Comparison of the antibacterial effect of aqueous, and hydro-alcoholic extracts of ripe and unripe *Morus nigra*

Farhad Sharifee[1], Leila Asadpour*[1], Shahab Shariati[2], Ali Salehzadeh[1]

[1] Department of Biology, Rasht Branch, Islamic Azad University, Rasht, Iran

[2] Department of Chemistry, Rasht Branch, Islamic Azad University, Rasht, Iran

* Corresponding authors: Department of Biology, Rasht Branch, Islamic Azad University, Rasht, Iran. Email: l.asadpour@yahoo.com

Abstract

The growing issue of microbial resistance to antibiotics has spurred significant efforts to develop new drugs, increasing the interest in plant-based treatments. *Morus nigra* is a commonly used fruit in medicine. This study aims to examine the antimicrobial properties of both ripe and unripe *M. nigra* fruits and compare their effects. In this experimental study, the antimicrobial effects of of ripe and unripe *M. nigra* extracts were evaluated by determining the zone of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) against *Staphylococcus epidermidis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans,* and *Streptococcus pyogenes.* These assessments were conducted using disk diffusion and broth macro-dilution methods. Statistical analysis was carried out using the ANOVA test with SPSS software. The antimicrobial effects of the unripe *M. nigra* fruit extracts with the MIC values of 0.78-1.5 mg/mL were stronger than ripe fruit extract against all tested bacteria. Also that the tested extracts were more effective against Gram-positive strains than Gram-negative strains Additionally, the antimicrobial properties of the unripe *M. nigra* extract were comparable to those of selected antibiotics including gentamicin. It can be concluded that unripe fruit exhibits stronger antimicrobial effects than ripe *M. nigra* fruit. This difference could be attributed to the variation in active compounds that are present at different stages of the fruit's development.

Key words: Herbal extract, Antibacterial resistance, *Morus nigra*, MIC, MBC

Introduction

There is a growing public concern regarding the harmful consequences of synthetic and chemical additives, which are utilized for their antibacterial, antioxidant, and flavor-enhancing capabilities. To address this concern, researchers have started investigating natural substitutes for these chemicals. An encouraging possibility is the utilization of natural botanical extracts, which encompass a diverse array of flavorful, antibacterial, antioxidant, and anticancer compounds (Ahmad Nejhad et al., 2023). According to an evaluation of data from 129 member states, the World Health Organization (WHO) predicts that the 'post-antibiotic' period will likely begin around the year 2050. This evaluation revealed widespread resistance to antimicrobial agents in every part of the world. Antimicrobial resistance (AMR) is a global problem that leads to the death of millions of people each year. This resistance occurs when opportunistic or primary pathogens become resistant through horizontal gene transfer (HGT) mechanisms and/or biofilm formation. It is a complex issue that has a devastating impact on humans, livestock, and the environment (Machado et al., 2023). As a result, the search for novel antibacterial compounds is a critical requirement that has enticed numerous scientists to find antimicrobial compounds from various sources, including plants. Historically, herbal plants have been widely employed for the treatment of various types of illnesses. Presently, there is an increasing fascination with utilizing plant derivatives because of their diminished occurrence of negative consequences. Various research have documented the therapeutic qualities of herbal plants or their constituents. The properties of numerous plant chemicals, such as neurotherapy (Noorbakhsh et al., 2022; Sherafatizangeneh et al., 2022), anti-oxidant (He et al., 2024), anti-diabetic (Behl & Kotwani, 2017), anti-viral (Shayan et al., 2021), anti-inflammatory (Lee et al., 2024), anti-cancer (Sharma et al., 2023), anti-analgesic (Rahimi et al., 2016), and anti-hepatotoxic (Rani et al., 2024) have been reported.

