A Biological Study on Quercus Bark as Antimicrobial Agent

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ABSTRACT

Quercus sp. [Oak] has been used in traditional medicine widespread around the word. in Iraq, It has been used widely as antiseptic after birth by women. The aim of this study was to evaluate the biological activity of the aqueous and alcoholic crude extracts of the bark of *Quercus* sp. against two types of bacterial reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 and assay the cytotoxicity of Oak crude extracts. The primary screening was carried by using well diffusion method. The both extracts [aqueous and alcoholic] showed a broad-spectrum effect on Gram positive and Gram negative reference strains. While alcoholic extracts showed the greatest effect, Gram negative were more sensitive than Gram positive. However, the minimum inhibition concentration was 100 mg/ml for both extracts against both bacterial strains. The two extracts showed bactericidal effect, the growth of the reference strains was monitored for 7 days and no growth was observed, except in the 100 mg/ml concentration of the aqueous extract, 2-3 colonies was growing back. The cytotoxic effect of the extracts was assayed on the red blood cells [RBCs], the result showed no cytotoxicity in the absence of alcohol. Thus, the bark of *Quercus* sp. may be used as a source of natural bactericidal agent safely.

Keywords: *Quercus* sp. [Oak], biological activity, cytotoxicity, aqueous and alcoholic extracts, Jaft

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INTRODUCTION

Many plants which regard as a gift of life, produce natural substances that synthesised to perform versatile biological function to the plant, those substances could be very beneficial in medicine, especially the products of medical plants [1]. The use of herbal medicines [alternative medicines], date back to [4000-5000] B.C. About 80% of the world population depend on the plant products to maintain their health and approximately 30% of prepared medicines are based on plants [2,3]. For many years, the researchers have focused on the antimicrobial effect of the plants, the extracts of different parts of some plants have been intensively studied, and the antimicrobial activity for many plants has been proved[4–6] According to the increase in the infectious diseases that causes the mortality of half the population of the tropical countries, the scientist focused on finding safe, cheap and available alternatives, 60-90% of the developing countries depending on alternative medicines to cure their infection disease [7].The overuse of the antibiotics leads to the bacterial multi-resistance, in addition to the side effects and the toxicity of most antibiotics[8],those reasons prompt to search for natural alternatives [9]. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* are the most causative agents if human infections [10,11].

The oak [*Quercus*] genus belongs to the family *Fagacae*, subfamily *Quercoideae*, which contains 400 species widespread in Europe, Asia and America. The bark of these trees has been used in traditional medicine to treat burns and wounds, used orally for gastrointestinal diseases, since medieval times [12]. The cut and dried bark of young branches and the lateral shoots of *Quercus robur*, *Quercus petraea* and/or *Quercus pubescens* are harvested in spring from March to April, saved to use as medicine [13]. The bark *Quercus cortex* used to treat the mild diarrhoea, minor inflammation of the oral mucosa or skin, whereas the powder of *Q. robur* used for prophylaxis of diarrhoea in cattle, horses, pigs, sheep and chicken [14].

The antibacterial activities of different parts of *Quercus* species have been studied intensively[15–17].The mechanisms of the antibiotic activity of many plants still unknown, but most of the antimicrobial activity was attributed to the active chemical compounds, which known as secondary metabolites, that found in the plant. In the *Quercus* the antibacterial activity might be due to presence of tannins in the plant extract [18].

The aromatic plants are economically important plants that provide the basic raw materials in pharmacology, food and cosmetic industries [19].

MATERIAL AND METHODS

Plant collection and preservation

The stem bark of Oak [Quercus sp.] was purchased from local markets, saved in dry sacs, then grounded by pistils to a powder and stored in a dark glass container at room temperature.

Preparation of aqueous extract

200 g of Oak powder was mixed well with 500 ml distilled water in a blender for 15 min, then it was left for one hour to settle. The mixture was filtered by using centrifuge [80 X for 10 min], followed by filtration by using Whatman No.1 filter paper. The filtrate was evaporated using rotary evaporator, and the rest of the filtrate was kept in freezer till used [20].

