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ICONST NST 2024

International Conferences on Science and Technology

Natural Science and Technology

September 4-6, 2024 in Durres, ALBANIA

ABSTRACTS
&
PROCEEDINGS BOOK

ICONST NST 2024

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Natural Science and Technology

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Published by

Association of Kutbilge Academicians, Isparta, Turkey
E-Mail: info@kutbilge.org

Publication Date: 20/10/2024
ISBN: 978-625-98911-3-2

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International Conferences on Science and Technology

Natural Science and Technology

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Scientific Committee

ICONST 2024

International Conferences on Science and Technology

Natural Science and Technology

September 4-6, 2024 in Durres, ALBANIA

Dear Readers;

The seventh of ICONST organizations was held in Durres/Albania between September 4-6, 2024 with the theme of '***science for sustainable technology***' again. In recent years, weather changes due to climate change have reached a perceptible level for everyone and have become a major concern. For this reason, scientific studies that transform technological progress into a sustainable one is seen as the only solution for humanity's salvation. Here we ask ourselves "which branch of science is responsible for sustainability?". Sustainability science is an interdisciplinary field of study that covers all basic sciences with social, economic, ecological dimensions. If we consider technology as the practical application of scientific knowledge, the task of scientists under these conditions is to design products that consume less energy, require less raw materials, and last longer.

ICONST organizations organize congresses on sustainability issues of three main fields of study at the same time in order to present different perspectives to scientists. This year, 194 papers from 21 different countries presented by scientists in **ICONST Organizations**.

27 papers from 6 countries (Albania, Italy, Kazakhstan, Kosovo. Poland, and Türkiye) presented in our **International Conference on Natural Science and Technology** organized under ICONST organizations. Turkey is the country with the highest participation with 48%, followed by Kazakhstan with 32%, Poland and Albania with 8%, Kosovo and Italy 4%. Outside of Türkiye participant rate is totally 52%.

As ICONST organizations, we will continue to organize organizations with the value you deserve in order to exchange ideas against the greatest threat facing humanity, to inspire each other and to contribute to science. See you at your future events.

ICONST Organizing Committee

ICONST NST 2024

International Conferences on Science and Technology

Natural Science and Technology

September 4-6, 2024 in Durres, ALBANIA

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Impact of Viscous Dissipation on Cu-Water Nanofluid Including Oxytactic Bacteria

Merve Gurbuz-Caldag*[1], Bengisen Pekmen[1]

Abstract: In this study, the two-dimensional, time independent natural convection flow of Cu-water nanofluid is numerically investigated in the presence of oxytactic bacteria. The effect of viscous dissipation is also taken into account. The dimensionless governing equations are simulated by global radial basis function (RBF) method. The vertical boundaries of the considered unit square cavity are differentially heated and horizontal walls are insulated. The numerical results are visualized in a variation of dimensionless numbers of Rayleigh number ($10^2 \leq Ra \leq 10^4$), bioconvection Rayleigh number ($1 \leq Ra_b \leq 100$), Peclet number ($0.1 \leq Pe \leq 10$), Lewis number ($1 \leq Le \leq 10$) and Eckert number ($10^{-5} \leq Ec \leq 10^{-2}$). The average Nusselt, Sherwood numbers and the density of microorganisms along different walls are also analyzed. The rise in the Eckert number reduces the convective heat transfer but enhances the mass transfer. The same effect is observed with an increase in the bioconvection Rayleigh number and a decrease in the Lewis number for a fixed $Ec=10^{-3}$. The average density of microorganisms at the left wall diminishes as the Peclet number decreases or the Rayleigh number increases. An augmentation in the Lewis number causes the bacteria to move down and form a circulation. However, an increment in the Peclet number leads the movement of the bacteria to be in the reverse direction.

Keywords: Cu-water, nanofluid, bioconvection, oxytactic bacteria, viscous dissipation.

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Investigation of the Conductivity Mechanisms of CdS/$C_{10}H_{10}N_2$ PN type Heterojunction

Ramazan Demir*[1], İsmet Kaya[2]

Abstract: In this research, a PN type heterojunction diode was fabricated using a P-type 4-amino-2-methylquinoline ($C_{10}H_{10}N_2$) on an N-type cadmium sulphide (CdS) film and its conductivity mechanism was studied. First, a thin CdS film was deposited on an ITO substrate by chemical deposition method (CDM). Then, a film $C_{10}H_{10}N_2$ was then deposited on the CdS film using spin coating technique. To investigate the transport mechanism, the ln (I) − ln (V) graph was plotted. The current-voltage characteristics were analyzed using the $I \sim V^m$ relationship, where V represents the voltage and m represents the power index. The m values were calculated for each region. We found m values from the slope of the graph in the first region, within the low voltage range of 0-0.36 volts, was 1.41, while in the second region, voltage 0.37-3.00 volts, it was 4.79 was. From these slope values we concluded that ohmic conduction predominates in the first area, while conduction limited by the trap charge predominates in the second area.

Keywords: CdS, 4-Amino-2-Methylquinoline, $C_{10}H_{10}N_2$, ohmic, trap charge limited.

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The Vıewpoınt of Pre-school Educators on Scıence, the Ways and Techniques They Use and the Issues They Encounter

Marsida Klemo*[1], Azem Hysa[1], Milidin Bakalli[1], Bruno Gorana[2]

Abstract: This study was conducted in the framework of a master's topic in Education Faculty, "Aleksander Moisiu", Durres, University. The purpouse of this study was to find out the views of pre-school educators about the scientific activities they carry out, the ways and techniques they use, and the issues they face while performing scientific activities. The study group consists of 47 pre-school educators who work in the kindergartens of Durrës city. An online questionnaire was used and completed for data collection. From the results achieved in the study it was found that most of the educators consider themselves qualified in educating science but declared that the kindergarten should be equipped and enriched with materials. Also, it was determined that educators try to evolve scientific process abilities in children through scientific activities. It was also been noticed that teachers use laboratory materials as well as common materials when performing these activities. According to the teachers' perspective, children prefer group work as well as individual work. These activities are performed at least once a week. Furthermore, it was highlighted that teachers use technics such as experiment, observation, demonstration more than other technics when conducting scientific activities. In addition, it turned out that the experiment was the most preferred technic by children, which is also easy to understand and implement. The main problem faced by educators during scientific activities was found to be the lack of teaching materials as well as classes filled with too many children.

Keywords: Science activities, pre-school educator, science materials, scientific physical environments

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Preparation and Characterization of Pomegranate Peel Added Magnetic Composite Material by Green Synthesis Method

Emine Belyurt[1], Aslıhan Arslan Kartal*[1]

Abstract: The production and use of biodegradable materials in various fields is important for the sustainability of living life. This study focuses on the preparation of magnetic biodegradable material with agricultural waste additives using green synthesis techniques. The peels of pomegranate fruit harvested in Denizli province were selected as agricultural waste. The basic biopolymer was calcium alginate and the biocomposite was prepared by encapsulating the other materials of the synthesis in these polymer capsules. Synthesis ratios of sodium alginate, nano iron (II,III) oxide and pomegranate powder were respectively: 3: 1/4 : 1. The characterisation of this material was performed by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) and presented. The process of attachment of heavy metals or ions in wastewater to the surface of biological organisms is called biosorption (Akdogan et al. 2023). This method is preferred in removal studies due to its selectivity, economy, applicability to existing methods and environmental friendliness (Lapo et al. 2019). Antimony is frequently used in various industrial products. Due to its toxic and carcinogenic effect, removal and determination of antimony from environmental media is required (Li et al. 2018). In this study, the conditions for the use of the newly prepared magnetic biocomposite in the removal of antimony (III) ion from aqueous solutions are investigated. In the model solution containing 20 ppm antimony (III), 76% removal was achieved with 0.1 g biocomposite. Research is underway to increase the removal rate and the proportion of biomaterial in the material. Studies are ongoing to increase the removal percentage of antimony ion with the biomaterial ratio in the material. Environmentalist techniques being developed for a sustainable life remain up-to-date.

Keywords: Green synthesis, biocomposite material, heavy metal, removal.

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Biologic Features Of Fruit Tree Cultivation In Aktobe Region

Issagali Altynzer

Abstract: Different ways of planting fruit trees in the Aktobe region are considered in this work. Currently, our republic has accumulated a lot of experience in landscaping, a rich assortment of plants has been created, and the addition of fruit trees to their creation enriches the aesthetic appearance of landscaping and shows their impact on the environment. Although horticulture and horticulture are one of the main branches of agriculture, they are of great importance in the consolidation. The relevance of the topic is to determine their biological features when planting fruit trees in the Aktobe region, enriching the environment with oxygen, giving people an aesthetic life, rich in vitamins necessary for human health, decorating with landscape design. The purpose of the study is to consider the biological features of growing fruit trees. In the course of research, apple varieties Golden, Renetka, brought from the Aksai forest of Ural, Western Kazakhstan, Talgarskaya krasavitsa, pear variety Delishes, cherry variety Shpanka were planted through seedlings. Apricot seedlings were grown in two ways. Planted seedlings were taken from the seeds of the first cultivated varieties. This is the main method of growing seedlings for planting apricot orchards. The second way is to grow apricot seedlings from seeds. In the research work, the biological features of the method of propagation of apricots by seeds were described, as well as the rules of their planting as seedlings were analyzed.2-year saplings of apple variety Ranetka, pear variety Talgarskaya krasavitsa, Shpanka variety were planted as seedlings. Here are the results of research on planting fruit tree seedlings in spring or autumn and their analysis. Determined biological conditions during spring and autumn planting of fruit tree seedlings.Considered ways of using effective methods of growing fruit trees in the university garden.

Keywords: fruit trees, spring cultivation of fruit trees, autumn cultivation of fruit trees, methods of cultivation of fruit trees.

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Ways To Increase Students' Interest In The Subject Using The Puzzle Method (Jigsaw Method) In The Biology Lesson

Balnur Makuyeva

Abstract: The relevance of the research work. Due to the daily development of modern educational innovations and the abundance of information waves, the student experiences difficulties with the full distribution of attention. In turn, this leads to a sharp decrease in interest and a deterioration in academic performance. In this question, I focus on the opinions of teachers and parents regarding the child's academic performance, the results of academic performance over the past year and, in addition, the features of memorizing information based on the approach of teaching biology in a traditional format. As an important aspect, I would like to note the emphasis on the fact that the studied data is fully remembered and put into effect, and not only for exams.

Students' participation in non-academic tasks and low motivation to complete school assignments, as well as refusal to study and lack of motivation to attend, as well as poor ability to communicate with teachers in the classroom lead to a low level of involvement in learning and, thus, force them not to engage in materials and perform them effortlessly, which leads to a decrease in at the scientific level, and students accordingly lose concentration in the classroom for reasons related to activity, the lesson constantly occurs at the same pace, as well as poor adaptation to the level of students and stages of development, all this increases the level of attention and coordination and allows them to enjoy those activities that go beyond learning. In dealing with these issues, it is necessary to experiment with modern strategies that increase engagement in the learning process and achievements in order to make the learning environment more favorable for students and help them avoid these fears without fear of cooperation, consistency and fun, biology and its difficulties. Working together and making the student the center of the learning process, for example, the Jigsaw strategy, which can contribute to improving academic performance and engagement in learning, given the above, may bring some benefit in solving the current research problem, which has led to the need to use the jigsaw collaborative learning strategy, in which students have little participation in biology lessons.
The purpose of the research work
Consideration of the Jigsaw method to improve student academic performance and interest in biology lessons.
Objectives of the research work:
1. Literature review based on the Jigsaw method
2. Analysis of the level of dust pollution of city neighborhoods using the Jigsaw method
3. Creation of a map of the dust pollution of the city of Aktobe to familiarize students with the performance of experimental work
Working methods
1) Theoretical (research information was obtained from various literature, a review and analysis of the literature was carried out);
2) Empirical (conducting experimental research);
3) Testing, comparative analysis of statistical data.
Scientific novelty and practical significance
Detecting the toxicity of dust in the air using the Jigsaw method and demonstrating to students the effectiveness of mapping pollution to increase students' interest in learning.

Keywords: jigsaw, biology, pedagogy, students' interest, the puzzle method, etc.

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Proper Nutrition Is A Guarantee Of Health

Orazgazy Balzhan Kurmangazykyzy

Abstract: The main condition for maintaining human health is proper nutrition. Therefore, special attention should be paid to nutrition. It is better to focus on the value of the food composition. Effective, proper nutrition contributes to maintaining health, normal growth and development of the body, increasing work capacity, and strengthening the body's resistance to various negative effects of the environment. Eating allows us to renew our cells and tissues. Proper nutrition is necessary for the prevention of cardiovascular diseases, health-limiting ways, diabetes, osteoporosis, cancer, for improving the functioning of the body, and for an active long life. You can prevent diseases, keep your health and attractive appearance, stay beautiful, slim and active by eating healthy food and high physical activity, doing sports and being active, being able to cope with stress and fighting, giving up smoking and alcohol.

What advice should be taken into account when starting a healthy diet? The main rule is not to stress the body. Because the body suffers from the sudden decrease in calories. Therefore, it is necessary to move to a healthy diet by gradually replacing unhealthy foods with healthy products. Nutritionists think that it is better to eat 4 meals a day. The interval between breakfast, lunch, afternoon meal and dinner should be 4-5 hours. According to the proper nutrition diet, you should eat lunch only at a certain time every day. Breakfast should be 25% of the daily amount, lunch - 50%, afternoon meal - 10%, dinner - 15%, dinner should be eaten 2 hours before bedtime. The number of calories used must compensate for the calories burned. You should not eat immediately after physical exertion, after hard work, or when your emotions are rising. Before meals, you should eat raw fruits and vegetables in the form of a salad or whole. Eating fruits and vegetables on an empty stomach improves the functioning of the digestive glands, gastrointestinal tract, and normalizes the intestinal microflora. Apples, cabbage, carrots, cucumbers, tomatoes, sweet peppers are especially useful.

