Antibacterial activity of Methanol and n-Hexane extracts of fruit casings of *Quercus rubra* against multi-drug resistance pathogenic bacteria isolated from patients with burn infection

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**ABSTRACT**
This study was designed to investigate the ability of methanol and n-hexane extracts of fruit casings of *Quercus rubra* against pathogenic bacteria isolated from patients with burn infection. Three concentrations of methanol and n-Hexane extracts of *Quercus rubra* were done (100, 200 and 400) mg/ml. Thirty eight samples were collected from Medical Burn Center in Najaf Governorate. Males and females in different age groups. The results indicated that there were 42% *Pseudomonas aeroginosa*, 22% *Staphylococcus aureus*, 18% *Klebsiella pneumonia*, 8% *E. coli*, 6% *Staphylococcus epidermis* and 4% *Proteus mirabilis*. Seventeen antibiotics were selected in antibiotics sensitivity test. Imipenem 10mg was the best antimicrobial effect. The results showed that all three concentrations had good antibacterial effect. It has also been shown that methanol solvent is more effective on bacteria than n-hexane solvent. Also, the results showed that *P. aeroginosa* and *S. aureus* have the highest rate of inhibition diameter with methanol solvent (38.123 ± 0.07 and 35.133 ± 0.092) mm respectively. While *K. pneumonia* was the highest rate of inhibition diameter by n-hexane solvent 32.4 ± 0.07mm. Methanol and n-hexane extracts of *Quercus rubra* can be regarded as a potential antibacterial against aerobic pathogenic bacteria isolated from patients with burn infection. Therefore, Fruit casings of *Quercus rubra* may be considered as a raw material for the manufacture of ointment for treatment of burns infections.

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**INTRODUCTION**

*Quercus robur* are among the most naturally adapted trees in northern Iraq. Its original habitat is the northeastern quarter of the United States and southeastern Canada, it is widely distributed in Greece, Asia Minor and Iran (Turner *et al.*, 2005; Umachigi *et al.*, 2008). Its pharmacological effects of *Q. robur* were evaluated and found to have had antibacterial, anti-inflammatory, anti-diabetic, and antioxidant effects (Uddin *et al.*, 2013; Skrypnik *et al.*, 2019). Its medical uses are wound healing, antibacterial, anti-MRSA, antivirals, antifungals, anti-inflammatory, and anti-digestive effects (Fernandez-Escobar *et al.*, 1999; Sati *et al.*, 2017). *Q. robur* have shown a broad spectrum factor, which can be used against Gram-positive and Gram-negative bacteria and fungi (Hamad *et al.*, 2017). It has an influential role in the growth of some microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Klebsiella pneumonia*, and *Proteus mirabilis* (Ahmed *et al.*, 2018). The main components found in *Q. robur* are tannins, polyphenols, sugar, starch and essential oils.
Also, it may be a good source to treat a number of diseases (Patra and Saxena, 2011; Bursal and Boğa, 2018). Q. robur fruits contain phenolic substances, such as quercetin and tannin, which are used in postpartum exercise, treatment of diarrhea, bleeding and skin diseases (Brown et al., 2017; Ricci et al., 2017).

**MATERIALS AND METHODS**

**Study design and patients**

This is a cross-sectional descriptive study, performed in Medical Burn Center in Al-Najaf City, Iraq, during the period from October 2019 to March 2020. A total of 83 samples were collected from patients with burn infections, females and males, age between 1 to 63 years old.

**Samples collection, culture and bacterial identification**

Eighty three swabs were collected from the burned area for patient with burn infections (2nd degree, with the signs of infection during the change of dressings) depended on (Aljanaby and Aljanaby, 2018). All emerged bacterial isolates were identified according to colony morphology and standard microbiological tests such as; colony morphology, gram stain, IMVIC test, motility test, coagulase test, oxidase test, catalase test and growth on MacConkey agar (Oxoid™). Finally, all isolates were identified by using Vitek2 system (BioMerieux, France).