Mulberries, which are part of the *Morus* genus in the Moraceae family, originate from the area spanning from India to China. These trees were cultivated millennia ago specifically for the purpose of rearing silkworms. They are highly adaptable to flourish in tropical, subtropical, and temperate regions, and possess the innate ability to grow in various topographical and soil conditions, spanning from sea level to elevations of 4000 m. The plant demonstrates a remarkable capacity to adjust to various soil and environmental conditions (Skrovankova et al., 2022). *M. nigra*, often known as black mulberry, has a significant amount of dietary polyphenols, which demonstrate a wide range of biological effects (de Pádua Lúcio et al., 2018). The Chinese Pharmacopoeia first recorded it as "Tang Materia Medica" and it has been used as a Traditional Chinese Medicine for many years. Several studies have shown that mulberry possesses several biological qualities, such as anti-obesity, antioxidant, neuroprotective, and anti-inflammatory effects (Wang et al., 2022).

This study aimed to compare the antibacterial effect of aqueous, hydroalcoholic and alcoholic extracts of ripe and unripe *M. nigra* on gram positive and gram negative bacteria.

Materials and methods

Plant material

On 06/06/2022, fruits from the *M. nigra* tree in Bahram village, Saqez, Iran, were collected. These fruits included both ripe and unripe fruits. The item was sent to the Faculty of Agricultural Sciences, Azad University, Rasht Branch, for botanical validation.

Microbial strain

Standard strains of *Staphylococcus aureus* ATCC 33591, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus mutans* ATCC 35668, *Streptococcus pyogenes* ATCC 19615, and *Pseudomonas aeruginosa* ATCC 9027 were used in this study. We obtained the strains from the Microorganisms Bank of the National Centre for Genetic and Biological Resources of Iran.

Extraction

The extraction process involved soaking the substance in a mixture of water and several solvents. This study utilized distilled water and 96% ethanol from Taghtirkhorasan, Iran, in a ratio of 1:20 to create an aqueous extract. Additionally, alcoholic and hydro-alcoholic extracts were prepared using a mixture of distilled water (30%) and ethanol (70%). To achieve this objective, 50 grams of dry powder were measured in a proportion of 1 to 20, and then 1000 cubic centimeters of the solvent were added to it. The glass bottle was sealed with a lid and covered with foil to protect the solution inside from exposure to light. The aqueous extract was immersed for a duration of 72 hours, while the alcoholic and aqueous-alcoholic extracts were immersed for a duration of 48 hours. The entire extract was subsequently passed through Whatman 2 filter paper. For the subsequent stage, a Buchi-R100 rotary device from Germany was employed to condense the extract. The rotation was carried out at a temperature of 40 °C and a speed of 60 rpm. At this temperature, the solvent underwent evaporation and was subsequently extracted from the solution. Ultimately, the extract was acquired with the intended level of purity. Next, the prepared extracts were placed into specialized containers, and the extracts were transformed into dry powder using a freeze dryer apparatus. This technique involves freezing the solvent in the sample and then subjecting it to sublimation. As a result, the material's physical and chemical properties will stay unaltered.

Antibacterial activity

In order to perform the disk diffusion test, the Müller Hinton agar culture medium (Merck, Germany) was first prepared and transferred to 8-cm plates, and the microbial suspension was then prepared according to 0.5 McFarland turbidity. After preparing the homogeneous solution with a sterile swab, the solution was stirred, and after dewatering (pressing the swab to the wall of the tube to eliminate its water), it was cultured on Müller-Hinton agar medium. After culturing, the dried discs (Padtan Teb, Iran) impregnated with the extract were selected and transferred to the medium under the hood and sterile conditions using sterile forceps. On each plate, three discs were placed on the medium at a distance of 4 cm. After placing the discs in the plate, they were closed and kept in an incubator at 37°C for 24 hours. Next, the plate was examined and the diameter of the growth inhibition zone was measured using an accurate ruler. This experiment was performed in four replications to calculate the mean and standard deviation. A one-way ANOVA test was used to analyze the mean growth inhibition zone.