Rules of healthy eating:
· food should be taken sitting at the table. If you do not pay attention to food, you will not know that you are full and you will overeat.
· you should eat slowly, chew your food carefully and maintain a regular diet. Food should be taken frequently and in small amounts, five times a day.

Currently, due to improper nutrition, overweight and obesity diseases are on the rise. Overeating, strict dieting and eating before bed almost all lead to the destruction of our health. Let's not forget that proper nutrition is a guarantee of health!

Keywords: At the same time, it contributes to the prevention of many diseases 80 percent of our health depends on how we eat.

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Trichoderma sp. as a Biological Control Agent Against *Pythium* sp.

Sultan Akyol[1], Anna Maria Vettraino[2], Refika Ceyda Beram[3]

Abstract: **Abstract**: *Pythium* genus belonging to Oomycetes include some dangerous species that poses a significant threat to both forest and urban tree ecosystems. These species typically infect the underground parts of plants, leading to root and collar rots that destabilize ecosystems. Chemical controls against diseases caused by *Pythium* species ecological concerns, underscoring the need for the development of biological control strategies. The application of biological control is crucial for combating *Pythium* sp. without harming natural ecosystems. This study aims to evaluate the *in vitro* evaluation of the biological control potential of *Trichoderma* sp. against isolate of *Pythium* sp. In the experimental setup, 4 plugs (1*1) of the pathogen were placed on 90 mm petri dishes containing PDA media. These samples were positioned equidistantly from the center and edges of the plates using a cork borer. Then, plug (add diameter) of *Trichoderma* sp. isolate was placed at the exact center of each Petri dish. This process was conducted in triplicate. A control plate was also prepared under identical conditions, using only *Pythium* sp., also in triplicate. All plates were incubated in the dark at 25°C. The growth rates of *Pythium* sp. were measured along the x and y axes on days 1, 3, and 7. The growth differences between the control and biological control groups were compared using the T-test. The results revealed that *Trichoderma* sp. significantly inhibited the growth of *Pythium* ($p<0.05$). This study demonstrates that *Trichoderma* sp. has significant potential as an effective biological control agent against *Pythium* sp. isolates. The findings suggest that *Trichoderma* sp. could be a promising solution for the biological control of *Pythium* sp. in urban environments.

Keywords: *Pythium* sp., *Trichoderma* sp., Biological control, Plant pathogen, T-test analysis

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Implementation of Ecolabels for Hygienic Products: A case study from Poland

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Abstract: Eco-labels have become an important tool for promoting sustainable production and consumption by providing environmental information on products and services. This article examines the effectiveness of eco-labels in supporting the development, production and sale of environmentally friendly products. While eco-labels have the potential to promote sustainable practices and raise consumer awareness, their impact varies across product groups. The study presents an analysis of mandatory and voluntary eco-labelling schemes and the data required to meet environmental targets, with a particular focus on the toilet tissue industry at a selected industry. The findings highlight the importance of integrating eco-labels with broader environmental policies and market strategies to ensure their effectiveness.

Keywords: Eco-labels, environmental targets, mandatory and voluntary schemes, sustainable production and consumption, toilet tissue industry

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Growing And Caring For Indoor Plants

Korkem Baktiyarova

Abstract: Caring for indoor plants is a pleasant activity that can turn into a real hobby. There are several important rules that allow you to keep your home garden in order and beauty. Each flower requires an individual approach: observe the soil and the well-being of the plant for several days. The main rule is to avoid extremes. Excessive watering or drying, excess sunlight or darkness - all this is destructive: flowers do not like extreme conditions, it is always better to stick to the golden mean. Do not forget to remove dried leaves and petals, spray and fertilize the plant.

The most important flower care parameters: temperature; light; humidity; nutrients. Watering, lighting and fertilization regimes may differ depending on the time of year, dormant or flowering periods. It's not for nothing that plants are called "indoor" - they are really comfortable at this temperature. Most flowers develop best at 13-24 degrees, and only exotic varieties require higher temperatures. Some, on the contrary, grow better in the cold. For them, the maximum heat mark is 16 degrees: at higher temperatures, the leaves begin to deteriorate.

Heat-loving plants bloom at temperatures from 16 degrees Celsius. Most flowers can withstand slight deviations from the norm without visible damage. They will be able to survive some period of cold or heat. The main thing is to avoid sudden temperature changes. Only cacti and succulents can cope with unexpected regime changes: in nature, they live in places where it is hot during the day and cool at night. In order for a flower to grow and develop, it is important to provide it with a suitable lighting regime. If this condition is not met, then the synthesis processes slow down, the flower does not receive the necessary nutrients and begins to die. The easiest way to provide access to light is to place the plant near a window. You can also use artificial lighting (at least 12 hours a day). Do not forget about such a parameter as lighting intensity. Some plants require and are drawn to full sunlight, while others grow better in the shade. You can also adjust the brightness of the lighting within the same room. It is enough to move a few steps away from the window and the light intensity will decrease by half. If you go deeper into the room, the light will be reduced by 95%. Having chosen a suitable place for the flower, you will see how it actively grows and blooms.

Keywords: Indoor plants, flowers, temperature changes, artificial lighting

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The Benefits And Harms Of Mushrooms

Madina Mamyrzhan

Abstract:Mushrooms are divided into two parts by wild and cultured cultivation. While the wild species grows independently in the forest, the cultivated species is grown annually on special farms. For example, the wild species include white, birch mushroom, poplar mushroom and Chanterelle. However, not all of this can be eaten. And the number of deaf people grown in special crops includes mushrooms such as suspension and others. Harm from fungus poisoning 95% of poisoning is caused by eating an improperly selected poisonous mushroom. It is best to use it only after you are completely sure that the base is not damaged by wild mushrooms. However, it is advisable to purchase a reliable and safe mushroom in stores. Collecting heavy metals forest mushrooms contain dangerous heavy metals-mercury, arsenic, cadmium and lead. Their presence depends on the type and place of growth of the fungus. It is better not to eat mushrooms that grow in places of general pollution, that is, around metal production plants. According to scientists, the content of mercury in mushrooms growing on unoccupied land has also increased. Therefore, special care should be taken when eating mushrooms.

The reason that can cause allergies is that allergies to fungus are rare in humans. Because it is associated with a common allergy and when the protein contained in the product is similar to the protein causing the allergy. Botulism causes Botulism, a disease caused by food poisoning. Botulism can occur when the oxygen content decreases, for example, when eating canned mushrooms. Therefore, be careful when eating canned mushrooms at home. Scientists recommend choosing canned mushrooms in a package with special holes in the store. And it is even better to store mushrooms in the refrigerator wrapped in paper.

The benefits of mushrooms
Fiber. One cup of roast lamb contains 1.9 grams of fiber, which means that it is 7.6% of the daily value. But the fungus loses to vegetables. Let's compare that one cup of broccoli contains 5.1 grams of fiber, which is 20% of the daily value. The protein of 100 grams of mushrooms contains 4 grams of protein. It's not that much, but in combination with other products, there will be a good result. Mushrooms that are low in fat and cholesterol contain very little fat and cholesterol. Excess sodium with low sodium content obviously leads to cardiovascular diseases, and its main source is table salt. Mushrooms have a unique taste due to their high content of glutamate. If you add mushrooms to the dish, then you can not use salt. Helps to control weight if you replace the red meat consumed daily with mushrooms, it is obvious that the amount of weight is different. Therefore, it is better to eat unusual foods that taste better than mushrooms than to eat meat with a high content of color.

Vitamin D. Vitamin D does not enter the body through foods. And the main source of this vitamin is fungus. Standing in direct sunlight, D accumulates vitamin. For example, the forest chanterelle mushroom is rich in vitamin D. Generally speaking, for the beloved brother of vegetarians who do not eat meat. The mushroom is rich in vitamin B and other vitamins such as selenium, potassium, honey, phosphorus. And some are rich in iron.

Keywords: mushrooms, benefits, harms, vitamin, product,

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Arctic And Alpine Plants

Nuray Kaspiyeva

Abstract: Arctic and alpine plants are essential to the stability and functionality of their ecosystems, demonstrating extraordinary adaptability to extreme environments characterized by low temperatures, brief growing seasons, and nutrient-poor soils. This research explores the physiological and ecological adaptations that enable these plants to survive and thrive under harsh conditions. Arctic plants, found in polar regions, and alpine plants, located in high-altitude areas, face similar environmental challenges but have evolved distinct strategies to address them. Arctic plants cope with extreme cold, permafrost, and intense UV radiation through adaptations such as compact growth forms, small needle-like leaves, thick waxy cuticles, and antifreeze proteins. Alpine plants, on the other hand, utilize growth forms like rosettes or dense mats, waxy coatings, reflective leaf surfaces, and extensive root systems to manage high UV exposure, rocky soils, and fluctuating temperatures. The study highlights how these plants' unique adaptations—ranging from reduced size and herbaceous growth in Arctic regions to deep or fibrous root systems in alpine environments—allow them to conserve heat, reduce water loss, and maximize nutrient uptake. Arctic plants often grow close to the ground to benefit from slightly warmer temperatures, while alpine plants employ strategies to trap heat and protect against frost. Both Arctic and alpine plants also exhibit rapid growth cycles that capitalize on short periods of favorable conditions, with their annual cycles of dormancy, carbohydrate storage, and flowering closely aligned with their harsh climates. Despite their remarkable resilience, Arctic and alpine plants are increasingly threatened by climate change. Rising temperatures, altered precipitation patterns, and shifts in ecological interactions pose significant risks, potentially disrupting growth, reproduction, and distribution patterns. These climate-induced changes can lead to shifts in plant communities and affect the overall stability of their ecosystems. Consequently, proactive conservation strategies are imperative to address these challenges. Effective conservation efforts must involve ongoing research, sustainable land management practices, and public education to ensure the continued survival of these plants and the stability of their ecosystems. Understanding and preserving Arctic and alpine plants is crucial for maintaining biodiversity and ecological balance in the face of a rapidly changing climate.

Keywords: arctic plants, alpine plants ,ecosystems ,adaptability, extreme environments, low temperatures Permafrost

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The Role of Plants in Climate Regulation

Shyryn Akhmetova

Abstract:Plants play a crucial role in regulating the Earth's climate through a series of complex and interdependent processes. Their involvement in carbon sequestration, atmospheric composition regulation, and hydrological cycles is fundamental to maintaining global temperature stability and mitigating climate change. Foremost, plants act as significant carbon sinks, absorbing carbon dioxide (CO_2) from the atmosphere during photosynthesis. This process not only reduces the concentration of greenhouse gases but also stores carbon in biomass and soil, thereby contributing to the long-term regulation of atmospheric CO_2 levels. Additionally, plants influence the albedo effect, where their presence in various ecosystems, such as forests and grasslands, affects the reflection of solar radiation back into space, subsequently influencing global temperatures. Through transpiration, plants release water vapor into the atmosphere, which contributes to cloud formation and precipitation patterns. This mechanism is essential in regulating local and global hydrological cycles, thereby influencing weather patterns and climate. Furthermore, vegetation plays a vital role in maintaining soil integrity, preventing erosion, and promoting water infiltration, which are critical for sustaining the balance of natural ecosystems. In summary, plants are integral to the Earth's climate system, contributing to carbon sequestration, atmospheric regulation, and the maintenance of the hydrological cycle.

In conclusion, the role of plants in climate regulation is indispensable, encompassing multiple processes that sustain the Earth's environmental equilibrium. By sequestering carbon, regulating atmospheric gases, influencing albedo, and managing the hydrological cycle, plants are vital to mitigating climate change and maintaining ecological stability. As global temperatures rise and ecosystems face increasing threats, preserving and restoring plant communities is essential for ensuring the resilience of the Earth's climate system. This underscores the critical need for conservation efforts and sustainable practices that protect and enhance plant biodiversity, which in turn supports the broader goal of climate stabilization.

Keywords: carbon sequestration, photosynthesis, greenhouse gases, hydrological cycles, atmospheric regulation, cloud formation, soil integrity, ecosystems.

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Gas Chromatography-Mass Spectrometry (GC–MS) Analysis of the Essential Oils of Common Daisy Flower (*Bellis perennis*) Obtained By Solvent-Free Microwave-Assisted Extraction Method

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Abstract: The common daisy flower (*Bellis perennis*) is a plant belonging to the Asteraceae family. Although daisy is naturally spread in Europe, it can be found in most parts of the world. This flower can be found up to 1600 meters above sea level and grows in moist areas and forests. In this study, the daisies collected from the Isparta and Aydın regions in Turkiye. Solvent-free microwave-assisted extraction was preferred as the method of oil extraction. Because, compared to other methods, this method is fast, no solvent is used, it is controllable, thermal degradation is prevented and the oils obtained can be analyzed directly by GC-MS without any processing. Daisy flowers were subjected to solvent-free microwave extraction at 800 W power for 45 minutes, and 0.82% oil content was obtained in the flowers of the Isparta region, while 0.74% oil content was obtained in the oils of the Aydın region. The essential oil components of these oils were then analyzed by GC-MS. According to the results obtained, the major compounds in daisy flowers collected from Isparta region were γ-muurolene (26.75%), germacrene-D (9.07%), n-tetracosane (6.24%), t-cadinol (4.78%), trans-α-farnesene (4.27%) and trans-caryophyllene (3.97%), while the major compounds in daisy flowers from Aydın region were germacrene-D (8.44%), n-tetracosane (7.93%), α-linalool (7.88%), β-pinene (6.21%), α-eudesmol (5.69%), trans-α-farnesene (5.19%) and n-heptacosane (5.18%). These findings show that daisy flowers has very different essential oil compounds in Aydın and Isparta regions. Therefore, it was determined that the environment of the plants has a very important effect on their essential oil compounds.

Keywords: *Bellis perennis* flower, Daisy Flower, Essential oils, Microwave distillation, GC/MS Analysis.