Preparation of alcoholic extract

100 g of Oak powder was mixed with 500 ml of ethanol [70%] in a magnetic stirrer for 48 hours. The extract was filtered by using Whatman No.1 filter paper, and the solvent was then removed by using a rotary evaporator, the residues were kept in freezer till used [20]

Phytochemical Analysis of the Plant Extract

Several qualitative tests for ethanol have been carried out to find out their general chemical composition. 1 gm of crude extract dissolved with 10 ml of distilled water, filtrated by Whatman No.1 filter paper, then 1 ml of filtrated crude extract was used for each reagent. Mayer, Dragendorff and Wagner reagent were used to detect alkaloids, precipitation and Turbidity indicate the positive result. A blue-green coloration on Whatman filter paper indicated the presence of phenol by using ferric chloride and folin reagent with the vapour of NH_4OH. Turbidity was an indicator of the presence of tannins by using lead acetate 1% reagent. To detect the existence of saponins, 1 ml of filtrated crude extract was shaken vigorously in test tube, the formation of stable foam was used as an indicator of the positive result, in addition, formation of the turbidity with mercury chloride reagent was a confirmation of saponins present. Alcoholic potassium hydroxide reagent was used to detect flavonoids, yellow precipitation indicated of positive result. Benedict's reagent was used to detect carbohydrates by mixing 1 ml of reagent with 1 ml of filtrated crude extract, then boiled, red participate indicated the presence of carbohydrate. Ninhydrin reagent was used to detect free amino group, a violet colour appearance indicated the positive result.[20–23].

Assay for antibacterial activity

The antibacterial activities of tested extracts were assayed using well diffusion method against *Escherichia coli*[ATCC 25922] and *Staphylococcus aureus*[ATCC 25923]. 0.1 ml of bacterial suspensions with concentration 3.0 x 10^8 CFU/ml [McFarland standard 1]of each of the test bacteria were cultivated on Muller-Hinton Agar [MHA, Merck] plates, and uniformly spread using L-shape spreader. The cork borer was used to make 6 mm diameter wells in the cultivated MHA agar, the wells then filled with 0.1 ml of each extract with concentration of 500 mg /ml. Sterile distilled water and ethanol [70%] were used as controls, petri dishes were then incubated at 37 °C for 24 hours, and the antibacterial activity was determined by measuring the mean of the inhibition zone diameters around each well. The experiment was repeated 3 times [24]

The Minimum Inhibitory Concentration [MIC]

The Minimum Inhibitory Concentration [MIC] was determined using well diffusion method as described previously, by using five concentrations [500, 400, 300, 200, 100 mg/ ml] of both alcoholic and the aqueous extracts [25].

Cytotoxicity of plant extracts assay

The cytotoxicity of the aqueous and alcoholic extracts was assayed on the human red blood cells [RBCs], according to [26], the Ringer solution was used to maintain the osmotic pressure of the RBC, 500, 400, 300, 200, 100 mg/ ml of the extracts were added to 1 ml of the RBCs with 19 ml of Ringer solution in serial test tubes, and the mixtures were monitored every hour for 12 hours.

RESULTS

The phytochemical analysis of ethanol extract of *Quercus sp.*fruits showed the presence of alkaloids, free amino group, Glycosides, phenols, saponins and tannins [Table 1].

Table 1: Phytochemical analysis of the ethanol extracts of *Quercus sp.*

Plant	Compounds						
	Alkaloids	Flavonoids	Free aminogroup	Glycosides	Phenols	Saponins	Tannins
Quercus sp.	+	-	+	+	+	+	+