**Antimicrobials susceptibility test**

Antimicrobials susceptibility testing was performed by disc diffusion method according to the Kirby-Bauer method onto Mueller Hinton agar (Oxoid™) surface (Bauer et al., 1966). Seventeen different antimicrobial discs were used in this study provide from Biomaxima, Poland is as follows: Ampicillin 10IU (AM), Penicillin 10μg (P), Amoxiclav 30μg (AMC), Vancomycin 30μg (VA), Ceftriaxone 30μg (CRO), Cefoxitin 30μg (FOX), Cefepime 30μg (FEP), tetracycline 30 μg (TE), ciprofloxacin 5μg (CIP), Norfloxacin 10μg (NOR), Amikacin 30μg (AK), Erythromycin 30μg (E), Trimethoprim 5μg (TM), Nitrofurantoin 300μg (F), Rifampin 5μg (RA), Chloramphenicol 30μg and Imipenem 10μg (IMP). The diameters of inhibition zones (mm) were measured using a caliper measure each zone with the unaided eye and compared with clinical and laboratory standards institute (CLSI) guideline (CLSI, 2019).

**Collection and identification of Q. rubra and preparation of plant extract**

Quercus rubra fruit casings of used were purchased from local markets in the city of Najaf in October of 2019, and have been diagnosed by taxonomists in the Botanical Laboratory for Graduate Studies at the College of Science - University of Kufa. After that it was cleaned and isolated from foreign materials, crushed by an electric mill, then the powder was collected in nylon bags and kept in the laboratory at room temperature until use (Harborne, 1984). Alcoholic extracts 30 grams of powder was taken and 300 ml of of alcohol solvent (Methanol 99% and n-Hexan 96%) was added in a 500 ml flask. The suspension was placed on a magnetic stirring plate for 48 hours at room temperature, then filtered with a piece of gauze and then by filter paper type filter paper Wattman, No. 1, and dried in the oven (40-30)°C. The extract was then stored in the refrigerator at 4° C for use (Yadav and Agarwala, 2011). The concentration was prepared by dissolving 100, 200, 400 mg of extracts with one mL of Dimethyl sulphoxide (DMSO) and thus the final concentration for each solvent would be 100 mg/mL, 200 mg/mL and 400 mg/mL that was used against bacteria (Tripathi et al., 2009; Aljanaby and Aljanaby, 2018).

**Antibacterial activity test**

The antibacterial activity of alcohol extract methanol and n-hexan of Quercus rubra fruit casings was done according to method by (Rauha et al., 2000; Radji et al., 2013) using well-speed agar methods. Sterile swabs (Bioanalyse, Turkey) were used to examine three or five new bacterial colonies with 0.5 McFarland turbidity on the surface of Muller-Hinton agar (Oxoid™). Three wells (6 mm diameter) were performed on each Muller-Hinton (Oxoid™) plate using a sterile cork drill (Himedia-India). 50 microlitres of each concentration was transferred to each well and left at 23-25 °C for 1 hour. Surface diffusion activation. Boards incubated at 37 °C for 18-24 hours. The inhibition zone around each well was measured in millimeters. All tests were carried out in triplicates (Aljanaby, 2013; Aljanaby and Gafil, 2013).

**Statistical analysis**

Fisher’s exact test was used in this study to compare samples using a padprismV.10 graph computer program. P values below 0.05 were considered statistically significant (Witwit et al., 2019; Aljanaby and Aljanaby, 2018).

**RESULTS AND DISCUSSION**

**Bacterial Isolates Percentage**

The results of microbial isolation from burn infection cases showed that the number of isolates is
about 83 isolates, 60 Gram negative (72.29%) and 23 Gram positive (27.71%) due to 6 bacterial types and the ratios were as follows: *P. aeruginosa* was (42%) followed by *S. aureus* bacteria (22%) and the lowest infection rate was *P. mirabilis* (4%), while the ratios of the following bacterial species, *K. pneumonia*, *E. coli* and *S. epidermis*, were 18%, 8% and 6%, respectively, (Figures 1 and 2).