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1. INTRODUCTION

Bellis perennis (Figure 1) is a common European species of daisy belonging to the Asteraceae family. Although it is also known as "English daisy", its common names include "meadow daisy" or "common meadow daisy ". It is also known as "koyungözü" among the people (Güner et al., 2012; Lüder, 2017). The word "Bellis", the genus name of the plant, originates from the Latin word "bellus" meaning "beautiful", and the word "perennis" means "eternal" in Latin and is considered as the flower of innocence (Güner et al., 2012). It is native to Europe and Western Asia and was introduced to North and South America (Brouillet, 2006). It is a perennial, usually rosette herbaceous plant. Leaves spoon-like, obtuse or broadly acuminate at the apex, abruptly narrowing at the base of the relatively winged petiole, entire or shortly toothed on each side near the apex, sparsely pubescent on all surfaces. Capitulum 10-25 cm on leafless stem. Involucre 0.5-1 cm wide; phyllaries ovate-lanceolate, blades 5-7 mm, tubular flowers 1.5-2 mm. Flowering time is between March and August. Habitat: Moist places at 0-2000 m altitude, often in forests (Davis, 1978).

Figure 1. *Bellis perennis*

Bellis perennis is used against eczema, eye diseases, stomach pain, tonsillitis, colds, skin boils, as a diuretic, diaphoretic and laxative (Yazıcıoğlu and Tuzlacı, 1995; Avato et al., 1997; Gudej and Nazaruk, 1997; Genç and Özhatay, 2006). In addition to the widespread use of *Bellis perennis* in traditional medicine, especially in recent years, researches have been carried out according to the biological activities of *Bellis perennis* and possible treatment pathways have been opened for diseases such as cancer or hyperlipidaemia (Karakaş et al., 2012).

Bellis perennis contains many metabolites including terpenes, polyphenols, various anthocyanins, flavonoids, essential oil, saponins and tannins (Al-Snafi, 2015). In this study, *Bellis perennis* samples were collected in Isparta and Aydın provinces of Turkey. The essential oil constituents contained in the samples were compared. Solvent-free microwave-assisted extraction was preferred as an oil extraction method. Because compared to other methods, this method is fast, solvent-free, controllable, thermal degradation is prevented and the obtained oils can be analysed directly by gas chromatography-mass spectrometry (GC-MS) without any processing (Figure 2).

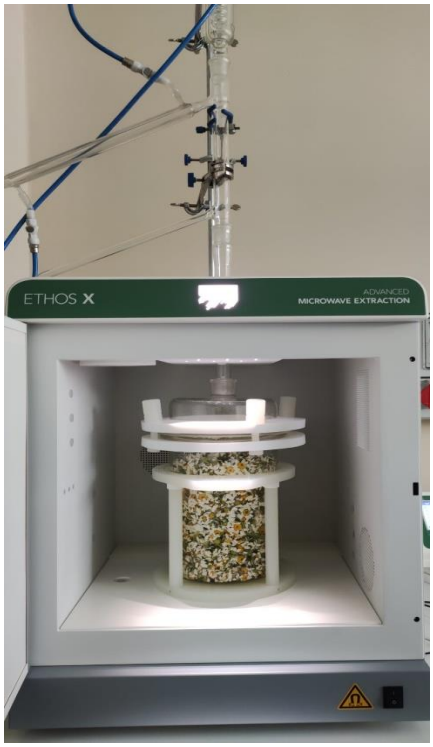

Figure 2. Solvent-free microwave assisted extraction instrument

2. MATERIAL AND METHOD

2.1. Plant material

Bellis perennis was collected during the flowering period in the central district of Isparta province (A) at an altitude of approximately 1050 m and in the central district of Aydın province (B) at an altitude of approximately 60 m in Turkey (Figure 3). In order not to lose the essential oil components of the plant, the collected flowers were separated from the stems and placed in vacuum bags. Afterwards, they were taken directly into the microwave-assisted extraction instrument without being treated with any solvent.

Figure 3. Geographical representation of collected plants A: Isparta region; B: Aydın region

2.2. Solvent-free microwave-assisted extraction of the essential oils

The samples in vacuum bags were weighed 1500 g without treatment with any solvent and placed directly into the sample container of the ETHOS X Advanced Microvawe Extraction instrument. Daisy flowers were subjected to solvent-free microwave-assisted extraction for 45 minutes at 800 W power. The oils obtained as a result of extraction were numbered and analysed for essential oil components by GC-MS.

2.3. GC–MS Analysis

Analysis of the essential oil compounds in *Bellis perennis* oil samples was performed using a GC-MS instrument. 50 µL of the obtained the essential oil sample was taken and diluted with 10 mL of n-hexane solvent. After the mixture was homogenized by vortexing for 3 minutes, a 1/40 dilution was made with acetone in a 2 mL volumetric flask and injected into the GC-MS instrument.

Analyses were carried out with Thermo Scientific Trace 1300 GC gas chromatograph instrument, Thermo Scientific-ISQ7000 single quadrupole mass spectrometer detector (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) system. Chromatographic evaluations were made using Xcalibur software. TraceGOLD TG-624SilMS GC (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) column was used as the analytical column for chromatographic separation. The inlet temperature of the instrument was 250 °C. The injection volume was 2 µL. 1/5 split ratio was used. Helium gas was used as the carrier gas and the gas flow was 1.5 mL/min. The oven temperature was programmed from 35 °C (2 min.) to 100 °C at a rate of 2 °C /min., then from 100 °C (1 min.) to 250 °C at a rate of 5 °C/min. The detector temperature was 280 °C.

2.4. Identification of Compounds

Essential oils compounds were identified by computer search using their mass spectra either with known components (Adams, 1989), or by comparison of received chemical substances mass-spectrum which are in essential oils composition and according to mass-spectrum library (Wiley, 2007).

3. RESULTS

3.1. Obtaining oils after extraction process

Oils were obtained each from 1500 g flower after solvent-free microwave-assisted extraction. Approximately 12.3 g of oil was obtained from flowers in Isparta region, while approximately 11.1 g of oil was obtained from flowers in Aydın region. According to these values, oil ratios were found to be 0.82% and 0.74%, respectively.

3.2. GC–MS analysis chromatograms

The comparative GC-MS analysis chromatogram of plants from Isparta and Aydın regions is shown in Figure 4. When the chromatograms are analysed, it is seen that there are significant differences in the relative abundance values of the peaks. The relative abundance values of the peaks are higher especially in the oils obtained from the flowers of Aydın region than the oils obtained from the flowers of Isparta region.

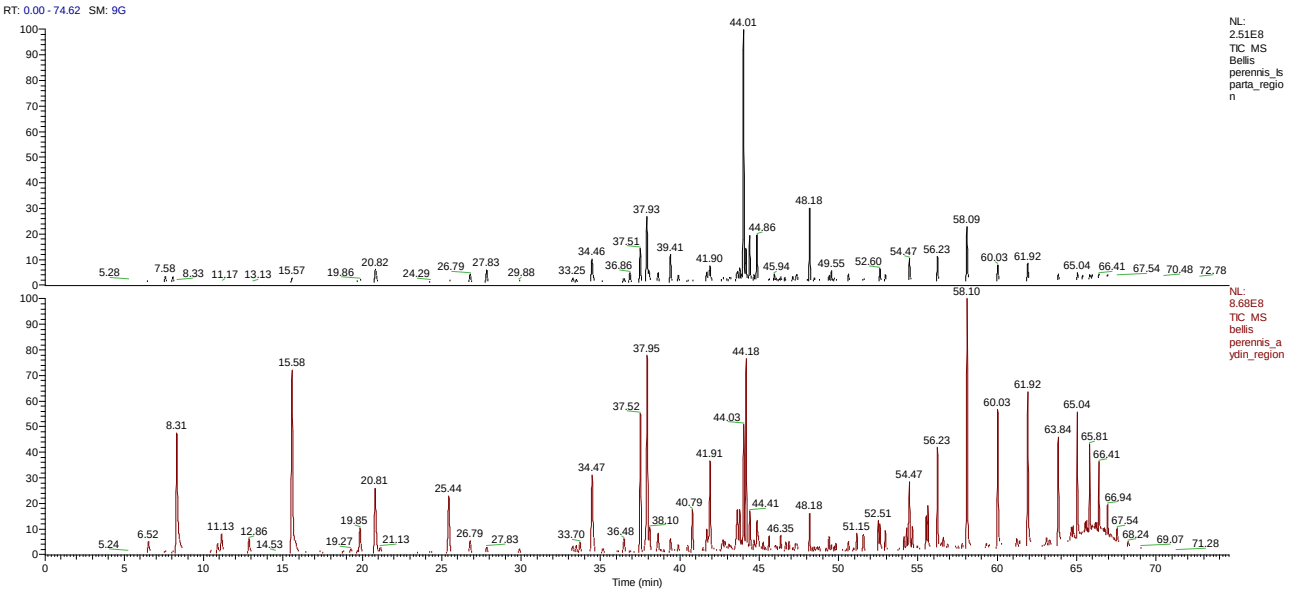

Figure 4. The chromatography at the above belongs to the Isparta region and at the bottom belongs to the Aydın region.

These peaks in the chromatograms were identified by library matching in the GC-MS instrument and the essential oil compounds of the oils are given in Table 1. According to the number of peaks in the chromatograms, a total of 40 essential oil compounds belonging to the oil obtained from the flowers of Isparta region were identified, while a total of 45 essential oil compounds belonging to the oil obtained from the flowers of Aydın region were identified. According to the results obtained, the major compounds in daisy flowers collected from Isparta region were γ-muurolene (26.75%), germacrene-D (9.07%), n-tetracosane (6.24%), t-cadinol (4.78%), trans-α-farnesene (4.27%) and trans-caryophyllene (3.97%). 97%), while the major compounds in daisy flowers from Aydın region were germacrene-D (8.44%), n-tetracosane (7.93%), α-linalool (7.88%), β-pinene (6.21%), α-eudesmol (5.69%), trans-α-farnesene (5.19%) and n-heptacosane (5.18%).

Table 1. The essential oil compositions of B. perennis samples

No	Compound	Molecular Formula	CAS No	A (%)	B (%)	Identified Methods
1	n-Octanol	$C_8H_{18}O$	111-87-5	tr	0.05	GC-MS
2	p-Cymene	$C_{10}H_{14}$	99-87-6	tr	0.32	GC-MS
3	α-Pinene	$C_{10}H_{16}$	80-56-8	tr	0.45	GC-MS
4	β-Pinene	$C_{10}H_{16}$	127-91-3	tr	6.21	GC-MS
5	α-Terpinene	$C_{10}H_{16}$	99-86-5	0.04	0.10	GC-MS
6	ç-Terpinene	$C_{10}H_{16}$	99-85-4	tr	0.61	GC-MS
7	trans-Anethole	$C_{10}H_{12}O$	4180-23-8	1.27	0.58	GC-MS
8	Pinocarvone	$C_{10}H_{14}O$	30460-92-5	-	0.13	GC-MS
9	Myrtenol	$C_{10}H_{16}O$	515-00-4	0.10	0.25	GC-MS
10	trans-Pinocarveol	$C_{10}H_{16}O$	547-61-5	tr	0.12	GC-MS
11	Camphor	$C_{10}H_{16}O$	76-22-2	tr	0.11	GC-MS
12	1,8-Cineole (Eucalyptol)	$C_{10}H_{18}O$	470-82-6	tr	0.76	GC-MS
13	3,3,6-Trimethyl-1,4-heptadien-6-ol	$C_{10}H_{18}O$	26127-98-0	0.04	tr	GC-MS
14	Linalool	$C_{10}H_{18}O$	78-70-6	0.62	7.88	GC-MS
15	(-)-Borneol	$C_{10}H_{18}O$	464-43-7	0.11	tr	GC-MS
16	Lavandulol	$C_{10}H_{18}O$	498-16-8	0.32	0.09	GC-MS
17	4-Terpineol	$C_{10}H_{18}O$	562-74-3	0.55	1.13	GC-MS
18	α-Terpineol	$C_{10}H_{18}O$	98-55-5	2.45	3.52	GC-MS

19	Myrtenol	$C_{10}H_{18}O$	515-00-4	0.10	tr	GC-MS
20	Grandisol	$C_{10}H_{18}O$	26532-22-9	0.16	tr	GC-MS
21	cis-Jasmone	$C_{11}H_{16}O$	488-10-8	tr	0.50	GC-MS
22	cis-3-Hexenyl-2-methyl butanoate	$C_{11}H_{20}O_2$	10094-41-4	0.29	tr	GC-MS
23	Chrysanthenyl acetate	$C_{12}H_{18}O_2$	54324-99-1	0.48	2.76	GC-MS
24	Lavandulyl acetate	$C_{12}H_{20}O_2$	25905-14-0	1.81	0.29	GC-MS
25	Neryl acetate	$C_{12}H_{20}O_2$	141-12-8	0.33	0.30	GC-MS
26	ar-Curcumene	$C_{15}H_{22}$	4176-06-1	0.38	0.72	GC-MS
27	Bicycloelemene	$C_{15}H_{24}$	32531-56-9	0.37	0.21	GC-MS
28	(−)-α-Cedrene	$C_{15}H_{24}$	469-61-4	tr	0.10	GC-MS
29	α-Elemene	$C_{15}H_{24}$	5951-67-7	0.67	0.35	GC-MS
30	trans-Caryophyllene	$C_{15}H_{24}$	87-44-5	3.97	3.73	GC-MS
31	α-Humulene	$C_{15}H_{24}$	6753-98-6	0.80	0.71	GC-MS
32	Aromadendrene	$C_{15}H_{24}$	25246-27-9	1.44	0.13	GC-MS
33	trans-α-Farnesene	$C_{15}H_{24}$	502-61-4	4.27	5.19	GC-MS
34	Germacrene-D	$C_{15}H_{24}$	23986-74-5	9.07	8.44	GC-MS
35	ç-Elemene	$C_{15}H_{24}$	674819-49-9	1.13	tr	GC-MS
36	Bicyclogermacrene	$C_{15}H_{24}$	24703-35-3	1.25	0.77	GC-MS
37	α-Amorphene	$C_{15}H_{24}$	483-75-0	3.36	0.52	GC-MS
38	α-Selinene	$C_{15}H_{24}$	473-13-2	tr	0.86	GC-MS
39	(+)-delta-Cadinene	$C_{15}H_{24}$	483-76-1	1.05	tr	GC-MS
40	γ-Muurolene	$C_{15}H_{24}$	30021-74-0	26.75	3.84	GC-MS
41	Caryophyllene oxide	$C_{15}H_{24}O$	1139-30-6	2.86	3.71	GC-MS
42	Cedr-8-en-15-ol	$C_{15}H_{24}O$	21441-72-5	1.10	1.10	GC-MS
43	Cubenol	$C_{15}H_{26}O$	21284-22-0	1.51	1.77	GC-MS
44	α-Eudesmol	$C_{15}H_{26}O$	473-16-5	3.20	5.69	GC-MS
45	t-Cadinol	$C_{15}H_{26}O$	5937-11-1	4.78	1.27	GC-MS
46	n-Heneicosane	$C_{21}H_{44}$	629-94-7	2.39	2.43	GC-MS
47	n-Docosane	$C_{22}H_{46}$	629-97-0	2.43	2.70	GC-MS
48	n-Tetracosane	$C_{24}H_{50}$	646-31-1	6.24	7.93	GC-MS
49	n-Hexacosane	$C_{26}H_{54}$	630-01-3	1.98	4.39	GC-MS
50	n-Heptacosane	$C_{27}H_{56}$	593-49-7	2.17	5.18	GC-MS
51	n-Octacosane	$C_{28}H_{58}$	630-02-4	0.87	3.48	GC-MS
52	n-Nonacosane	$C_{29}H_{60}$	630-03-5	0.70	3.00	GC-MS
	Others			6.65	5.63	GC-MS
	Identified components			40	45	

tr: trace (< 0.1), A: Oil obtained from *Bellis perennis* flowers in the Isparta region, B: Oil obtained from *Bellis perennis* flowers in the Aydın region

