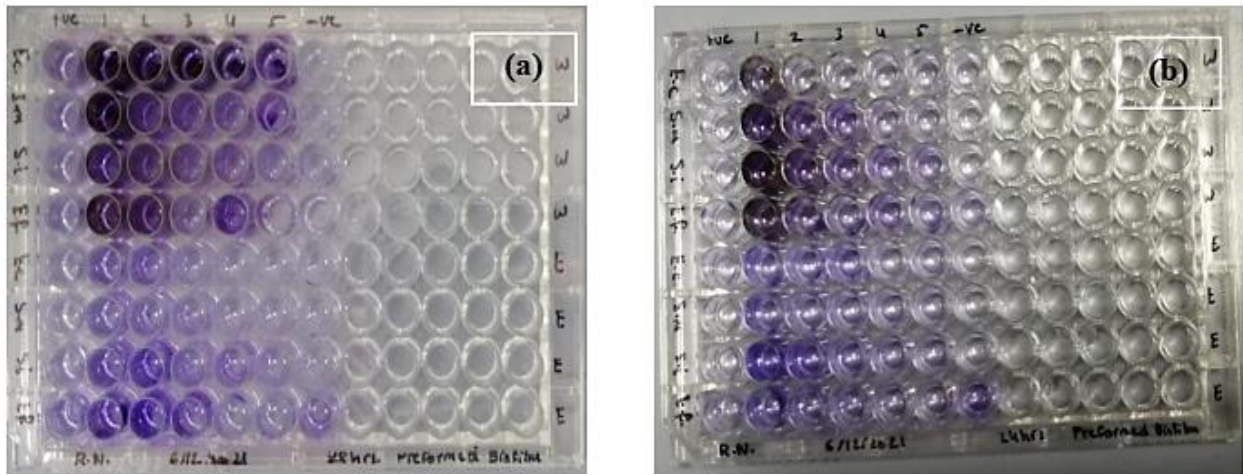


Figure S5 The dispersal effect of *Quercus cerris* L. leaf extracts on the diverse pre-formed bacterial biofilms at (a): 24 hrs and (b): 48 hrs.