![Figure 1: Numbers and percentages of aerobic pathogenic bacteria isolated from patients with burn infections.](image1)

Antimicrobial susceptibility test was conducted (Table 1) for 35 *Paeruginosa* isolates. The results of the present study demonstrated that the antimicrobial resistance rates of the 35 isolates to *Paeruginosa* of Chloramphenicol, Augmantin, Rifampin, Tetracycline and Trimethoprim (100%),

![Figure 3: Effect of Q.rubra concentrations on bacteria](image3)

Antibiotics Sensitivity Test

Antimicrobial susceptibility test was conducted (Table 1) for 35 *Paeruginosa* isolates. The results of the present study demonstrated that the antimicrobial resistance rates of the 35 isolates to *Paeruginosa* of Chloramphenicol, Augmantin, Rifampin, Tetracycline and Trimethoprim (100%),

![Figure 4: Effect of Q.rubra on bacteria growth.](image4)

![Figure 5: Diameters of inhibition zones mm of Q.rubra extracts in 400 mg/ml against three pathogenic bacteria isolated from patients with burn infection. R=3.](image5)
Comparison of the effect of crude extracts of *Q. rubra* on burn bacteria

From Table 2, all extracts were very effective in the growth of bacteria isolated from burns and decreased with increasing concentration (Figures 3 and 4). The effect of the plant on the bacterium *P. aeruginosa* has been observed. There were statistically significant differences when comparing concentrations 100 mg/mL with 200 mg/mL and n-hexane. When the concentration is compared to 200 mg/mL with 400 mg/mL, there are also differences in the solvent n-hexane and no differences with respect to methanol. At a concentration of 400 mg/mL, the methanol solvent gave a higher result of inhibition with an average (37.233 ± 0.092 mm) while the n-hexane solvent had a higher result of inhibition (32.400 ± 0.07 mm). The effects of crude extracts (methanol and n-hexane) was more effective at a rate of inhibition (38.123 ± 0.07 mm) than Imipenem (32.400 ± 0.05 mm), while methanol and n-hexane was more effective by a rate of inhibition (38.123 ± 0.07 mm) than Imipenem (32.400 ± 0.05 mm) (Figure 5).

In this study, as *P. aeruginosa* has been found in most patients, *bacteria S. aureus* and *K. pneumoniae* has been found in some patients. Initially, the burn wounds are sterile and free of any microbe. However, bacteria may reach a burning area through hair follicles and sweat glands, which may survive thermal injury, as in the Gram-positive or after hospitalization. The ability to eradicate burn wounds (Sperandio et al., 2013). Or, this may be a high percentage of bacteria due to its natural presence in the body as a result of hospital acquired infection due to its high rate in most hospitals (Peleg and Hooper, 2010). I believe the spread of these bacteria may be due to their resistance to many of the antibiotics and disinfectants used in hospitals, and thus the combustion area turns into an appropriate means for the growth of these bacteria because of the weak resistance to the skin tissues subject to burning and damage. One of the reasons for this high prevalence of *Paeruginosa* may be its simple nutritional requirements which, as demonstrated by its ability to grow in distilled water and withstand a wide range of external conditions and ultimately have an effective opportunistic role (Hamszah et al., 2011) or because of the inhibitory effects of immunity.
### Table 1: Resistance pattern of most dominant aerobic pathogenic bacteria isolated from patients with burn infections