4. DISCUSSION AND CONCLUSIONS

Bellis perennis plants were collected at an altitude of approximately 1050 m in the central district of Isparta province and at an altitude of approximately 60 m in the central district of Aydın province during the flowering period. All analytical studies were carried out at Suleyman Demirel University Natural Products and Research Center (SUDUM). Solvent-free microwave-assisted extraction was used in the oil extraction of the collected plants, especially because it is fast, solvent-free, controllable, preventable against thermal degradation and the extracts obtained can be analysed directly. It was determined that the flowers in Isparta region had 0.82% oil content and the flowers in Aydın region had 0.74% oil content. The oils obtained after extraction were analysed by GC-MS. In the chromatogram results obtained, it was determined that the major peak and relative abundance values of the oils were significantly different from each other. According to the findings obtained, it is thought that these changes may be caused by the characteristics of the regions where the plants are located, weather conditions in the season of collection, soil type. Further researches can be carried out based on the results of this study.

Acknowledgements

N/A

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: T.E.; Investigation: O.B.; Material and Methodology: O.B., T.E.; Supervision: O.B., T.E.; Visualization: O.B.; Writing-Original Draft: O.B., T.E.; Writing-review & Editing: O.B., T.E.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Adams, R.P. (1989). Identification of essential oils by ion trap mass spectroscopy. Academic Press, New York, USA.

Al-Snafi, A. (2015). The Pharmacological importance of *Bellis perennis*-A review. International Journal of Phytotherapy, 5, 63-69.

Avato, P., Vitali, C., Mongelli, P., Tava, A. (1997). Antimicrobial activity of polyacetylenes from *Bellis perennis* and their synthetic derivatives. Planta Medica, 63, 503-507.

Brouillet, L. (2006). *Bellis perennis*. In Flora of North America North of Mexico, 1st ed. Flora of North America Editorial Committee, Flora of North America Association, New York, USA, 20, 2006, 23.

Davis, P.H. (1978). Flora of Turkey and East Aegean Islands. Vol. 6. Edinburgh. University of Edinburgh Press, Edinburgh, 128-135.

Genç, E.G., Özhatay, N. (2006). An Ethnobotanical Study in Çatalca (European part of Istanbul) II. Turkish Journal of Pharmaceutical Scienes, 3, 73-89.

Gudej, J., Nazaruk, J. (1997). Apigenin glycosidoesters from flowers of *Bellis perennis* L.. Acta Poloniae Pharmaceutica, 54, 233-235.

Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T. (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, s. 121. ISBN: 978-605-60425-7-7.

Karakas, F.P., Şöhretoğlu, D., Liptaj, T., Štujber, M., Ucar Turker, A., Marák, J., Çalış I., Yalçın, F.N. (2014). Isolation of an oleanane-type saponin active from *Bellis perennis* through antitumor bioassay-guided procedures. Pharm. Biol., 52, 951–955.

Lüder, R. (2017). Grundkurs Pflanzenbestimmung: Eine Praxisanleitung für Anfänger und Fortgeschrittene. 8th Ed.; Quelle & Meyer: Wiebelsheim, Germany.

Wiley, (2007). Registry, 8th ed. with NIST 05 MS Spectra, Revision 2005 D.06.00. s.l. Agilent Technologies.

Yazıcıoğlu, A., Tuzlacı, E. (1995). Folk Medicinal Plants of Trabzon (Turkey). Marmara Üniversitesi Eczacılık Dergisi, 11, 333-342.

Omparıson Of The Physıco-Chemıcal Properties Of Vınegar, Whıch Is Tradıtıonally Produced From Wıld Apples (*Malus sylvestris* L.) In Prızren, Wıth Vınegar Sold In The Market

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Abstract: Vinegar is a plant product consumed all over the world and ranked among food products. This study aims to determine the physico-chemical properties of naturally produced vinegars and industrially prepared vinegars in the Prizren region of Kosovo. Vinegar samples: Industrially produced vinegars in Prizren were obtained from the market, while naturally produced vinegars at home were obtained from certain producers. Vinegar samples were analyzed in three samples: industrial, natural and household vinegar. pH, acidity, brix, TDS, electrical conductivity, turbidity test with turbidimeter and spectrophotometric analysis were performed for the obtained vinegar samples. As a result of the analysis, it was found that the pH values of the Natural and industrial vinegar samples were 2.2 and 2.12, and the pH value of the household vinegar was 3.33. After that, it was found that the acidity values were consistent with the pH values. The acidity value of natural and industrial vinegar samples was found to be 6.3 to 7.6, and the acidity of household vinegar is 4.6. The electrical conductivity values of Natural and industrial vinegar samples were 3431 and 1961, and the electrical conductivity of household vinegar was 894. TDS values The TDS values of natural and industrial vinegar samples were 1721 and 978, and the TDS value of household vinegar was 69. Brix Values: The Brix scale of natural and household vinegar samples was 0, and the Brix scale of industrial vinegar was determined to be 3.8. The degrees of turbidity were measured with a turbidimeter and the turbidity value of the samples of Natural and domestic vinegar was found to be the highest (13.3 and 20.6, while the turbidity of the industrial vinegar sample was 0.26. As a result of the study, it was confirmed that the vinegar is an important food ingredient for human health Prizren data on the general chemical structure of household and industrial vinegars produced in the region were discovered..

Keywords: Vinegar, wild apple, physico-chemical analysis.

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1. Introduction

Human interaction with their environment continues, and the exploration of its resources is still ongoing. It is very difficult to determine the right time for the first use of traditional medicinal plants, a common practice in rural communities. According to the World Health Organization, approximately 80% of the world's population still use medicinal plants as the first line of health care for their ailments (Ashworth and Cloatre, 2022).

Vinegar is a liquid composed mainly of acetic acid and water. It is usually produced through the fermentation of ethanol by acetic acid bacteria. Vinegar has been used for thousands of years as a condiment, preservative and cleaning agent. Apple cider vinegar is very healthy. Vinegar is a popular fermented food that has been used since ancient times in both the East and the West. Vinegars are widely useful for medicinal and cosmetic purposes. They have an alkaline characteristic that breaks down lactic acid in body tissues and helps relieve fatigue, and the sour flavor stimulates appetite, digestion and absorption. In addition, vinegar promotes the secretion of sodium and silicic acid and is known to be effective in preventing or treating fever, swelling, stomach pain, and metabolic complications, such as high blood pressure, atherosclerosis, insulin resistance, and hyperlipidemia (Budak et al. al., 2014).

The quality of vinegar depends on fermentation, production methods, raw materials and additives used. In addition, the content of acetic acid, the smell component and the composition of organic acid and free amino acids affect the quality of vinegar. Vinegars undergo oxidation after they are opened, and the oxidation processes trigger a series of chemical and enzymatic reactions that change the vinegar.

This study is devoted to different aspects of the physico-chemical analysis and their comparison between traditionally produced and commercially produced wild apple vinegar.

2. Material and Method

The approach to this research is prospective quantitative, making the statistical analysis of data from the results obtained in the laboratory and their generation in numerical values.

This research is mainly based on primary and secondary data.

1. The primary data were obtained through the use of electronic libraries of different universities, studies of our country and foreign ones regarding the physico-chemical properties of vinegar.

2. While the secondary data is based on the samples obtained from traditional vinegar and that which is found in the markets in our country.

2.1. Analyzing tests such as: pH, acidity, smell, appearance, density, sensory evaluation, volatile components, it is intended to perform sensory analysis which does not require any tools and equipment, etc. Samples will be analyzed in triplicate.

2.2. Preparation of solutions from 10 to 60 mg/L from the base solution with the same solvent.
The analyzes were carried out in the laboratories of the UBT innovation center.

Figure 1. Types of vinegar (natural, domestic-household and industrial vinegar)

2.1. Determination of pH

The pH measurement was done with a pH meter from the Ariko digital company. Measurements in the pH meter were made before and after the addition of the enzyme solutions to the liquid, preceding the calibration with a buffer in the environment with pH 4.00 provided by the Fluka company.

2.2. Determination of Total Acidity

The determination of total acidity or titratable acidity is done by the titrimetric method. NaOH solution with a concentration of 0.5 mol/dm3 (0.5 N), known as standard solution or titrant, is placed in the burette. While in the Erlenmeyer, a precisely measured volume of the solution is placed that is analyzed in the presence of the phenolphthalein indicator. With this, a chemical reaction known as a titration reaction is carried out between the solution being analyzed and the standard solution. Dilute 20 ml of vinegar sample with approximately 25 ml of clean water. Titrate with 0.5 N NaOH until pink color using phenolphthalein indicator. The amount of acetic acid in the sample is calculated as g/L.

$$M_{CH3COOH}V_{CH3COOH} = M_{NaOH}V_{NaOH} \quad \Rightarrow \quad M_{CH3COOH} = (M_{NaOH}V_{NaOH})/ V_{CH3COOH}$$

2.3. Determination of Turbidity With a Turbidimeter

The determination of turbidity was recorded on the device 2100AN IS TURBIDIMETER from the manufacturer Hach. The turbidity was determined in the turbidimeter device, the analysis was carried out before the application of the enzyme solutions to the apple juice and after the apple juices were treated with the enzyme solutions at different concentrations and intervals of time and temperature.

2.4. Determination of Apple Juice Solutions With a Spectrophotometer

The spectra of the vinegars were read for a certain time in the range of 190 to 700 nm. The goal is to determine which wavelengths give the most peaks.

2.5. Determining the Capacity

Determination of specificity was analyzed with Ariko digital equipment. The purpose of conducting conductivity analyzes and their effects on the quality parameters of vinegar samples are examined.

2.6. Determination of TDS

TDS refers to the total amount of dissolved substances in water. The concentration of TDS in water is measured in parts per million (ppm) or milligrams per liter (mg/l). A high TDS indicates that there are more dissolved substances in the water, while a low TDS indicates a lower number of dissolved substances. Dissolved substances in water can be minerals, organic compounds, inorganic compounds and salts. Common ions that lead to increased TDS levels in water include magnesium, calcium, sodium, and potassium, among others.

2.7. Determination of the ˚BRIX scale

The determination of the °Brix degree in the apple juice samples was done with a refractometer of the "ISO LAB Laborgerate GmbH" type. The determination of °Brix was done before at room temperature three times.

3. Results and discussion

Three vinegar samples were taken for the master thesis: home-made vinegar, industrially produced vinegar in the market and naturally produced vinegar. The studies were carried out in the UBT laboratory in Prizren. Assays were repeated 3 times and the mean was added to the results.

Figure 2. Types of vinegars used

By means of the titration method, titrating acidity, also known as general acidity, was also determined. The analysis was applied to each sample separately and was performed three times for each one of them.

$$M_{CH3COOH}V_{CH3COOH} = M_{NaOH}V_{NaOH} \quad \Rightarrow \quad M_{CH3COOH} = (M_{NaOH}V_{NaOH})/ V_{CH3COOH}$$

Determination of turbidity was done using the turbidimeter device in the vinegar samples. With the refractometric method, the dry matter was also determined. The refractometric method is based on the relationship that exists between the concentration of the dissolved substance and the light refraction coefficient. The sample to be analyzed is mixed well to make it as homogeneous as possible, and if necessary, the sample is also filtered. A few drops of the liquid are placed on the prism of the refractometer (checked in advance, with distilled water) and the reading is taken. If the measurement is performed at a temperature other than 20 °C, the correction is made according to the information in the literature. Analyzes were performed on vinegar samples.