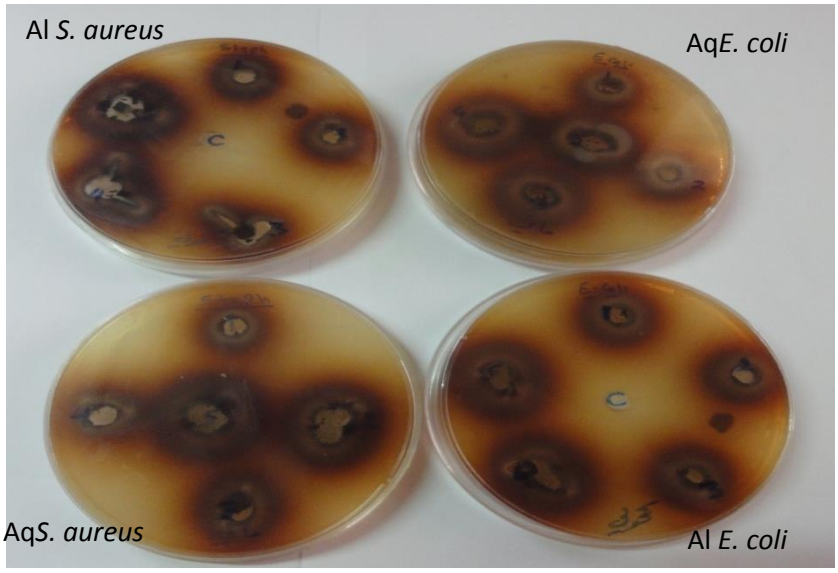
Primary screening

The result of screening the antimicrobial activity of Oak bark on the Gram positive and negative reference by using 500 mg/ml of each aqueous and alcoholic extracts, showed a broad spectrum effect on both Gram positive and Gram negative reference strains, in the Gram positive strain the inhibition zone was 16 mm for the alcoholic extract and 15 mm for the aqueous extract, whereas on the Gram negative reference strain, using the same concentration [500 mg/ml] of the aqueous and alcoholic extracts showed 17.5 and 19 mm inhibition zones respectively.

The minimum inhibition zone [MIC] assay

The minimum inhibition zone results showed that the minimum inhibition concentration for both extracts [aqueous and alcoholic] was 100 mg/ml, which give 10.5 and 11 mm inhibition zone for the aqueous and alcoholic extracts respectively on Gram negative strain, and 10.3 and 10.8 mm for the aqueous and alcoholic extracts respectively on Gram positive strain [Figure 1, table 2].

Figure 1: The figure showed the result of the minimum inhibitory concentration [MIC] for the aqueous and



alcoholic Oak extracts on *Escherichia coli* ATCC 25922 and *Staphylococcusaureus* ATCC 25923, the figure shows the inhibition zones around the wells in the MHA plates. Aq= aqueous extract, Al= alcoholic extract.

Table 2: Shows the results of the MIC assay, the table shows the diameter of the inhibition zone measured in mm for both aqueous and alcoholic extracts on *Staphylococcus aureus* and *Escherichia coli* reference strains.

Concentration strain	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml
Aqueous extracts					
Staphylococcus aureus	10.3 mm	11 mm	12.5 mm	14 mm	15 mm
Escherichia coli	10.5 mm	12 mm	13.8 mm	15.9 mm	17.5 mm
Alcoholic extract					
Staphylococcus aureus	10.8 mm	12.2 mm	13.5 mm	15 mm	16 mm

Escherichia coli	11 mm	13 mm	15 mm	17 mm	19 mm

The antimicrobial effect

The two extracts [alcoholic and the aqueous] showed bactericidal effect, the growth of the reference strains was monitored for 7 days and no growth was observed, except in the 100 mg/ml concentration of the aqueous extract, 2-3 colonies was growing back.

The cytotoxicity trails

The results of assaying the cytotoxic effect of the extracts on red blood cells [RBCs], showed No lysis for the RBCs in 100,200,300,400 mg/ml of the aqueous extract, the only lysis was observed in the 500 mg/ml concentration tube, the RBCs cells where examined under microscope to ensure the rapture of the cells, whereas the control distil water showed no lysis effect on RBCs [Figure 2A]. Alcoholic extract showed hemolysis of the RBCs in all concentrations [100, 200, 300, 400 and 500 mg/ml], the same results were observed in the control [alcohol] tubes.

DISCUSSION

Global prevalence of infectious diseases caused by bacteria is a major public health problem, for years, scientists were search for any available, low cost, safe to use medicines, many attempts have been made to synthesis antibiotics which has above features, but the obstacles was always the side effects of any synthesis compound.

The use of plant was the most abundant way to treat different infections, as anaesthetics, disinfectants or food preservatives, although, some shows a distinct side effect. However, over the years of study, only a small number of plants, out of 400,000 plant species on the earth, have been proven to have antimicrobial activity [27]. The secondary metabolites of plants, including the tannins, flavonoids and alkaloids possess antimicrobial properties *in vitro* [28].