MIC and MBC determination

Following the completion of the qualitative stage of disk diffusion and the confirmation of antibacterial properties in the plant extracts, the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the tested bacteria was carried out using the following procedure: Initially, 100µL of serial dilutions of 0.78-100 mg/mL of the extract were prepared. Subsequently, 100µL of a microbial suspension was generated with a concentration of 1.5×10^6 CFU/mL for each bacteria and were added to each dilution. Subsequently, all of the plates were subjected to incubation at a temperature of 37 °C for a duration of 24 hours. After a duration of 24 hours, the samples were cultured on the surface of a nutrient agar medium and subsequently were incubator for 24 hours at a temperature of 37 °C. The plate with half the number of colonies compared to the control plate was designated as the MIC, whereas the plate with 5 or fewer colonies was designated as the MBC.

Statistical analysis

The results were inputted into SPSS V23 software and analyzed using one-way ANOVA. Significance was attributed to values of $p < 0.05$.

Results

Antimicrobial results of *M. nigra* unripe fruit extracts were reported in our previous study (Sharifee et al., 2022). Here, the results of the *M. nigra* ripe fruit extract are compared with the previous study. The mean diameter and standard deviation of the inhibition zone for the antibiotics penicillin, cefixime, gentamicin and tetracycline, as well as the prepared extracts are provided in Table 1.

Table 1. The average diameter of the inhibition zone of ripe and unripe *M. nigra* extract and standard antibiotics for standard bacterial strains

Bacteria / **Extracts**	***Staphylococcus epidermidis***	***Staphylococcus aureus***	***Streptococcus mutans***	***Streptococcus pyogenes***	***Pseudomonas aeruginosa***
Penicillin (10 µg)	17.5 ± 0.5	40.33 ± 1.5	17.67 ± 0.5	15.67 ± 0.5	R
Cefixime (5 µg)	12.67 ± 1.1	18.33 ± 0.58	21.83 ± 1.61	23.33 ± 0.5	R
Gentamicin (10 µg)	21.33 ± 1.1	18 ± 1	15.83 ± 0.76	17 ± 0.5	16.83 ± 0.29
Tetracycline (30 µg)	R	29.67 ± 1.53	R	R	17 ± 0.5
Aqueous extract Ripe *M. nigra* (40 µl)	16.17 ± 0.29	12.83 ± 1	15 ± 1	15 ± 1	8.33 ± 0.58
Hydro-alcoholic	13.17 ± 1	10.17 ± 1.26	12.5 ± 0.5	12.5 ± 0.5	7.67 ± 0.58

Extract Ripe *M. nigra* (40 µl)					
Aqueous extract Unripe *M. nigra* (40 µl)	22.0 ± 0.8	18.75 ± 0.5	22.25 ± 0.6	22.25 ± 0.5	13.5 ± 1.3
Hydro-alcoholic Extract Unripe *M. nigra* (40 µl)	18.75 ± 0.9	17.5 ± 1.3	18.75 ± 0.5	17.75 ± 0.5	10.5 ± 0.6

Disk diffusion, MIC, and MBC results for *S. epidermidis*

According to the data in Table 1, the aqueous extract of ripe *M. nigra* fruits has demonstrated an antibacterial action with a diameter of inhibition zone measuring 16.17 mm against *S. epidermidis*. Similarly, the aqueous extract of unripe *M. nigra* fruits has shown even stronger antibacterial activity, with a diameter of inhibition zone measuring 22 mm against the same bacteria. Also, the hydro-alcoholic extract of ripe *M. nigra* fruits exhibited a result of 13.17 mm against *S. epidermidis*, while the extract of unripe *M. nigra* fruits showed a result of 18.75 mm.