| Antimicrobials   | *P. aeruginosa* (35 isolates) | *S. aureus* (18 isolates) | *K. pneumonia* (15 isolates) |
|------------------|-------------------------------|---------------------------|-----------------------------|
| Amikacin 30mg    | 13 (37.1)                     | 7 (38.8)                  | 5 (33.3)                    |
| Ampicillin 10mg  | -                             | 18 (100)                  | -                           |
| Amoxiclav 30mg   | 35 (100)                      | 18 (100)                  | 15 (100)                    |
| Cefepime 20mg    | 28 (80)                       | -                         | 12 (80)                     |
| Cefoxitin 30mg   | 29 (82.8)                     | -                         | 13 (86.6)                   |
| Ceftriax 30mg    | -                             | 15 (83.3)                 | -                           |
| Chloramphenicol 30mg | 35 (100)           | 17 (94.4)                 | 14 (93.3)                   |
| Ciprofloxac 5mg  | 30 (85.7)                     | -                         | 10 (66.6)                   |
| Erythromycin 10mg| 18 (51.4)                     | 9 (50)                    | 11 (73.3)                   |
| Imipenem 10mg    | 3 (8.5)                       | 1 (5.5)                   | 1 (6.6)                     |
| Nitrofurant 300mg| 28 (80)                       | 15 (83.3)                 | 13 (86.6)                   |
| Norfloxacin 10mg | 20 (57.1)                     | 9 (50)                    | 9 (60)                      |
| Penicillin 10u   | -                             | 18 (100)                  | -                           |
| Rifampin 5mg     | 35 (100)                      | 10 (55.5)                 | 15 (100)                    |
| Tetracycline 30mg| 35 (100)                      | -                         | 14 (93.3)                   |
| Trimethoprin 5mg | 35 (100)                      | 18 (100)                  | 15 (100)                    |
| Vancomycin 10mg  | -                             | 16 (88.89)                | -                           |

Data presented as numbers and percentage of pathogenic bacterial isolates that were resistant to antimicrobials: no. (%). (-) = not used.

### Table 2: Evaluation of antibacterial activity of Q.rubra extracts against pathogenic bacteria isolated from patients with burn infection

| Pathogenic bacteria | 100 mg/ml Methanol extracts, M±SE mm | 200 mg/ml | 400 mg/ml | A | B | P value |
|---------------------|---------------------------------------|-----------|-----------|---|---|---------|
| *P. aeruginosa*     | 34.450 ± 0.028                        | 38.120 ± 0.072 | 38.123 ± 0.072 | <0.0001*** | 0.9751 |
| *S. aureus*         | 30.400 ± 0.057                        | 33.170 ± 0.117 | 35.133 ± 0.092 | <0.0001*** | 0.0002*** |
| *K. pneumonia*      | 24.133 ± 0.088                        | 28.140 ± 0.087 | 29.233 ± 0.120 | <0.0001*** | 0.0018** |
| *P. aeruginosa*     | 35.133 ± 0.088                        | 35.567 ± 0.233 | 37.233 ± 0.145 | 0.0045** | 0.0037** |
| *S. aureus*         | 26.067 ± 0.883                        | 31.383 ± 0.252 | 32.267 ± 0.185 | 0.0044** | 0.0478* |
| *K. pneumonia*      | 29.167 ± 0.120                        | 31.167 ± 0.545 | 32.400 ± 0.057 | 0.0232* | 0.0879 |

M±SE: Mean ± standard error of diameters of inhibition zone in millimeters, A: Compare between concentration 100 and 200, B: Compare between concentration 200 and 400.

### Table 3: Comparison between imipenem 10mg and Q.rubra extracts in 400 (mg/ml) against three pathogenic bacteria isolated from patients with burns infections. R=3.

| Pathogenic bacteria | Diameters of inhibition zone (mm) ± Slandered error | p value |
|---------------------|-----------------------------------------------------|---------|
|                     | Methanol | n-Hexan | Imipenem 10mg | A | B |
| *P. aeruginosa*     | 38.123 ± 0.07 | 37.233 ± 0.14 | 33.292 ± 2.17 | 0.0903 | 0.1445 |
| *S. aureus*         | 35.133 ± 0.09 | 32.267 ± 0.18 | 25.357 ± 0.07 | <0.0001*** | <0.0001*** |
| *K. pneumonia*      | 29.233 ± 0.12 | 32.400 ± 0.05 | 29.333 ± 0.66 | 0.8898 | 0.0102* |

A: compare between Imipenem 10mg and methanol B: compare between Imipenem 10mg and n- hexan
For patients with burns in addition to multiple drug resistance as indicated (Sydnor and Perl, 2011). The current study showed that most P. aeruginosa, S. aureus, and K. pneumoniae isolates have high resistance to most of the antibiotics used, which may reach 100%, while other isolates showed sensitivity to antibiotics, but at a different rate. It can be believed that one of the main reasons for antibiotic resistance is the wrong or random use of the antibiotic without relying on Sensitivity examination as indicated or because of the ability of these bacteria to develop easily through acquired resistance or transfer of genes or through genes that mutate the chromosome or may have possession of virulence factors or some enzymes that work to destroy Antibiotics (Fridkin et al., 2014).