The Oak named locally in Iraq as Jaft, and it is prominent used by Iraqi women, after soaking or boiling in water, as antiseptic following birth. Many studies have been revealed the antimicrobial activity of different parts of *Quercus species* [12,29–32]. Phytochemical analysis of *Quercus* sp. extracts showed the presence of most of the secondary metabolic alkaloids, free amino group, Glycosides, phenols, saponins and tannins, which agreed with other study[1,33].

In this study, the bark of Oak was extracted in ethanol and distil water, and the crude extracts were applied for antimicrobial screening on Gram positive and negative reference strains. The result showed that Oak has a broad spectrum antimicrobial effect on both Gram positive and negative reference strains. The Gram positive bacteria are less sensitive than gram negative strain, as shown in table 1. The MIC [mg/ml] shows 10.3 mm and 10.5 mm inhibition zone on *S. aureus* and *E. coli* respectively for the aqueous extract. The same results were observed for the alcoholic extract which showed 10.8 and 11 mm inhibition zones on *S. aureus* and *E. coli* respectively. This result was disagreed with most studies on Oakextract which showed that Gram positive bacteria are more sensitive than Gram negative [12,29,30,32]. However, Puupponen-Pimiä conclude that different bacterial species exhibit different sensitivities towards phenolic compounds and their study showed that the phenolic extract of berry appear to have the same effect of our extract which was more effective on Gram negative than Gram positive bacteria, which support our results [34]. It has been found that that the most abundant compounds in the oak bark extract were phenolic and flavonoids compounds [35]. In addition, Deryabin. and Tolmacheva observed that phenolic compounds: 1,2,3-trihydroxybenzene and 4-propyl-1,3-benzenediol are important *Quercus cortex* constituent responsible for both antibacterial activity [12].

The activity of phenolic compounds might be due to the fact of the differences in the cell surface components between Gram positive and negative bacteria,the outer membrane of Gram negative bacteria functions as a barrier against hydrophobic compounds[36]. Ferreira *et al*. also stated that the antimicrobial effect of phenolics may be due to the potential mutagenic activity of these compounds. Stam-mati *et al*. showed other mechanisms that might involve in the antimicrobial action of phenolics, which is genotoxicity [37]. Helander *et al*. [1998] have studied effect of plant-derived essential oil components on Gram negative bacteria, they found that small phenolic compounds such as carvacrol and thymol inhibited *E. coli* and *Salmonella*, they attributed the inhibitory effects to the disruptive action of these compounds on the outer membrane. The other antimicrobial mechanism of the phenolic substances is the decomposition of the membrane of microbes [38]. The phenolic substances have also an important role to discourage the growth of bacteria through inhibition of the enzymes responsible for the metamorphosis of the protein [39].

Al-Manhel and Niamah study on the effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes that causing spoilage of food showed that the alcoholic extract has much more activity on investigated fungi and bacteria than aqueous extract, they attributed that to the way of the extraction and the solvent that used in the extraction process both effect the final product of the extraction [40]. Alcoholic

extraction showed highly percentage of phenolic substances, which is the most active product from *Quercus* as shown by Ferreira *et al*. the phenolic extract also effects on the permeability of the cell membrane [20]. The superiority of the alcoholic extract than that of the aqueous one might be due to the presence of phenol compound and the absence of this compound in the aqueous solution.

The cytotoxicity of the ethanolic and aqueous of oak extracts has been assayed in our study, the results showed that the aqueous extract showed RBCs hemolysis in only high concentration [500 mg/ml] of the extract, whereas the alcoholic extract showed hemolysis in all concentrations used, in addition to the control [ethanol]. Lang-Ming Chi and Wen-guey Wu in 1991 found that the leakage of the small cation K^+ from the cells caused the hemolysis for the ethanol-treated RBCs due to the disruption in the osmotic pressure , thus, they suggested that the creation of membrane pores might involve in the defected the cytoskeletal network of ethanol-treated RBC, this finding support our result [41]

CONCLUSION

The aqueous and alcoholic crude extracts of Quercus sp. [oak] exhibit a brood antimicrobial activity on Gram positive and negative reference strains with minimum inhibition concentration of 100 mg/ml, with no cytotoxicity in the absence of alcohol. The locally uses plant [Jaft] can be used safely as disinfectant as it uses locally.