S. epidermidis exhibits resistance to tetracycline and demonstrates a high susceptibility to gentamicin. Based on the statistical analysis, the ripe *M. nigra* aqueous and hydro-alcoholic extracts exhibit more antibacterial activities than tetracycline ($p<0.05$). On the other hand, the unripe *M. nigra* aqueous extract showed significantly more antimicrobial effect on *S. epidermidis* than all the antibiotics in Table 1, except gentamicin. Also, the difference between the results of unripe *M. nigra* hydro-alcoholic extract and the results of cefixime and tetracycline is significant ($p<0.05$).

The MIC and MBC results for the extracts are given in Table 2. The MIC and MBC of both aqueous and hydro-alcoholic extracts derived from ripe *M. nigra* for *S. epidermidis* were determined to be 25 and 50 mg/ml, respectively. In contrast, the MIC and MBC of unripe *M. nigra* extract were found to be much lower in concentration.

Table 2. MIC and MBC of Ripe and unripe *M. nigra* extracts against standard bacterial strains.

Extracts / **Bacteria**	**Aqueous extract Ripe *M. nigra* (mg/mL)**		**Hydro-alcoholic Extract Ripe *M. nigra* (mg/mL)**		**Aqueous extract Unripe *M. nigra* (mg/mL)**		**Hydro-alcoholic Extract Unripe *M. nigra* (mg/mL)**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus epidermidis	25	50	25	50	1.56	12.5	1.56	12.5
Staphylococcus aureus	25	50	25	50	1.56	12.5	1.56	12.5

Streptococcus mutans	12.5	25	12.5	25	1.56	12.5	1.56	25
Streptococcus pyogenes	25	50	25	50	1.56	12.5	1.56	12.5
Pseudomonas aeruginosa	12.5	25	12.5	25	0.78	3.12	1.56	6.25

Disk diffusion, MIC, and MBC results for *S. aureus*

Based on the information shown in Table 1, the aqueous extract of ripe *M. nigra* fruits has exhibited antibacterial properties, as evidenced by an inhibition zone diameter of 12.83 mm against *S. aureus*. In a similar manner, the aqueous extract derived from unripe *M. nigra* fruits has demonstrated a more potent antibacterial effect, as evidenced by an inhibition zone measuring 18.75 mm against *S. aureus*. The hydro-alcoholic extract of ripe *M. nigra* fruits demonstrated an inhibition zone of 10.17 mm against *S. aureus*, but the extract of unripe *M. nigra* exhibited an inhibition zone of 17.5 mm. However, the antibacterial activity of the aqueous and hydro-alcoholic extracts of unripe *M. nigra* on *S. aureus* was much weaker compared to penicillin and tetracycline ($p<0.05$). Nevertheless, the antimicrobial action of these extracts was statistically equivalent to that of cefixime and gentamicin antibiotics ($p > 0.05$). The results of MIC and MBC show that the unripe *M. nigra* extract shows inhibitory and bactericidal properties at a lower concentration compared to the ripe *M. nigra* extract against *S. aureus.*

Disk diffusion, MIC, and MBC results for *S. mutans* and *S. pyogenes*

The results show that the immature extract of *M. nigra* has more antimicrobial properties than its mature extract in the *S. mutans* and *S. pyogenes* standard strain. Based on the statistical analysis, the ripe *M. nigra* aqueous and hydro-alcoholic extracts exhibit more antibacterial activities than tetracycline ($p<0.05$). Although aqueous ripe *M. nigra* extract showed similar effects compared to gentamicin, cefixime, and penicillin ($p > 0.05$).

The unripe *M. nigra* aqueous extract showed significantly more antimicrobial effects on *S. mutans* and *S. pyogenes* than all the antibiotics in Table 1, except Cefixime. Also, the difference between the results of unripe *M. nigra* hydro-alcoholic extract and the results of tetracycline is significant ($p<0.05$), and the unripe *M. nigra* hydro-alcoholic results are similar to those of gentamicin, cefixime, and penicillin ($p>0.05$). The findings of MIC and MBC tests indicate that the unripe *M. nigra* extract exhibits inhibitory and bactericidal capabilities at a lower concentration than the ripe *M. nigra* extract when tested against *S. mutans* and *S. pyogenes*.