The results revealed that Imipenem was the best choice antibiotic for treatment of P. aeruginosa, S. aureus and K. pneumoniae infection, since the effect of Imipenem appeared in most bacterial isolates 91.5%, 94.5% and 92.4%, respectively. This result agrees with the study of (Aljanaby, 2018). The lowest percentage of antibiotic resistance for bacteria isolated from burns was Imipenem. Antibiotic resistance of P. aeruginosaby outer membrane semi permeability, produce enzymes like-(lactamases and cephalosporinase), Efflux Pumps, genetic resistance and mutation in chromosomal genes (Breidenstein et al., 2011). Antibiotic resistance of K. pneumonia by alternative pass pathway not inhibited by the drugs, reduced membrane permeability of the drug, drug inactivation by degradation or enzyme modification such as (beta lactamases and transferase) and alteration of drug targets (Warjri et al., 2015). Antibiotic resistance of S. aureus by cell wall that is impermeable to the drugs, B-lactamase activity, altered penicillin-binding proteins (PBPs) and genes modified present some strains of S. aures (Foster, 2017).

In the previous studies and research on the effect of Q. robur on bacteria, the reason for inhibition phenolic acids, flavonoids and tannins compound (Hamad et al., 2017; Skrypnik et al., 2019), which gave a stronger effect on the bacteria had the ability to destroy cell membranes by forming complexes with proteins on the outer membrane of bacteria or by tearing the cell membranes by bonding with lipophilic substances present in the membrane as well, and this was confirmed by (Ribechnini et al., 2015).

Q. robur contains tannins. It has antibacterial and anti-inflammatory properties. Pharmacological studies show the effectiveness of Q. robur in purulent dermatitis (Dawid-Pač, 2013).

Imipenem, the first of a new class of carbapenem antibiotics, has dynamic activity against most negative, positive and clinically important bacterial species, including bacterial isolates that are resistant to other antibiotics. The medicine is well distributed to most tissues and fluids shortly after intravenous administration. Also, this medicine may be especially useful in treating infections caused by bacteria mixtures (El-Gamal and Oh, 2010).

Q. robur all extracts were better as a result of Imipenem against all bacteria against P. aeruginosa and K. pneumoniae respectively, In general, the methanol solvent is better than the n-hexane, because it contains many active plant compounds compared to n-hexane (Ameera et al., 2016) and in particular the methanol extract has a greater effect against P. aeruginosina and S. aureus, while the n-hexane extract has the greatest effect against bacteria K. pneumonia. These results indicate that Q. robur have better antibacterial activity for burn patients than antibiotics, so, it is recommended that making an ointment from these plants may be beneficial for treatment.

CONCLUSIONS

Methanol and n-hexane extracts of Quercus rubra can be regarded as a potential antibacterial against aerobic pathogenic bacteria isolated from patients with burn infection. Therefore, Fruit casings of Quercus rubra may be considered as a raw material for the manufacture of ointment for treatment of burns infections.

Conflict of Interest
None.

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REFERENCES

Ahmed, Z. F., Hama, B. A., Abdullah, R. M. 2018. The Effect of Methanolic Extract of Quercus infectoria Against Some Causative Agents of Diarrhea and Eliminate their Antimicrobial Resistance. Diyala Journal For Pure Science, 14(2):235–249.

Aljanaby, A. A. J. 2018. Antibacterial activity of an aqueous extracts of Alkanna tinctoria roots against drug resistant aerobic pathogenic bacteria isolated from patients with burns infections. Russian Open Medical Journal, 7(1):e0104–e0104.