REFERENCES

1. Omar S, Rahman F Magbool F AL, Ibrahim Elnima E, E SM, Eldin Omar Hussein S. PRELIMINARY PHYTOCHEMICAL SCREENING OF QUERCUS INFECTORIA GALLS PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF STEM BARK AND LEAVES EXTRACTS FROM FICUS SYCOMORUS View project World Journal of Pharmacy and Pharmaceutical Sciences PRELIMINARY PHYTOCHEMICAL SCREENING OF QUERCUS INFECTORIA GALLS. www.wjpps.com [Internet]. 2018;7[1]. Available from: www.wjpps.com
2. Shinwari M.I. and Khan MA. Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. Pak. J For. 1998;48[1–4]:63–90.
3. Gulfraz , M.; Abdul-Waheed; Mehmood S., and Ihtisham M. Extraction and purification of various organic compounds in selected medicinal plants of Kotli Sattian, District Rawalndindi, Pakistan. Ethnobot Leafl. 2006;10:13–23.
4. Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot. 2001;64[7]:1019–24.
5. Muyibi A, Yusuf U. Antibacterial activity of some medicinal plants against pathogenic bacteria. Available from: https://www.researchgate.net/publication/337185158
6. Pârvu M, Pârvu AE, Roca-Casian O, Vlase L, Groza G. Antifungal activity of Allium obliquum [Internet]. Vol. 4, Journal of Medicinal Plants Research. 2009. Available from: http://www.academicjournals.org/jmpr
7. Malini M, Abirami G, Hemalatha V AG. Antimicrobial activity of ethanolic and aqueous extracts of medicinal plants against waste water pathogens. Int J Res Pure Appl Microbiol. 2013;3[2]:40–2.
8. Eggleston K, Zhang R, Zeckhauser RJ. The global challenge of antimicrobial resistance: Insights from economic analysis. Int J Environ Res Public Health. 2010 Aug;7[8]:3141–9.
9. Alviano DS, Alviano CS. Plant Extracts: Search for New Alternatives to Treat Microbial Diseases. Vol. 10, Current Pharmaceutical Biotechnology. 2009.
10. Cheesbrough M. Medical Laboratory Manual for Tropical Countries. In Oxford, United Kingdom: Oxford Publishers; 1984. p. 32–3.
11. Peirano G. Multi resistant enterobacteriaceae new threat to an old prob; expect review of anti-infective therapy. Expert Rev Anti Infect Ther. 2008;6:657–69.
12. Deryabin DG, Tolmacheva AA. Antibacterial and anti-quorum sensing molecular composition derived from quercus cortex [Oak bark] extract. Molecules. 2015 Sep 1;20[9]:17093–108.
13. Council of Europe: Strasbourg F. European Pharmacopoeia. 7th ed. France; 2011.
14. EMEA [European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit]. Tylosin: Summary Report [3]; Committee for Veterinary Medicinal Products: London, UK, 1997; pp. 205–212.
15. Basri, D.F., and Fan SH. The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian J Pharmacol. 2004;73[2]:26–9.
16. Ghaderi Ghahfarokhi, M., Sadeghi Mahoonak, A., Alami,M., Khomeiri, M., and Mamashloo S. Evaluation of Antimicrobial Activity of the Ethanolic Extracts from Q.brantiand Q.castaneifoliaFruitAgainst Some Food-borne Pathogens by Microdilution Method. Food Technol Nutr. 2012;9[1]:81–95.