Disk diffusion, MIC, and MBC results for *P. aeruginosa*

The results of *P. aeruginosa* show that this strain was resistant to penicillin and cefixime. Therefore, the extracts prepared from these two antibiotics have shown a better antimicrobial effect. However, statistically, none of the extracts were better than gentamicin and tetracycline.

The aqueous extract of unripe fruit, with a diameter of 13.5 mm, has the highest antimicrobial effect on this gram-negative bacterium. MIC and MBC tests show that unripe *M. nigra* extract inhibits and kills *P. aeruginosa* at lower concentrations than ripe extract.

Discussion

Antibiotic resistance is an escalating public health concern (Farshadfar et al., 2020). Over time, existing antibiotic treatments lose their effectiveness or are insufficient to combat infections. According to the WHO, this challenge leads to extended hospital stays, increased healthcare costs, and higher mortality rates. Therefore, the need for discovering new antibiotics is critical. Promising approaches to combating antibiotic resistance include naturally derived products (Atanasov et al., 2021). Plants, which have been used to treat various infectious diseases for centuries, continue to play a significant role in traditional medicine for preventing and managing health issues. Inspired by traditional practices and grounded in scientific research, many drugs have been developed from medicinal plants, with some naturally occurring molecules serving as the foundation for new drug synthesis (Kakouri et al., 2023). In our research, we evaluated the antibacterial properties of *M. nigra* extract against gram positive and gran negative bacteria.

For an efficient extraction process, a high yield is typically desired, though this doesn’t necessarily mean that the extract will contain high concentrations of bioactive components. Since some bioactive compounds are highly sensitive to heat and oxygen, it is important to protect them from oxidation and thermal degradation. Therefore, both the extraction yield and the properties of the bioactive components should be carefully considered when choosing an extraction method. While traditional healers often use water as the primary solvent, studies have shown that organic solvents can sometimes yield more potent extracts than water-based ones. In this study, the extractive yield was significantly higher with water compared to the organic solvent ethanol. This suggesting that the plant parts studied contained more water-soluble phytoconstituents. Ahmad et al. (2016) reported that the aqueous extract of blackberry fruit exhibited no antimicrobial activity (Ahmad & Ahmed, 2016). This finding is in stark contrast to the results of our current study, which demonstrated that the aqueous extract had a stronger antimicrobial effect than the aqueous-alcoholic extract. The discrepancy between these studies may be attributed to differences in the extraction methods used and the geographical variations in the growth of *M. nigra* plants.

The results of the present study indicate that *M. nigra* extract had a significant impact on both Gram-positive and Gram-negative bacteria. This outcome may be due to the lipophilic nature of extract, which enabled it to interact with the lipids in the bacterial cell membrane, thereby enhancing membrane permeability (Radünz et al., 2019). In Gram-positive bacteria, the cell wall consists of a single layer of peptidoglycan, which has limited capacity to block the invasion of antibacterial substances (Wang et al., 2017; Zhao et al., 2021). Conversely, the cell wall structure of Gram-negative bacteria is more complex, comprising outer membrane proteins, peptidoglycan layers, and lipopolysaccharides. This complexity significantly restricts the diffusion of harmful external molecules into the bacterial cells (Kim et al., 2019). In the results of the study, it was observed that unripe *M. nigra* fruit extract has better antimicrobial effects than ripe fruit extract. The results of disk diffusion method as well as MIC and MBC confirmed the greater antimicrobial effects of unripe *M. nigra* fruit extract. In a study by Kumar et al., 2015, the inhibition zones of *M. nigra* ethanolic extract against *E. coli, S. aureus, P. aeruginosa, Klebsiella pneumoniae,* and *Salmonella typhi* measured 10 mm, 16 mm, 14 mm, 12 mm, and 14 mm, respectively (Kumar et al., 2015). In our current study, the inhibition zones of ripe fruit extract against *S. aureus* and *P. aeruginosa* were 15 mm and 8.33 mm, respectively. Additionally, the inhibition zones for the unripe *M. nigra* fruit extract against *S. aureus* and *P. aeruginosa* were 18.75 mm and 13.5 mm. Similar to Kumar's findings, our study also showed that the extract was more effective against Gram-positive strains than Gram-negative strains. Moreover, the unripe *M. nigra* fruit extract demonstrated stronger antimicrobial activity than the ripe fruit extract. Budiman et al. (2017) examined the effect of *M. nigra* fruit extract on *Staphylococcus epidermidis*. Their findings indicated that at a concentration similar to that used in our current study, the inhibition zone for this bacterium measured 15.33 mm (Budiman et al., 2017). In our study, the inhibition zone against *S. epidermidis* was 16.17 mm, showing comparable results. Additionally, our findings revealed that the unripe *M. nigra* fruit extract produced 18.75 mm inhibition zone against the bacteria, indicating stronger antimicrobial properties compared to the ripe *M. nigra* fruit extract.