Aljanaby, A. A. J., Aljanaby, I. A. J. 2018. Prevalence of aerobic pathogenic bacteria isolated from patients with burn infection and their antimicrobial sus-
ceptibility patterns in Al-Najaf City, Iraq- a three-year cross-sectional study. 7:1157.

Aljanaby, A. A. J., Gafil, F. A. A. 2013. Effect of different antibiotics on aerobic pathogenic bacteria and urinary tract infection in Al-Manathera City, Iraq: a comparative study. Research on Chemical Intermediates, 39(8):3679–3687.

Aljanaby, A. A. J. 2013. Antibacterial activity of an aqueous extract of Petrocellinum crispum leaves against pathogenic bacteria isolated from patients with burns infections in Al-najaf Governorate, Iraq. Research on Chemical Intermediates, 39(8):3709–3714.

Ameera, O. H., Ghaida, J. M., Mohammed, Y. H., and, I. H. H. 2016. Phytochemical screening of methanolic dried galls extract of Quercus infercudia using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). Journal of Pharmacognosy and Phytotherapy, 8(3):49–59.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turck, M. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology, 45(4 ts):493–496.

Breidenstein, E. B., de la Fuente-Nuñez, C., Hancock, R. E. 2011. Pseudomonas aeruginosa: all roads lead to resistance. Trends in Microbiology, 19(8):419–426.

Brown, N., Jeger, M., Kirk, S., Williams, D., Xu, X., Pattasso, M., Denman, S. 2017. Acute Oak Decline and Agrilus biguttatus: The Co-Occurrence of Stem Bleeding and D-Shaped Emergence Holes in Great Britain. Forests, 8(3):87–87.

Bursal, E., Boğa, R. 2018. Polyphenols analysed by UHPLC-ESI-MS/MS and antioxidant activities of molasses, acorn and leaves of oak (Quercus robur subsp. pedunculiflora). Progress in Nutrition, 20:167–175.

CLSI 2019. Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement M100-S21. Clinical and laboratory standards institute.

Dawid-Pač, R. 2013. Medicinal plants used in treatment of inflammatory skin diseases. Advances in Dermatology and Allergology, 3(3):170–177.

El-Gamal, M. I., Oh, C.-H. 2010. Current Status of Carbapenem Antibiotics. Current Topics in Medicinal Chemistry, 10(18):1882–1897.

Fernandez-Escobar, R., Gallego, F. J., Benloch, M., Membrillo, J., Infante, J., de Algaba, A. P. 1999. Treatment of oak decline using pressurized injection capsules of antifungal materials. Forest Pathology, 29(1):29–38.

Foster, T. J. 2017. Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS Microbiology Reviews, 41(3):430–449.

Fridkin, S., Jamesbaggs, R., Fagan, S., Magill, L. A. P. 2014. Vital Signs: Improving Antibiotic Use Among Hospitalized Patients. Morbidity and Mortality Weekly Report, 63(09):194–200.

Gauglitz, G. G., Shahrokhhi, S., Williams, F. N. 2017. Burn wound infection and sepsis.

Hamad, H. O., Mehmet, A., Gulcin, I., Yilmaz, M., karaogul, E. 2017. Evaluation of phenolic contents and bioactivity of root and nutgall extracts from iraqian Quercus infercudiaolivier. Records of Natural Products, 11:205–210.

Hamszah, A. M., Lafta, I., Alwan, M. J. 2011. Bacterial isolation from burn wound infections and studying their antimicrobial susceptibility. Kufa Journal for Veterinary Medical Sciences, 2(1):121–131.

Harborne, J. B. 1984. Phytochemical methods. New York: In Chapman and Hall, pages 4–5.

Patra, A. K., Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and rumenant nutrition. Journal of the Science of Food and Agriculture, 91(1):24–37.

Peleg, A. Y., Hooper, D. C. 2010. Hospital-Acquired Infections Due to Gram-Negative Bacteria. New England Journal of Medicine, 362(19):1804–1813.