17. Khosravi, A.D. and Behzadi A. Evaluation of the Antibacterial Activity of the Seed Hull of Quercus Branti on some Gram Negative Bacteria. Pakistanian J Med Sci. 2006;22:429–32.
18. Ebrahimi A, khayami M nejati V. Comparison of Antimicrobial effect of different parts of Quercus persica against Escherichia coli O157:H7. Horiz Med Sci. 2012;17[4]:11–7.
19. Najafi, Sh., Sadeghi Nejad, B., Deokule, SS., and Estakhr J. Phytochemical screening of Bidaria khandalense[Sant.] Loranthus capitellatusWall., Viscum articulatumburm. F. and Vitex negundoLinn. Res J Pharm Biol Chem Sci. 2010;1:388–91.
20. Harborne JB. Phytochemical Methods. Phytochemical Methods. Springer Netherlands; 1980.
21. Agarwal O. Chemistry of Organic Natural Products. 3rd ed vol. Subhash, Bazar,India: Geol Publishing House; 1975. 490p p.
22. Akinpelu DA, Aiyegoro OA, Okoh. In vitro antimicrobial and phytochemical properties of crude extract of stem bark of Afzelia africana [Smith]. African J Biotechnol [Internet]. 2008;7[20]:3665–70. Available from: http://www.academicjournals.org/AJB
23. Silva,G.L; Lee,I-K. and Kinghorn AD. , In: Cannell, R.J.P.[Eds.][vol.4]. . In: Natural Products Isolation Methods in Biotechnology. Totowa, New Jersey.: Humana Press; 1998. p. pp:343-633.
24. Al-Manhel AJ, Niamah AK. Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food. Vol. 25, Pakistan Journal of Food Sciences. 2015.
25. JAN NEXSON PARHUSIP A, BOING SITANGGANG A. Antimicrobial Activity of Melinjo Seed and Peel Extract [Gnetum gnemon] Against Selected Pathogenic Bacteria. Microbiol Indones. 2011 Jun;5[2]:103–12.
26. Nair, M.G.; Mishar, A.R.; Muks, M.H.; Taft, W.H.; Kesller, J.E.; Miller, J.R.; Zhn, P.P.; Meinhart, J.D. & Lynm D. Faerifungin, a new broad spectrum antibiotic from *Streptococcus griseus* var *autotrophicus*. J Nat Prod. 1989;52:797–809.
27. Varahalarao, V. and Chandrashekhar N. In vitro bioactivity of Indian medicinal plan Calotropis procera. J Glob Pharma Technol. 2010;2[2]:43–5.
28. Dahanukar SA, Kulkarni RA, Rege NN. PHARMACOLOGY OF MEDICINAL PLANTS AND NATURAL PRODUCTS. PLANTS [NATURAL PRDUCTS] Indian Journal of Pharmacology. 2000.
29. Güllüce, M.; Adıgüzel, A.; Öğütçü, H.; Şengül, M.; Karaman, I. and Şahin F. Antimicrobial effects of Quercus ilex L. extract. Phyther Res. 2004;18[3].
30. Hussein RA. Extraction and Identification of a Flavonoid compound from Oak Plant[Quercus infectoria Oliv.] and study of Its Antibacterial Activity, in vitro.
31. Nareen Qadr Faqi Abdulla. The Effect of Aqueous and Alcoholic Extracts of Galls of Quercus infectoria on the Growth of Some Pathogenic Fungi. ZANCO J PURE Appl Sci. 2018 Dec 25;30[6].
32. Bajalan I, Javadian M, Zarinkoob S, Dalvand H. Bulletin of Environment, Pharmacology and Life Sciences O OR RI IG GI IN NA AL L A AR RT TI IC CL LE E Antibacterial Activity of the Extract of Oak [Quercus persica] Fruits. Vol. 3, Env. Pharmacol. Life Sci. 2014.
33. Ghafour, N. H.; Aziz, H. A.; Almolla RM. Determination of some chemical constitutes of Oak plants [Quercus spp] in the mountain Oak forest of Sulaimani governorate. J Zankoy Sulaimani. 2010;13[1].
34. Puupponen-Pimia R, Nohynek L, Meier C, Ka M, Hko È Nen È, Heinonen M, et al. Antimicrobial properties of phenolic compounds from berries. 2001.
35. Ferreira JPA, Miranda I, Sousa VB, Pereira H. Chemical composition of barks from Quercus faginea trees and characterization of their lipophilic and polar extracts. PLoS One. 2018 May 1;13[5].
36. Helander IM, Alakomi H-L, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, et al. Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. 1998.
37. Stammati, A., Bonsi, P., Zucco, F., Moezelaar, R., Alakomi, H.-L. andvon Wright A. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. Food Chem Toxicol. 1999; 37:813–23.
38. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12[4]:564–82.
39. Newman DJ CG. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012;75[3]:311–35.
40. Al-Manhel AJ, Kareem Niamah A. Effect of Aqueous and Alcoholic Plant Extracts on Inhibition of Some Types of Microbes and Causing Spoilage of Food. J Nutr Food Sci. 2012;s5.
41. Chi L-M, Wu W-G. Mechanism of hemolysis of red blood cell mediated by ethanol. Vol. 1062, Biochimica et Biophysica Acta. 1991.