Issa et al. (2017) explored the antimicrobial properties of various *M. nigra* fruit extracts, using both standard and clinical strains, similar to our current study. Their findings revealed that only the ethanolic extract at a concentration of 10 mg/ml produced a 14 mm inhibition zone against clinical *S. aureus*, while the other *M. nigra* extracts showed no antimicrobial activity against clinical strains (Issa & Abd-Aljabar, 2017). In contrast, the extract used in our study demonstrated significant antimicrobial effects on strains. The differences in results may be attributed to the higher concentration and different extraction methods used in our study.

The unripe *M. nigra* extract at a concentration of 1.56 mg/ml has inhibitory properties on different bacterial strains, on the other hand, the ripe extract at higher concentrations (25 and 12.5 mg/ml) has an inhibitory effect on the strains. The bacteriostatic properties of the extracts also show the same high difference between ripe and unripe *M. nigra* fruit extracts.

Conclusion

It can be concluded that unripe fruit exhibits stronger antimicrobial effects than ripe *M. nigra* fruit. This difference may be due to the variation in active compounds present at different stages of the fruit's growth. Notably, the antimicrobial effects of the unripe fruit extract surpass those of certain antibiotics, such as penicillin and cefixime (against *P. aeruginosa*) and tetracycline for Gram-positive bacteria (*S. epidermidis*, *S. mutans*, and *S. pyogenes*). Also, the antimicrobial properties of the unripe *M. nigra* extract show the same results compared to gentamicin.

References

Ahmad Nejhad, A., Alizadeh Behbahani, B., Hojjati, M., Vasiee, A., & Mehrnia, M. A. (2023). Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of Calotropis procera leaf aqueous extract. *Scientific Reports*, *13*(1), 14716. https://doi.org/10.1038/s41598-023-42086-1

Ahmad, R., & Ahmed, A. (2016). Evaluation of antibacterial activity of Morus nigra & Citrus limon. *Journal of Ayurvedic and Herbal Medicine*, *2*(6), 213-217.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature reviews Drug discovery*, *20*(3), 200-216.

Behl, T., & Kotwani, A. (2017). Chinese herbal drugs for the treatment of diabetic retinopathy. *Journal of Pharmacy and Pharmacology*, *69*(3), 223-235. https://doi.org/10.1111/jphp.12683

Budiman, A., Aulifa, D. L., Kusuma, A. S. W., & Sulastri, A. (2017). Antibacterial and antioxidant activity of black mulberry (Morus nigra L.) extract for acne treatment. *Pharmacognosy Journal*, *9*(5).