Table 1. Results of all performed analyses

	NATURAL	INDUSTRIAL	HOUSEHOLD
pH	2,2	2,12	3,33
Acidity (g/L)	40,5	49,5	37,5
BRIKS	0	3,8	0
NTU	13,3	0,26	20,6
EQ (ms/cm)	3431	1961	894
TDS	1721	978	69

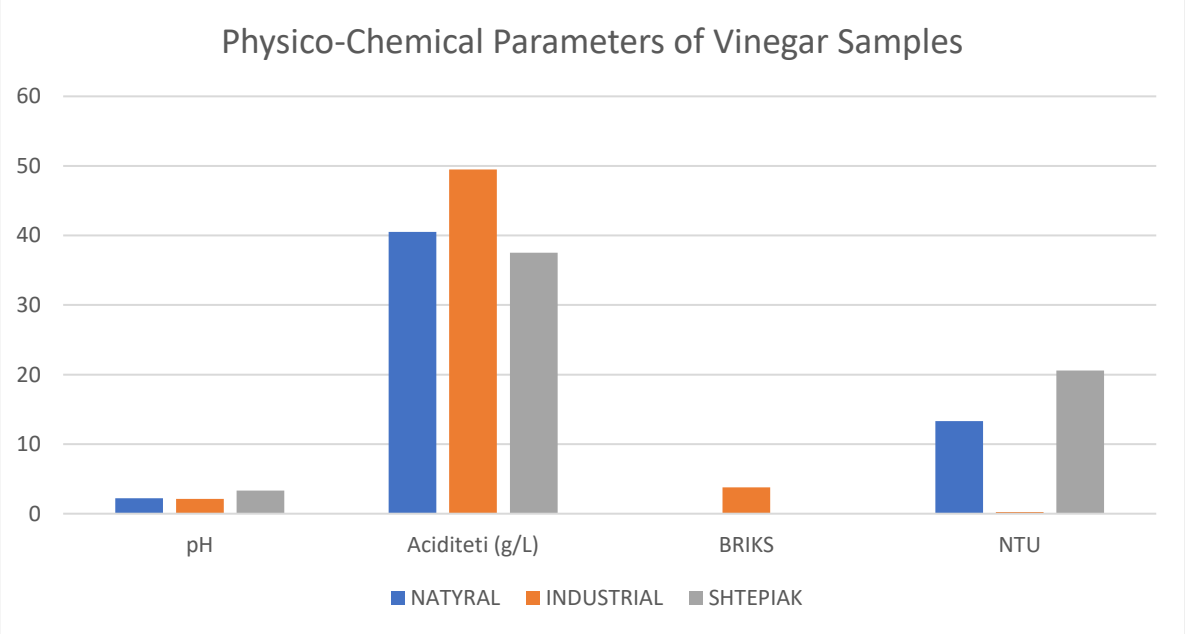

Figure 3. Physico-Chemical Parameters of Vinegar Samples

In this study, the physico-chemical analyzes of the natural domestic vinegars produced in Prizren and the industrial vinegars sold were examined. The examined vinegars were tested in various aspects to see if they comply with the TSE 1880 EN 13188 vinegar standard and if they are contrary to it. To summarize, according to the data obtained, differences were detected in the physico-chemical analyzes of the vinegars. In the TS 1880 EN 13188 standard, the total acid content in the vinegar composition (in terms of free acetic acid in water) must not be less than 40 g/L. No value is stated in the standard for pH. As a result of the measurements, the total acidity was determined between 37.5 and 49.5. The pH value was found to be in the range of 2.12-3.33. During the fermentation stage in the production of vinegar, the sugar is reduced and first converted into ethyl alcohol and then into acetic acid. It has been reported that acetic acid, which is formed by fermentation of acetic acid, in addition to the natural acid in apples, increases the amount of acidity in the environment. Gerbi et al., (1998) in their research with 65 types of vinegar samples, reported that the total acidity of wine and apple vinegars ranged between 54-66 g/L. The pH values for the vinegar samples ranged between 2.63 and 3.27. Gerbi et al. (1998) reported that the pH value of 65 types of vinegar samples varied between 2.36-3.0. It can be seen that our work is in accordance with the standards and is similar to scientific studies.

According to the acidity of the vinegars analyzed in this study, it can be seen that the results vary from 37.5 g/L to household vinegar, 40.5 g/L to natural vinegar and 49.5 g/L to industrial vinegar. In a study by Dini et al. (2020) developed at the University of California, acidity levels of natural vinegars ranged from 20 g/L to 50 g/L, while for household vinegars 5-10 g/L. By comparing the results of our study and data published in other research, it appears that the high levels of vinegars used in the food processing industry make a significant difference to those of natural and household vinegars.

Regarding the TDS (Total Dissolved Solids) of the vinegar samples in our study, there are differences where in natural vinegar it was 1721, in industrial vinegar 978, while in domestic vinegar it was 69. These results are similar to other studies. The study conducted by Dini et al. (2020) at the University of California, recorded different levels of TDS for natural vinegars 500 mg/L - 3000 mg/L, for vinegars used in the food industry 300-700 mg/L. and household vinegars that typically have lower TDS levels, around 50-200 mg/L. Comparing TDS results from the Kosovo study with data from other publications shows different attitudes in TDS levels for natural, industrial and household vinegars. These differences can affect the quality and use of vinegars in different fields such as health, gastronomy, or the chemical and food industries. Regarding the Brix analysis (sugar content) in our study, they were found only in industrial vinegar (3.8), while in natural and household vinegar the values were 0. From these data it can be concluded that industrial vinegar usually has an increased sugar content, as a result of processing and adding ingredients to improve taste and consistency while natural and household vinegars tend to have low or zero sugar values, being more authentic and less processed. In comparison to other studies, similar values are seen, for example, a study in China by Li and Wang (2017) results showed that industrial vinegars had a high sugar content (between 3-7% Brix), while traditional vinegars had a low content of sugar or zero. Another study in the USA by Johnson and Nichols (2020) showed that commercial vinegars, especially sweetened and flavored ones, had significant sugar content (about 5-10% Brix), while simple household or natural vinegars did not contained added sugar and had very low or zero Brix values.

Acknowledgements
Thanks to the University of Business and Technology, Pristina, Kosovo, for providing the necessary research facilities.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

4. References

Ashchyan, H., Jen, M., Elenitsas, R., & Rubin, A. I. (2018). Surreptitious apple cider vinegar treatment of a melanocytic nevus: newly described histologic features. Journal of Cutaneous Pathology, 45(4), 307-309.

Atik, D., Atik, C., & Karatepe, C. (2016). The effect of external apple vinegar application on varicosity symptoms, pain, and social appearance anxiety: a randomized controlled trial. Evidence-Based Complementary and Alternative Medicine, 2016, 6473678,

Bourgeois, J. & Barja, F. (2009). The history of vinegar and of its acetification systems. Archives des Sciences. 62. 147-160.

Budak, N. H., Aykin, E., Seydim, A. C., Greene, A. K., & Guzel-Seydim, Z. B. (2014). Functional properties of vinegar. Journal of Food Science, 79(5), R757-R764.

Bray, G. A. 2014. Medical treatment of obesity: The past, the present and the future. Best Practice and Research Clinical Gastroenterology 28(4): 665-684

Briggs M. “Alles Essig” 2007, München, Germany

Casale, M., Sáiz Abajo, M.-J., González Sáiz, J.-M., Pizarro, C., & Forina, M. (2006). Study of the aging and oxidation processes of vinegar samples from different origins during storage by near-infrared spectroscopy. Analytica Chimica Acta, 557(1-2), 360-366.

Cusano, E.; Cagliani, L.R.; Consonni, R.; Simonato, B.; Zapparoli, G. NMR-based metabolic profiling of different yeast fermented apple juices. LWT 2020, 118

Chen, F., Li, L., Qu, J., & Chen, C. (2009). Cereal vinegars made by solid-state fermentation in China (pp. 243-259). Milano

Chen, H., Chen, T., Giudici, P. and Chen, F. 2016. Vinegar functions on health: Constituents, sources, and formation mechanisms. Comprehensive Reviews in Food Science and Food Safety 15(6): 1124-1138

,Dini, I.; Di Lorenzo, R.; Senatore, A.; Coppola, D.; Laneri, S. Validation of Rapid Enzymatic Quantification of Acetic Acid in Vinegar on Automated Spectrophotometric System. *Foods* 2020, *9*, 761. https://doi.org/10.3390/foods9060761

Food and Agriculture Organization. FAOSTAT. Available online: http://www.fao.org/faostat/en/#data/QL
Gerbi , V., Zeppa, G., Beltramo, R., Carnacini, A., Antonell, A. (1998). Characteriza- tion of white vinegars of different sources with artificial neural networks. Journal of the Science of Food and Agriculture, 78, 415-425.

Grierson, B. 2009. Malt and distilled malt vinegar Vinegars of the World, p. 135-143. Milano

Hickey Richard J, Vaughn Reese H. 1954. Industrial fermentation: Chapter 17-acetic acid (vinegar). Vol 1. New York: Chemical Publishing Co., Inc. p 498-535

Ishak, I., George, P., Ibrahim, F. W., Yahya, H. M., & Fara, N. (2018). Acute modulatory effects of apple cider vinegar, garlic, ginger, lemon and honey mixture, with and without exercise on postprandial glycemia in non-diabetic females. Journal of Health Sciences, 16, 105-111.

Isham, N. K. M., Mokhtar, N., Fazry, S. and Lim, S. J. 2019. The development of an alternative fermentation model system for vinegar production. Lebensmittel Wissenschaft Technologie 100: 322-327

Jeong, E. J., & Cha, Y. J. (2016). Development of an onion vinegar beverage containing Yuza (Citrus junos Siebex Tanaka) and its biological activity. Journal of Life Science, 26(5), 563-570

Kim, B. E., Turski, M. L., Nose, Y., Casad, M., Rockman, H. A., & Thiele, D. J. (2010a). Cardiac copper deficiency activates a systemic signaling mechanism that communicates with the copper acquisition and storage organs. Cell Metabolism, 11(5), 353-363

Kılıçgün H,; Yangilar, F.; 2023, Total Polyphenolic, Flavonoid Contents and Antioxidant Capacity of Household Kara Sakı Apple Vinegar Produced by Traditional Method Using Different Yeast Materials

Lee, S. M., Choi, Y. M., Kim, Y. W., Kim, D. J., & Lee, J. S. (2009). Antioxidant activity of vinegars commercially available in Korean markets. Food Engineering Progress, 13, 221-225.

Li, Y.; Zhang, X.; Nie, J.; Bacha, S.A.S.; Yan, Z.; Gao, G. Occurrence and co-occurrence of mycotoxins in apple and apple products from China. Food Control. 2020, 118, 107354

Liu Q, Tang GY, Zhao CN, Gan RY, Li HB. Antioxidant Activities, Phenolic Profiles, and Organic Acid Contents of Fruit Vinegars. Antioxidants (Basel). 2019 Mar 27;8(4):78. doi: 10.3390/antiox8040078. PMID: 30934715; PMCID: PMC6523695.

Mas, A., Torija, M. J., García-Parrilla, M. D. C., & Troncoso, A. M. (2014). Acetic acid bacteria and the production and quality of wine vinegar. The Scientific World Journal, 2014, 1-6.

Mohebbi, S.; Babalar, M.; Zamani, Z.; Askari, M.A. Influence of early season boron spraying and postharvest calcium diptreatment on cell-wall degrading enzymes and fruit firmness in ‘Starking Delicious’ apple during storage. Sci. Hortic. 2020,

Ousaaid, D.; Mechchate, H.; Laaroussi, H.; Hano, C.; Bakour, M.; El Ghouizi, A.; Conte, R.; Lyoussi, B.; El Arabi, I. Fruits Vinegar: Quality Characteristics, Phytochemistry, and Functionality. Molecules 2022, 27, 222. https://doi.org/10.3390/molecules27010222.

Petsiou, E. I., Mitrou, P. I., Raptis, S. A., & Dimitriadis, G. D. (2014). Effect and mechanisms of action of vinegar on glucose metabolism lipid profile, and body weight. Nutrition Reviews, 72(10), 651-661.

Saha, P. and Banerjee, S. 2013. Optimization of process parameters for vinegar production using banana fermentation. International Journal of Research in Engineering and Technology 2: 501-514

Solieri, L., Landi, S., De Vero, L. and Giudici, P. 2006. Molecular assessment of indigenous yeast population from traditional balsamic vinegar. Journal of Applied Microbiology 101(1): 63-71

Torri, L., Jeon, S. Y., Piochi, M., Morini, G., & Kim, K. O. (2017). Consumer perception of balsamic vinegar: A cross-cultural study between Korea and Italy. Food Research International, 91, 148-160.

Zhang, L.; Hu, J.; Han, X.; Li, J.; Gao, Y.; Richards, C.; Zhang, C.; Tian, Y.; Liu, G.; Gul, H.; et al. A high-quality apple genome assembly reveals the association of a retrotransposon and red fruit colour. Nat. Commun. 2019, 10, 1–13.

San Chiang Tan. “Vinegar Fermentation”, Master Thesis in University of Louisiana at Lafayette 2003

Effects of *Laurus nobilis* and Rifampicin on Motor Function and Oxidative Stress in *Drosophila melanogaster*

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Abstract: This study evaluates the effects of rifampicin and *Laurus nobilis* extract on *Drosophila melanogaster*, focusing on mortality rates, climbing performance, oxidative stress parameters, and phytochemical components. Adult flies were treated with rifampicin 25 mg, 12.5 mg, and *L. nobilis* extract 10 mg, with mortality assessed after 72 hours. Climbing ability was measured, and oxidative stress was evaluated through MDA levels, PO activity, and CAT activation. GC-MS analysis identified key components in *L. nobilis* extract, and molecular docking studies assessed binding with the 8UUQ protein. Mortality rates were 2.22% for 25 mg rifampicin, 1.11% for 12 mg rifampicin, and 6.66% for the combination of 25 mg rifampicin and 10 mg *L. nobilis* extract. Rifampicin improved climbing ability index 0.167 for 25 mg, 0.367 for 12.5 mg, while 10 mg *L. nobilis* extract resulted in the lowest climbing index 0.111. The combination treatment had the highest MDA levels, with *L. nobilis* alone showing the lowest MDA levels. PO activity was most inhibited in the combination group, and CAT activity increased with decreasing rifampicin doses. GC-MS analysis identified Pinene, Sabinene, and Terpinyl acetate as key components, and molecular docking revealed varying binding affinities with the 8UUQ protein. Acute toxicity tests classified the components as low toxicity, with neurotoxic effects observed for Pinene and Sabinene. Rifampicin significantly enhanced climbing ability, while *L. nobilis* extract may increase oxidative stress when combined with rifampicin. The study provides insights into the therapeutic and toxicological properties of these compounds and their effects on motor performance and oxidative stress.