Radji, M., Agustama, R. A., Elya, B., Tjampakasari, C. R. 2013. Antimicrobial activity of green tea extract against isolates of methicillin-resistant Staphylococcus aureus and multi-drug resistant Pseudomonas aeruginosa. Asian Pacific Journal of Tropical Biomedicine, 3(8):663–667.

Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., Vuorela, P. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology, 56(1):3–12.

Ribechni, E., Mangani, F., Colombini, M. P. 2015. Chemical investigation of barks from broad-leaved tree species using EGA-MS and GC/MS. Journal of Analytical and Applied Pyrolysis, 114:235–242.

Ricci, A., Parpinello, G. P., Palma, A. S., Teslić, N., Brilli, C., Pizzi, A., Versari, A. 2017. Analytical profiling of food-grade extracts from grape (Vitis vinifera sp.) seeds and skins, green tea (Camellia sinensis) leaves and Limousin oak (Quercus robur) heartwood using MALDI-TOF-MS, ICP-MS and spectrophotometric methods. Journal of Food Composition and Analysis, 59:95–104.
Sati, A., Sati, S. C., Sati, N., Sati, O. P. 2017. Chemical composition and antimicrobial activity of fatty acid methyl ester of Quercus leucotrichophora fruits. *Natural Product Research*, 31(6):713–717.

Skrypnik, L., Grigorev, N., Michailov, M., Antipina, M., Danilova, M., Pungin, A. 2019. Comparative study on radical scavenging activity and phenolic compounds content in water bark extracts of alder (Alnus glutinosa (L.) Gaertn.), oak (Quercus robur L.) and pine (Pinus sylvestris L.). *European Journal of Wood and Wood Products*, 77(5):879–890.

Sperandio, F., Huang, Y.-Y., Hamblin, M. 2013. Antimicrobial Photodynamic Therapy to Kill Gram-negative Bacteria. *Recent Patents on Anti-Infective Drug Discovery*, 8(2):108–120.

Sydnor, E. R. M., Perl, T. M. 2011. Hospital Epidemiology and Infection Control in Acute-Care Settings. *Clinical Microbiology Reviews*, 24(1):141–173.

Tripathi, Y. B., Tiwari, O., Nagwani, S., Mishra, B. 2009. Pharmacokinetic-interaction of Vitex negundo Linn. & paracetamol. *The Indian Journal of Medical Research*, 130(4):479–483.

Turner, K., Leϑler, L., Freedman, B. 2005. Plant communities of selected urbanized areas of Halifax, Nova Scotia, Canada. *Landscape and Urban Planning*, 71(2-4):191–206.

Uddin, G., Rauf, A., Gul, S., Saleem, M., Umar, S., Khan, A. 2013. Phytochemical screening, antimicrobial and antioxidant activities of aerial parts of Quercus robur. *Journal of Medicinal Plants Research*, 1(1):1–4.

Umachigi, S. P., Jayaveera, K. N., Kumar, C. K. A., Kumar, G. S., swamy, B. M. V., Kumar, D. V. K. 2008. Studies on Wound Healing Properties of *Quercus infectoria*. *Tropical Journal of Pharmaceutical Research*, 7(1):913–919.

Warjri, I., Dutta, T. K., Lalzampuia, H., Chandra, R. 2015. Detection and characterization of extended-spectrum β-lactamases (blaCTX-M-1 and blaSHV) producing Escherichia coli, Salmonella spp. and Klebsiella pneumoniae isolated from humans in Mizoram. *Veterinary World*, 8(5):599–604.

Witwit, I. N., Mubark, H. M., Al-Labban, H. M. Y., jabbar jaloob Aljanaby, A. A. 2019. synthesis and characterization of new imidazole azo ligand with some of transition metal ions, and their biological effect on two pathogenic bacteria of burn patients. *International Journal of Research in Pharmaceutical Sciences*, 10(3):1847–1856.

Yadav, R. N. S., Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *J Phytol*, 3(12):10–14.