de Pádua Lúcio, K., Rabelo, A. C. S., Araújo, C. M., Brandão, G. C., de Souza, G. H. B., da Silva, R. G., de Souza, D. M. S., Talvani, A., Bezerra, F. S., Cruz Calsavara, A. J., & Costa, D. C. (2018). Anti-Inflammatory and Antioxidant Properties of Black Mulberry (<i>Morus nigra</i> L.) in a Model of LPS-Induced Sepsis. *Oxidative Medicine and Cellular Longevity*, *2018*, 5048031. https://doi.org/10.1155/2018/5048031

Farshadfar, C., Mollica, A., Rafii, F., Noorbakhsh, A., Nikzad, M., Seyedi, S. H., Abdi, F., Verki, S. A., & Mirzaie, S. (2020). Novel potential inhibitor discovery against tyrosyl-tRNA synthetase from Staphylococcus aureus by virtual screening, molecular dynamics, MMPBSA and QMMM simulations. *Molecular Simulation*, *46*(7), 507-520.

He, Y., Yu, J., Song, Z., Tang, Z., Duan, J.-A., Zhu, H., Liu, H., Zhou, J., & Cao, Z. (2024). Anti-oxidant effects of herbal residue from Shengxuebao mixture on heat-stressed New Zealand rabbits. *Journal of Thermal Biology*, *119*, 103752.

Issa, N. K., & Abd-Aljabar, R. S. (2017). Phytochemical analysis, antioxidant (DNA Nicking Assay) and antibacterial activities of Morus nigra L. fruits secondary metabolites. *Science Journal of University of Zakho*, *5*(2), 198-204.

Kakouri, E., Daferera, D., Trigas, P., Charalambous, D., Pantelidou, M., Tarantilis, P. A., & Kanakis, C. D. (2023). Comparative Study of the Antibacterial Activity, Total Phenolic and Total Flavonoid Content of Nine Hypericum Species Grown in Greece. *Applied Sciences*, *13*(5), 3305. https://www.mdpi.com/2076-3417/13/5/3305

Kim, N.-N., Kim, W. J., & Kang, S.-S. (2019). Anti-biofilm effect of crude bacteriocin derived from Lactobacillus brevis DF01 on Escherichia coli and Salmonella Typhimurium. *Food control*, *98*, 274-280.

Kumar, A., Kumari, M., Mazumdar, R. S., & Dhewa, T. (2015). In vitro antibacterial activity of ethanolic extracts of Viola serpens and Morus nigra against pathogens isolated from patients suffering from jaundice. *World J. Pharm. Res*, *4*, 889-898.

Lee, J.-W., Kim, E.-N., & Jeong, G.-S. (2024). Anti-Inflammatory Herbal Extracts and Their Drug Discovery Perspective in Atopic Dermatitis. *Biomolecules & Therapeutics*, *32*(1), 25.

Machado, A., Zamora-Mendoza, L., Alexis, F., & Álvarez-Suarez, J. M. (2023). Use of Plant Extracts, Bee-Derived Products, and Probiotic-Related Applications to Fight Multidrug-Resistant Pathogens in the Post-Antibiotic Era. *Future Pharmacology*, *3*(3), 535-567. https://www.mdpi.com/2673-9879/3/3/34

Noorbakhsh, A., Hosseininezhadian Koushki, E., Farshadfar, C., & Ardalan, N. (2022). Designing a natural inhibitor against human kynurenine aminotransferase type II and a comparison with PF-04859989: a computational effort against schizophrenia. *Journal of Biomolecular Structure and Dynamics*, *40*(15), 7038-7051.

Radünz, M., da Trindade, M. L. M., Camargo, T. M., Radünz, A. L., Borges, C. D., Gandra, E. A., & Helbig, E. (2019). Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (Syzygium aromaticum, L.) essential oil. *Food chemistry*, *276*, 180-186.