Keywords: Drosophila melanogaster, Laurus nobilis, molecular docking, motor function, oxidative stress, rifampicin

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1. INTRODUCTION

Drosophila melanogaster was recognized as a model organism for immunology research in the early 21st century due to its significant similarities with the innate immune system in mammals. This species plays a central role in basic biological research due to its low cost, rapid reproduction cycle, and extensive genetic resources. *D. melanogaster* is widely used as a model for genotoxicity and systemic toxicology studies. It is also commonly employed in disciplines such as biochemistry, cell biology, and developmental biology (Pam et al., 2021).

Valued for its aromatic and cleansing properties for millennia, *L. nobilis* is widely used as a culinary herb. The plant's various parts, including its essential oil, have notable applications in agriculture, medicine, food, and pharmaceuticals. *L. nobilis* essential oil is recognized for its antimicrobial, insecticidal, antibacterial, antifungal, and antioxidant properties, and is utilized in cosmetics and as a food preservative. Additionally, the seeds have demonstrated antiulcer and antidiabetic effects. The increasing demand for natural products has led to the use of bay laurel berries as a natural source of anthocyanins, replacing synthetic dyes in various industries (Paparella et al., 2022).

Insecticide resistance and excessive pesticide use can have negative effects on human health, show toxic effects on non-target insects, and cause environmental contamination (Barathi et al., 2024; Simões et al., 2022). Antibiotics also show promise as synergists because they can significantly alter the toxicity of certain insecticides against insects (Soh & Veera Singham, 2022; Tang et al., 2021).
Rifampicin is a widely used antibiotic for treating bacterial infections in humans. However, recent studies have shown that rifampicin could also serve as a potential synergist for insecticides. Specifically, this antibiotic selectively inhibits bacterial endosymbionts like *Buchnera* found within insects such as aphids *Myzus persicae*. These symbiotic bacteria can play a significant role in the insects' development of resistance to certain insecticides. By disrupting these symbiotic

bacteria, rifampicin can enhance the effectiveness of insecticides against pests, making the insects more susceptible to these chemical treatments (Li et al., 2024).

Reactive oxygen species (ROS) are produced in cells as part of normal physiological and metabolic processes, and cells possess universal antioxidant protection mechanisms to help maintain redox balance (Mailloux, 2018; Sies, 2017). However, this balance can be disrupted under stress conditions, leading to oxidative stress. In cases of oxidative stress, antioxidants obtained from foods, plants, or nutraceutical and pharmaceutical supplements can support antioxidant defense and mitigate the effects of stress. Antioxidants can enhance the cells' natural defense mechanisms by neutralizing free radicals or strengthening existing antioxidant protection systems (Nimse & Pal, 2015).

This study investigates the biochemical and protective responses in *D. melanogaster* exposed to leaf extracts of *L. nobilis*. The research involves acute toxicity tests, climb assays, survival assays, and biochemical analyses on the flies. The goal is to highlight the potential toxic effects of *L. nobilis* leaf extracts by demonstrating their ability to induce increased mortality and cellular stress markers in *D. melanogaster*. Additionally, a molecular docking study has been conducted to explore the potential biological interactions of the plant.

2. MATERIAL AND METHOD

2.1 Plant extraction

Extraction was carried out according to (Jang et al., 2013) with minor modifications. *L. nobilis* leaves were initially dried in an oven at 40°C and then ground into a powder using a blender. The obtained powder was subjected to ultrasonic treatment in pH 7.4 phosphate buffer at 20 kHz frequency for 10 minutes using a sonicator. Centrifugation was performed at 5,000 g for 10 minutes at 5°C using a refrigerated centrifuge. Subsequently, the supernatants were collected into capped tubes and stored at -25°C.

2.2. The procurement and propagation of *D. melanogaster*

The *D. melanogaster* strain used in our study was cultured at 25°C in incubators to facilitate mating and reproduction. The culture medium prepared using the standard medium developed by (Bozcuk, 1976) consisted of 52 g corn flour, 47 g powdered sugar, 9.5 g brewer's yeast, 3 g agar agar, and 3 ml propionic acid.

2.3. Acute toxicity test of *D. melanogaster*

Six groups were established in the study:
•Control Group: Normal culture medium
•*L. nobilis* Group: 10 mg *L. nobilis* leaf extract
•Rifampicin Group: 25 mg Rifampicin
•Rifampicin Group: 12.5 mg Rifampicin
•*L. nobilis* + Rifampicin Group: 10 mg *L. nobilis* leaf extract + 25 mg Rifampicin
•*L. nobilis* + Rifampicin Group: 10 mg *L. nobilis* leaf extract + 12.5 mg Rifampicin
Thirty *D. melanogaster* were placed in each group and observed for 72 hours. Mortality rates were recorded at 72 hours, and LC50 values were determined by Probit analysis. At the end of the experiment, *D. melanogaster* was collected from each culture, homogenized in phosphate buffer, and sacrificed. Samples were stored at -20°C for analysis. The analyses were conducted in triplicate.

2.4. Climbing Assay

The climbing assay was conducted as described by (Pendleton et al., 2002). Thirty flies were placed in an empty glass vial. A horizontal line was drawn 5 cm above the bottom of the vial. After the flies acclimated for 10 minutes at room temperature, both control and treated groups were tested randomly, with a total of 3 trials for each. Mean values were calculated and then averaged to obtain the group mean and standard error.

2.5. Enzyme activity assays

2.5.1. Malondialdehyde (MDA) activity

Lipid peroxidation activity was analyzed according to the methods of (Draper & Hadley, 1990) For this analysis, 10% trichloroacetic acid (TCA) and 0.675% thiobarbituric acid (TBA) solutions were used. Malondialdehyde (MDA), a

product of lipid peroxidation, forms a pink complex with TBA when incubated at 90°C. The absorbance of this complex was measured at 532 nm. The MDA values were calculated in nmol/ml using the extinction coefficient of the MDA-TBA complex at 532 nm (n = 1.56 x 10^5 cm^-1M^-1).

2.5.2. Phenol oxidase (PO) activity

Phenol oxidase activity was determined using the method of (Hung & Boucias, 1996) The extract was added to a phosphate buffer containing 20 mM L-DOPA, and the change in absorbance was measured at 492 nm. The amount of peroxidase in the sample was calculated in PO units, where one unit is defined as the amount of enzyme that increases the absorbance by 0.001 per minute.

2.5.3. Catalase (CAT) activity

Catalase activity was determined using the method of (Luck, 1965). This method is based on the spectrophotometric measurement of the decrease in absorbance at 240 nm due to the breakdown of $H2O_2$ by catalase. For the assay, a phosphate buffer (pH 7, 1/15 M) was prepared by mixing $Na_2HPO_4.H_2O$ and KH_2PO_4. To 100 ml of this buffer, 160 µl of H_2O_2 was added. The reaction was initiated by adding 20 µl of the extract, and the change in absorbance was monitored for 2 minutes. The enzyme unit was calculated based on the change in absorbance, where one unit of catalase activity is defined as the amount of enzyme that catalyzes the decomposition of 1 µmol of H_2O_2 per minute.

2.6. Gas chromatography-mass spectroscopic (GC-MS) analysis

For gas chromatography-mass spectrometry (GC-MS) analysis following the methodology outlined by (Aytar, 2024). Following this, the samples underwent centrifugation at 3500 revolutions per minute for 10 minutes, and the resulting supernatant was utilized for GC-MS analysis. The GC-MS analysis was conducted in accordance with the protocol, utilizing the NIST Standard Reference Database.

2.7. Molecular docking studies

Molecules were drawn in the Chem-Draw Ultra 18.0 program, and their minimal energy forms were obtained in the Chem 3D 18.0 program and saved in Mol2 format. The Protein Data Bank was used to record the enzymes (PDB). Measles virus Fusion protein in the pre-fusion conformation with bound [FIP-HRC]2-PEG11 (8UUQ) (2.34 Å) were chosen and preserved in PDB format. Molecule-enzyme interactions using AutoDock Vina 1.5.7 software and binding energies (kcal/mol) were determined (Trott & Olson, 2010). 2D and 3D visuals are demonstrated by BIOVIA Discovery Studio Visualizer software (Biova, 2015).

2.8. Toxicity Prediction

The 3D structures of six phytocompounds were saved in SMILES format and uploaded to the PROTOX-II (https://tox-new.charite.de/protox_II/) web servers (Charite University of Medicine, Institute for Physiology, Structural Bioinformatics Group, Berlin, Germany) (Banerjee et al., 2018; Rolta, Salaria, et al., 2021; Rolta, Yadav, et al., 2021; Salaria et al., 2020)

3. RESULTS

The results of the mortality assessment for adult *D. melanogaster* after 72 hours of treatment with various substances are detailed in Table 1. The control group exhibited no mortality. The group treated with 25 mg of Rifampicin had a mortality rate of 2.22%. In contrast, the 12 mg Rifampicin group showed a lower mortality rate of 1.11%. Treatment with 10 mg of *L. nobilis* extract resulted in no mortality. The combination of 25 mg Rifampicin and 10 mg *L. nobilis* extract led to a mortality rate of 6.66% Similarly, the combination of 12.5 mg Rifampicin and 10 mg *L. nobilis* extract also resulted in no mortality. The LC50 value 6.64 µg/mL indicates the concentration at which 50% mortality occurs This value helps to quantify the extract's toxicity, showing that a concentration of around 6.64 mg/mL is required to kill half of the *D. melanogaster* population.

Table 1. Mortality percentage of adult *D. melanogaster* after 72 hours of the application of *L. nobilis* extract

Mortality %		
Concentration (mg/mL)	72h	LC50 (mg/mL)
Control	0 ± 0	6.64
25 mg Rifampicin	2.22 ± 3.845	
12 mg Rifampicin	1.11 ± 1.923	
10 mg Laurus nobilis	0 ± 0	
25 mg Rifampicin + 10 mg *Laurus nobilis*	6.66 ± 5.774	
12,5 mg Rifampicin + 10 mg *Laurus nobilis*	0 ± 0	

The climbing test results conducted on *D. melanogaster* reveal the effects of various treatment groups on the motor abilities of the organisms in Table 2. The application of 25 mg Rifampicin resulted in an average climbing index of 0.167 ± 0.033, indicating a certain improvement in climbing ability with this dose. Interestingly, the application of 12.5 mg Rifampicin resulted in a higher climbing index of 0.367 ± 0.033. This finding suggests that lower doses of Rifampicin might optimize the motor functions of *D. melanogaster*, while higher doses could potentially impair performance. The application of 10 mg *L. nobilis* leaf extract resulted in the lowest climbing performance, with an average climbing index of 0.111 ± 0.019. This suggests that *L. nobilis* leaf extract may have an inhibitive effect on motor abilities. Similarly, the combination of 25 mg Rifampicin and 10 mg *L. nobilis* leaf extract resulted in an average climbing index of 0.144 ± 0.019, while the combination of 12.5 mg Rifampicin and 10 mg *L. nobilis* leaf extract resulted in an average climbing index of 0.122 ± 0.051. Although these combinations exhibited higher climbing indices compared to the *L. nobilis* leaf extract alone, they showed lower performance compared to Rifampicin alone. The control group had an average climbing index of 0.244 ± 0.084. The higher climbing performance of the control group compared to the L nobilis leaf extract and combination treatments further emphasizes the potential negative effects of *L. nobilis* leaf extract. In conclusion, Rifampicin significantly enhanced the climbing ability of *D. melanogaster*, whereas *L. nobilis* leaf extract appeared to reduce this effect. Rifampicin was more effective at lower doses, and the addition of *L. nobilis* leaf extract might negatively impact motor performance. These findings support the positive effects of Rifampicin on the neuromuscular system while highlighting the potential toxic or inhibitive effects of *L. nobilis* leaf extract. Future research could further explore the interactions and dose-response relationships of these compounds to determine optimal combinations and doses for motor function enhancement.

Table 2. Climbing test results

Treatment	Climbing index (Mean ± Standard Deviation)
25 mg Rifampicin	0.167 ± 0.033
12.5 mg Rifampicin	0.367 ± 0.033
10 mg Laurus nobilis leaf	0.111 ± 0.019
25 mg Rifampicin + 10 mg *Laurus nobilis* leaf	0.144 ± 0.019
12.5 mg Rifampicin + 10 mg *Laurus nobilis* leaf	0.122 ± 0.051
Control	0.244 ± 0.084

It was observed that the MDA level in the Rifampicin Group (25 mg) was approximately 20% lower, and in the Rifampicin Group (12.5 mg), it was approximately 25% lower compared to the control group. In the *L. nobilis* (10 mg) + Rifampicin Group (25 mg), the MDA level was observed to be approximately 15% higher, while in the *L. nobilis* (10 mg) + Rifampicin Group (12.5 mg), it was observed to be approximately 6% lower. Additionally, the MDA formation in the *L. nobilis* (10 mg) + Rifampicin Group (25 mg) was 44% higher compared to the Rifampicin Group (25 mg). In the *L. nobilis* (10 mg) + Rifampicin Group (12.5 mg), the MDA formation was 22% higher compared to the Rifampicin Group (12.5 mg) (Figure 1A).

When the PO activity was examined, inhibition was observed in all groups compared to the control group. The inhibition rates were 59% in the Rifampicin Group (25 mg), 50% in the Rifampicin Group (12.5 mg), 65% in the *L. nobilis* (10 mg) + Rifampicin Group (25 mg), 30% in the *L. nobilis* (10 mg) + Rifampicin Group (12.5 mg), and 20% in the *L. nobilis* (10 mg) group. The *L. nobilis* (10 mg) + Rifampicin Group (12.5 mg) was found to be 26% more active compared to the Rifampicin Group (12.5 mg). However, the *L. nobilis* (10 mg) + Rifampicin Group (25 mg) showed 20% inhibition compared to the Rifampicin Group (25 mg) (Figure 1B).