Rahimi, A., Razmkhah, K., Mehrnia, M., Mohamadnia, A., Sahebjamee, H., Salehi, S., Asl, E. A., Tahmasebi, H., Shandiz, S. A. S., Davouodbeglou, F., Ghasemi, S., Ardalan, N., & Kordkandi, Z. G. (2016). Molecular docking and binding study of harpagoside and harpagide as novel anti-inflammatory and anti-analgesic compound from Harpagophytum procumbens based on their interactions with COX-2 enzyme. *Asian Pacific Journal of Tropical Disease*, *6*(3), 227-231. https://doi.org/https://doi.org/10.1016/S2222-1808(15)61019-2

Rani, J., Dhull, S. B., Rose, P. K., & Kidwai, M. K. (2024). Drug-induced liver injury and anti-hepatotoxic effect of herbal compounds: a metabolic mechanism perspective. *Phytomedicine*, *122*, 155142.

Sharifee, F., Asadpour, L., Shariati, S., & Salehzadeh, A. (2022). Evaluation of Antibacterial Effect of Aqueous, Hydro-Alcoholic and Alcoholic Extracts of Morus nigra on Gram-Positive and Gram-Negative Bacteria. *Journal of Advances in Environmental Health Research*, *10*(3), 225-234.

Sharma, A., Sharma, L., Nandy, S. K., Payal, N., Yadav, S., Vargas-De-La-Cruz, C., Anwer, M. K., Khan, H., Behl, T., & Bungau, S. G. (2023). Molecular Aspects and Therapeutic Implications of Herbal Compounds Targeting Different Types of Cancer. *Molecules*, *28*(2), 750. https://www.mdpi.com/1420-3049/28/2/750

Shayan, S., Jamaran, S., Askandar, R. H., Rahimi, A., Elahi, A., Farshadfar, C., & Ardalan, N. (2021). The SARS-Cov-2 Proliferation Blocked by a Novel and Potent Main Protease Inhibitor via Computer-aided Drug Design. *Iran J Pharm Res*, *20*(3), 399-418. https://doi.org/10.22037/ijpr.2021.114846.15061

Sherafatizangeneh, M., Farshadfar, C., Mojahed, N., Noorbakhsh, A., & Ardalan, N. (2022). Blockage of the Monoamine Oxidase by a Natural Compound to Overcome Parkinson's Disease via Computational Biology. *Journal of Computational Biophysics and Chemistry*, *21*(03), 373-387. https://doi.org/10.1142/s2737416522500156

Skrovankova, S., Ercisli, S., Ozkan, G., Ilhan, G., Sagbas, H. I., Karatas, N., Jurikova, T., & Mlcek, J. (2022). Diversity of Phytochemical and Antioxidant Characteristics of Black Mulberry (Morus nigra L.) Fruits from Turkey. *Antioxidants*, *11*(7), 1339. https://www.mdpi.com/2076-3921/11/7/1339

Wang, E., Wang, N., Zou, Y., Fahim, M., Zhou, Y., Yang, H., Liu, Y., & Li, H. (2022). Black mulberry (Morus nigra) fruit extract alleviated AD-Like symptoms induced by toxic Aβ protein in transgenic Caenorhabditis elegans via insulin DAF-16 signaling pathway. *Food Research International*, *160*, 111696. https://doi.org/https://doi.org/10.1016/j.foodres.2022.111696

Wang, F., Wei, F., Song, C., Jiang, B., Tian, S., Yi, J., Yu, C., Song, Z., Sun, L., & Bao, Y. (2017). Dodartia orientalis L. essential oil exerts antibacterial activity by mechanisms of disrupting cell structure and resisting biofilm. *Industrial Crops and Products*, *109*, 358-366.

Zhao, M., Bai, J., Bu, X., Tang, Y., Han, W., Li, D., Wang, L., Yang, Y., & Xu, Y. (2021). Microwave-assisted aqueous two-phase extraction of phenolic compounds from Ribes nigrum L. and its antibacterial effect on foodborne pathogens. *Food control*, *119*, 107449.