Regarding CAT activity, while the Rifampicin Group (25 mg) showed approximately 40% inhibition compared to the control group, activation was observed in all other groups. This activation was approximately 145% in the Rifampicin Group (12.5 mg), 60% in the *L. nobilis* (10 mg) + Rifampicin Group (25 mg), 35% in the *L. nobilis* (10 mg) + Rifampicin

Group (12.5 mg), and 200% in the *L. nobilis* (10 mg) group, showing the maximum value. When L. nobilis (10 mg) was added to the Rifampicin Group (25 mg), the CAT activation in the *L. nobilis* (10 mg) + Rifampicin Group (25 mg) increased by 150% compared to the Rifampicin Group (25 mg). When *L. nobilis* (10 mg) was added to the Rifampicin Group (12.5 mg), an 80% inhibition was observed in the *L. nobilis* (10 mg) + Rifampicin Group (12.5 mg) compared to the Rifampicin Group (12.5 mg) (Figure 1C).

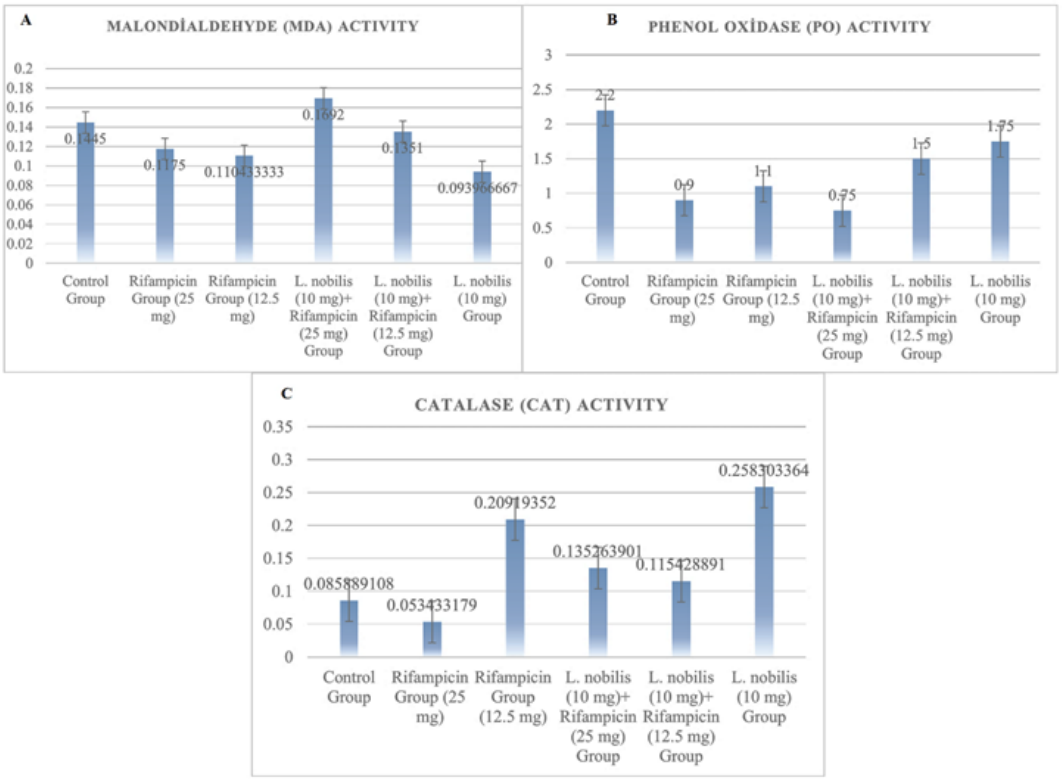
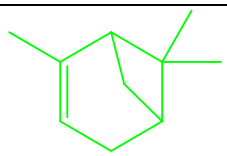
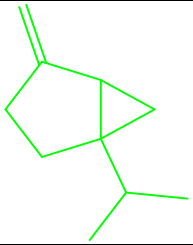
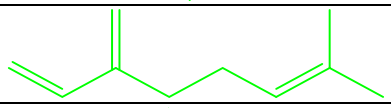
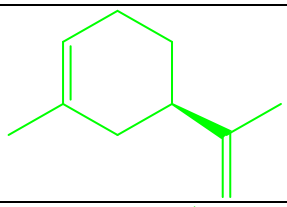
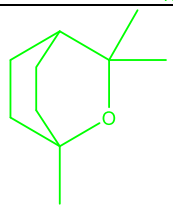
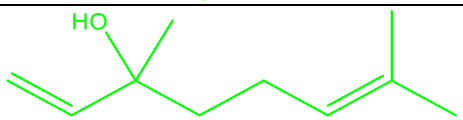
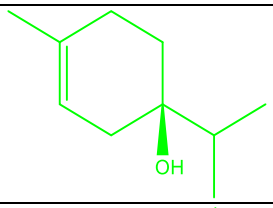
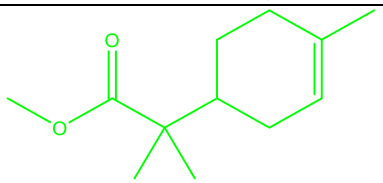
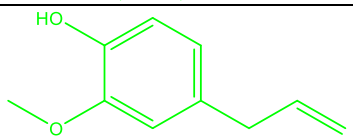
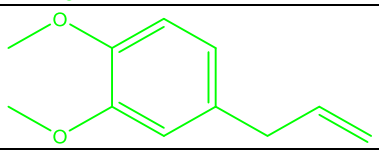


Figure 1. The activities of A) MDA, B) PO, and C) CAT at various concentrations of Rifampicin and *Laurus nobilis* in *Drosophila melanogaster*.

In conclusion, rifampicin affects PO and CAT activities differently depending on the dose. When the bay leaf extract was added to rifampicin, the amount of MDA, a marker of cell damage, increased. While the *L. nobilis* leaf extract combined with a high dose of rifampicin led to CAT activation, it caused PO inhibition. The results obtained from the groups that received only *L. nobilis* leaf extract indicate that the bay leaf possesses an antioxidant role by suppressing MDA formation, thereby reducing oxidative stress, protecting cells from damage, and stimulating the activities of the PO and CAT enzymes.

The methanol extract of *L. nobilis* was analyzed using GC-MS. The analysis identified a total of 10 different compounds within the sample (Table 3). The major constituents identified included Pinene, Sabinene, Myrcene, and 2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl, with notable area percentages of 9.79%, 13.28%, 2.21%, and 33.78%, respectively. Other significant compounds detected were Linalool, 4-Terpineol, Terpinyl acetate, Eugenol, and Methyl-eugenol, contributing to the overall chemical profile of the extract with area percentages of 2.61%, 1.29%, 14.33%, 2.50%, and 4.11%. These findings indicate the rich and diverse phytochemical composition of *L. nobilis* methanol extract, which may underlie its potential therapeutic properties.

Table 3. GC-MS analysis of *Laurus nobilis* methanol extract

No	Retention Time (minutes)	Compound Name	Area (%)	Molecular Structure
1	9.050	alpha-PINENE	10.01	
2	10.455	Sabinene	13.28	
3	11.077	Myrcene	2.21	
4	12.380	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	3.21	
5	12.469	2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl	3.25	
6	14.6788	Linalool	2.61	
7	17.366	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	1.29	
8	22.527	alpha-Terpinyl acetate	14.33	
9	22.747	Eugenol	2.50	
10	24.009	Methyleugenol	3.11	

The molecular docking study of major components with the 8UUQ protein in Drosophila reveals key insights into their binding affinities and interactions with specific amino acids (Table 4). Pinene displayed a binding energy of -5.8 kcal/mol, interacting primarily through alkyl interactions with LEU454 and PRO350 in chains E and G, and forming Pi-alkyl interactions with TYR349 in chain G (Figure 2A and 3A). Similarly, Sabinene exhibited a binding energy of -5.8 kcal/mol, with alkyl interactions involving amino acids such as PRO86, ILE87, and LEU257 in chains F and E, suggesting a stable binding affinity with the protein (Figure 2B and 3B). 2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl showed a stronger binding energy of -6.3 kcal/mol, establishing a carbon hydrogen bond with PRO350 in chain G, along with multiple alkyl and Pi-alkyl interactions, indicating a robust ligand-protein complex (Figure 2C and 3C). Lastly, Terpinyl acetate recorded a binding energy of -5.1 kcal/mol, forming a conventional hydrogen bond with GLY455 in chain E and engaging in alkyl interactions with LEU370 and PRO350 in chains E and G, alongside Pi-alkyl interactions with TYR349 (Figure 2D and 3D). These findings suggest that the binding interactions and energies of these compounds could play a significant role in modulating the activity of the 8UUQ protein in Drosophila, contributing to a deeper understanding of their molecular effects.

This study's findings offer valuable insights into the potential impact of various ligands on D. melanogaster in experimental settings. The acute toxicity profile of the phytochemical components in Laurus nobilis extract was assessed, with the LD50 values determined as follows: Pinene at 3700 mg/kg, Sabinene at 5000 mg/kg, 2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl at 3080 mg/kg, and Terpinyl acetate at 900 mg/kg. All components were classified under toxicity class 5, indicating low toxicity.

Table 4. Molecular docking study of major component with *Measles virus Fusion protein in the pre-fusion conformation with bound [FIP-HRC]2-PEG11 (*8UUQ)

Ligand	**Protein**	**Binding Energy (kcal/mol)**	**Amino acid**	**Interacting**	Distance
α-Pinene	8UUQ	-5.8	E: LEU454-: [001	Alkyl	5.26
			G:PRO350-: [001	Alkyl	4.74
			: [001:C8- G:PRO350	Alkyl	3.63
			: [001:C10- G:PRO350	Alkyl	4.11
		-	G: TYR349-: [001	Pi-Alkyl	4.97
			G: TYR349-: [001:C10	Pi-Alkyl	5.02
Sabinene	8UUQ	-5.8	F:PRO86-: [001	Alkyl	4.09
			G:PRO219-: [001	Alkyl	4.28
			: [001:C3- F:PRO86	Alkyl	3.54
		-	: [001:C3- F: ILE87	Alkyl	4.13
			: [001:C7- E: LEU257	Alkyl	4.53
			: [001:C7- F: VAL83	Alkyl	4.11
			: [001:C7- F:PRO86	Alkyl	4.08
			: [001:C7- G:PRO219	Alkyl	4.34
			: [001:C8- F: ILE87	Alkyl	5.30
			: [001:C8- G:PRO224	Alkyl	4.59
2-Oxabicyclo [2.2.2] octan-6-ol, 1,3,3-trimethyl-	8UUQ	-6.3	G: PRO350:HD2-: [001: O1	Carbon Hydrogen Bond	2.26
			G:PRO350-: [001	Alkyl	4.95
			: [001- E: LEU454	Alkyl	4.72
			: [001:C8- G:PRO350	Alkyl	4.73
			: [001:C10- G:PRO350	Alkyl	3.46
			G: TYR349-: [001	Pi-Alkyl	4.99
			G: TYR349-: [001:C8	Pi-Alkyl	4.53

Terpinyl acetate	8UUQ	-5.1	E: GLY455:HN-: [001: O2	Conventional Hydrogen Bond	2.27
			E: LEU370-: [001	Alkyl	5.39
			G:PRO350-: [001	Alkyl	4.13
			: [001:C9- E: LEU370	Alkyl	4.09
			: [001:C9- G:PRO350	Alkyl	5.32
			G: TYR349-: [001:C12	Pi-Alkyl	4.16

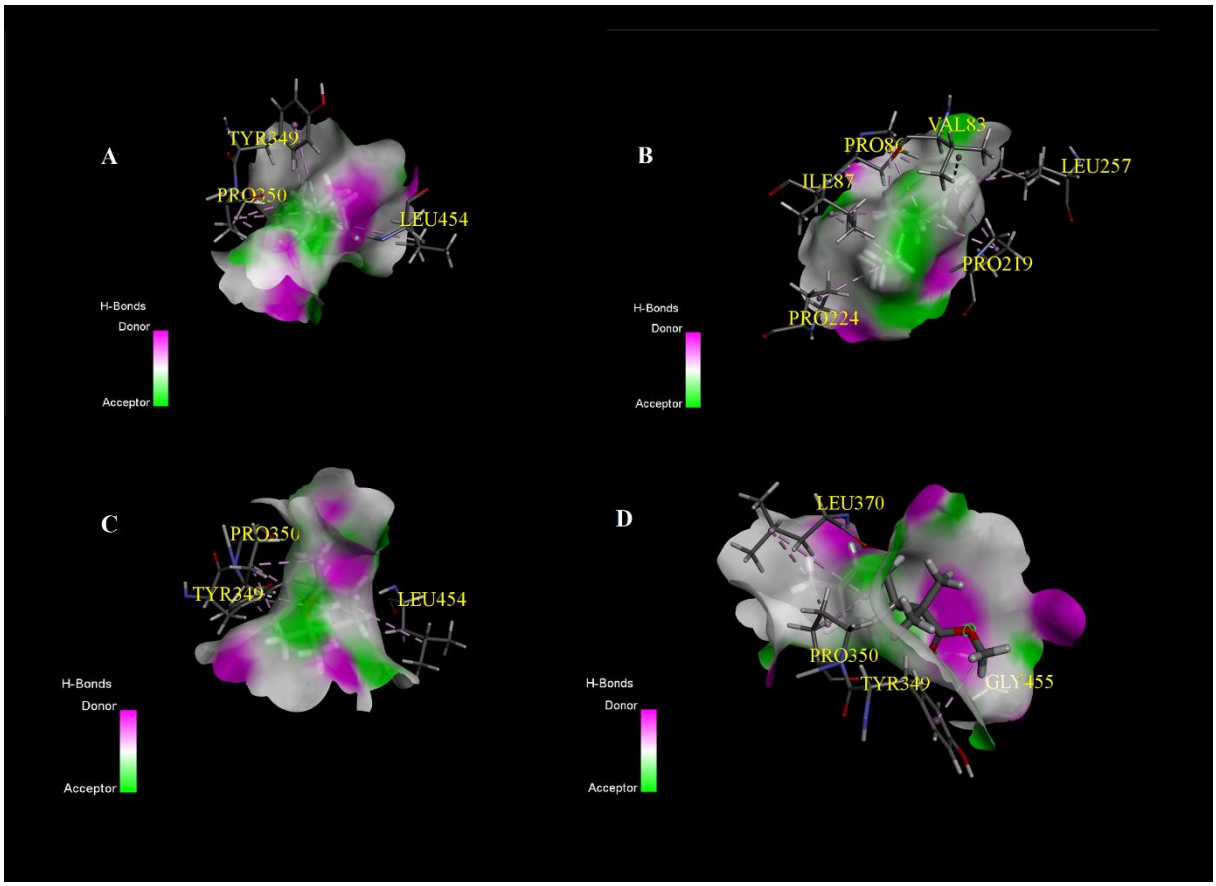


Figure 2. Interaction diagram of A) α-Pinene B) Sabinene C) 2-Oxabicyclo [2.2.2] octan-6-ol, 1,3,3-trimethyl- D) Terpinyl acetate with *H-bond*

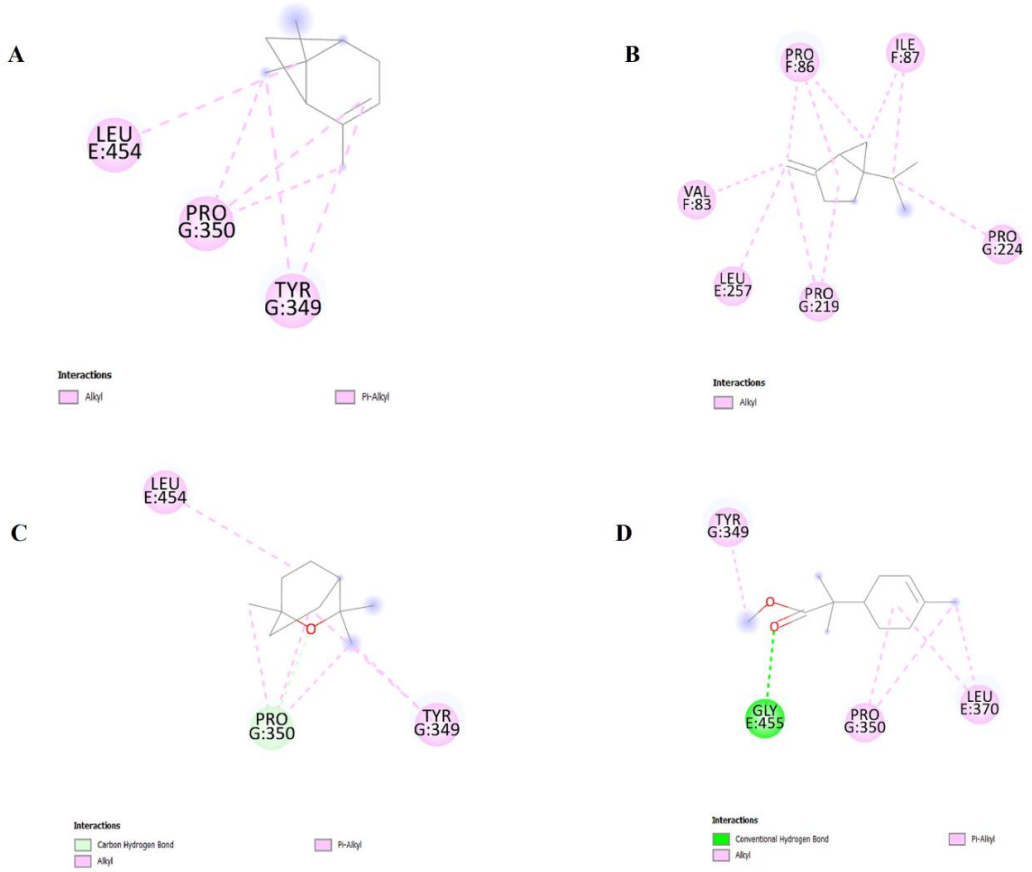


Figure 3. Molecular docking process of A) α-Pinene B) Sabinene C) 2-Oxabicyclo [2.2.2] octan-6-ol, 1,3,3-trimethyl- D) Terpinyl acetate with Measles virus Fusion protein in the pre-fusion conformation with bound [FIP-HRC]2-PEG11 (8UUQ)

Regarding neurotoxicity, Pinene and Sabinene exhibited active neurotoxic effects, whereas 2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl, and Terpinyl acetate did not show such activity (Table 5 and Figure 4). None of the components demonstrated immunotoxicity or cytotoxicity, and clinical toxicity was absent across all tested components. Furthermore, none of the components impacted the Nuclear Factor (Erythroid-Derived 2)-Like 2/Antioxidant Responsive Element (Nrf2/ARE), Heat Shock Factor Response Element (HSE), Mitochondrial Membrane Potential (MMP), Tumor Suppressor p53, or ATPase Family AAA Domain-Containing Protein 5 (ATAD5). Overall, the components of *Laurus nobilis* extract displayed low toxicity profiles and exhibited specific effects depending on the assessed toxicity model.

Table 5. Acute toxicity profile values of phytochemical components in *Laurus nobilis* extract

Toxicity Model	α-Pinene	Sabinene	2-Oxabicyclo [2.2.2] octan-6-ol, 1,3,3-trimethyl-	Terpinyl acetate
LD_{50} (mg/kg)	3700	5000	3080	900
Toxicity class	5	5	5	5
Neurotoxicity	Active	Active	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive
Clinical toxicity	Inactive	Inactive	Inactive	Inactive
Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	Inactive	Inactive	Inactive

Heat shock factor response element (HSE)	Inactive	Inactive	Inactive	Inactive
Mitochondrial Membrane Potential (MMP)	Inactive	Inactive	Inactive	Inactive
Phosphoprotein (Tumor Supressor) p53	Inactive	Inactive	Inactive	Inactive
ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	Inactive	Inactive	Inactive

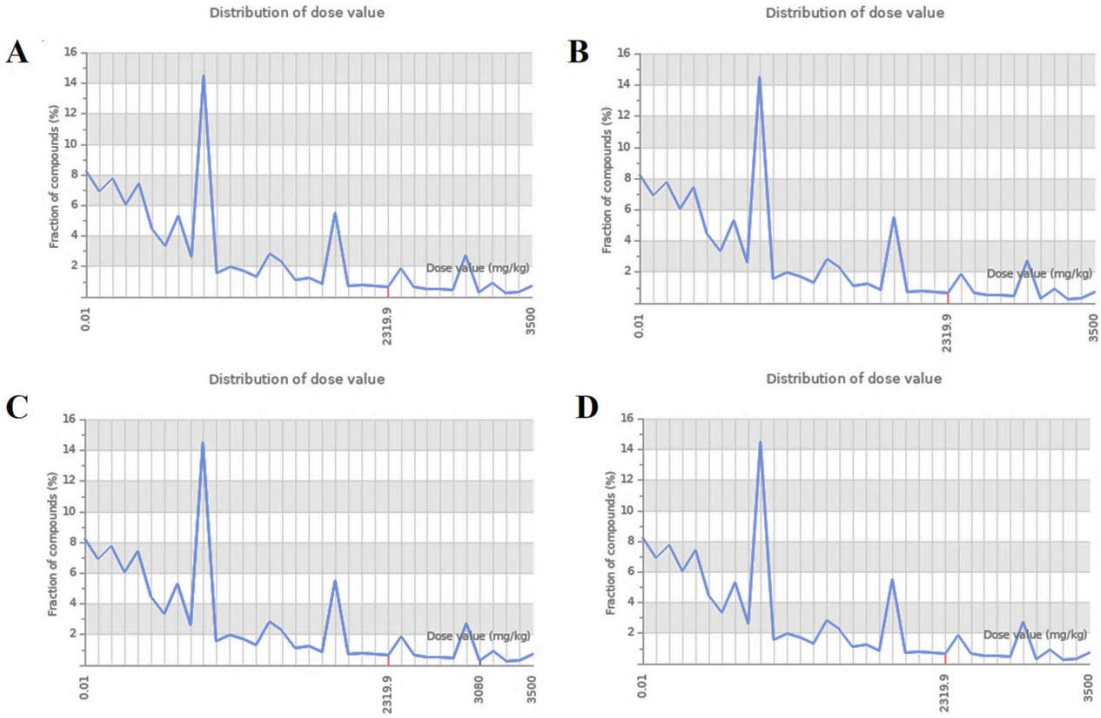


Figure 4. Acute toxicity profile and distribution of dose values for various compounds in Drosophila melanogaster A) α-Pinene B) Sabinene C) 2-Oxabicyclo [2.2.2] octan-6-ol, 1,3,3-trimethyl- D) Terpinyl acetate

4. DISCUSSION AND CONCLUSIONS

Recent studies have explored the effects of various plant extracts and their components on oxidative stress and survival rates in different model organisms, revealing both positive and negative impacts. Specifically, our research highlights the significant effects of *Laurus nobilis* leaf extract on oxidative stress markers and survival rates, as well as the interactions between *L. nobilis* extracts and the antibiotic rifampicin. These findings provide valuable insights into the complex effects of phytochemicals on biological responses and their potential applications.

In studies by (Chintalchere et al., 2021), exposure of *Musca domestica* larvae to *Laurus nobilis* essential oil for 24 hours resulted in an increase in MDA levels, indicating enhanced lipid peroxidation caused by the oil. Additionally, *L. nobilis* essential oil significantly elevated the activities of superoxide dismutase and glutathione peroxidase. In terms of glutathione reductase activity, *L. nobilis* essential oil exhibited a notable effect with a value of 0.0150 ± 0.001 U/mg protein. These findings suggest that *M. domestica* has developed an antioxidant defense mechanism to counteract the reactive oxygen species generated by short-term exposure to LC50 concentrations of the tested essential oils. In studies by (Santos et al., 2023) *L. nobilis* essential oil, 1,8-cineole at a dose of 125 µg/µL resulted in 67.6% mortality in *Chrysomia megacephala* larvae, while linalool at 30 µg/µL resulted in 100% mortality. These results indicate that these components significantly contribute to the insecticidal activity of *L. nobilis* essential oil.

In the study by Park et al. (2012), the LC50 value of paraquat for male *D. melanogaster* was reported as 24.7 mM within 24 hours. Dietary administration of curcumin, quercetin, *Sanguisorba officinalis*, and *Zedoariae rhizoma* extracts prior to paraquat exposure extended the lifespan and enhanced motor activities of the flies. Additionally, these treatments

improved other oxidative stress index factors such as ROS levels and superoxide dismutase. However, no significant changes in catalase activities were observed.

In the study by Valéria et al. (2014), research on *D. melanogaster* showed that samples treated with the extract exhibited a higher survival rate at the lowest dose compared to the control group fed only with the diet. This finding suggests that a slight increase in ROS levels at low concentrations of the extract enhances the biomarker activities in D. melanogaster, leading to an improvement in antioxidant capacity. In the study by Hosamani (2009), *D. melanogaster* was fed a standard *Bacopa monnieri* powder diet for 7 days. The study reported that Bacopa monnieri significantly reduced levels of endogenous oxidative markers, including malondialdehyde and catalase. Additionally, *Bacopa monnieri* extended the lifespan of the flies, reduced mortality rates, and improved motor performance. Furthermore, *Bacopa monnieri* provided substantial protection against oxidative stress induced by paraquat.

In studies by (BouzidI et al., 2019.), the effects of *L. nobilis* essential oil on *Culex longiareolata* larvae were compared with the control group, and it was observed that these effects were not significant. Mortality rates were recorded at different time intervals (1st, 3rd, 5th, and 7th days) after treatment. The intoxicated larvae exhibited behavioral changes by sinking to the bottom of the jar and remaining motionless until death. These findings suggest that *L. nobilis* essential oil and its active components could be developed as effective control agents against mosquito larvae. In the study by Amagon et al. (2023), the LC50 value for *Curcuma longa* and *Zingiber officinale* extracts was reported as 512.6 mg/5 g diet, while the LC50 value for isoniazid was determined to be 4.813 mg/5 g diet. The survival assay results showed a survival rate of 78.2% in *D. melanogaster* at the lowest dose of isoniazid (0.0012 g/5 g diet). The survival rates were 66.5% at 0.0024 g/5 g diet, 58.1% at 0.0036 g/5 g diet, and 25% at the highest dose of 0.0048 g/5 g diet. For the extract-treated *D. melanogaster*, the survival rate was 90% at the lowest dose (0.0641 g/5 g diet). Survival rates were 71.6% at 0.128 g/5 g diet, 44.5% at 0.1922 g/5 g diet, and 25% at the highest dose of extract (0.2563 g/5 g diet).
In the groups treated with rifampicin alone, it was observed that the MDA levels were lower than in the control group, with the amount varying depending on the dose. However, when *L. nobilis* leaf extract was added to the rifampicin groups, an increase in MDA levels was observed compared to the rifampicin-only groups. In the group receiving only *L. nobilis* leaf extract, MDA levels were found to be lower than in all other groups. This suggests that *L. nobilis* leaf extract suppresses oxidative stress, thereby exhibiting antioxidant effects.

Rifampicin at a high dose significantly inhibited PO activity, while this inhibition decreased as the dose was reduced. When *L. nobilis* leaf extract was added to rifampicin, the inhibition level in the high-dose rifampicin group (25 mg) increased further, whereas the opposite effect was observed with the lower dose. Specifically, the addition of *L. nobilis* leaf extract to the low-dose rifampicin group increased PO activity. *L. nobilis* leaf extract alone, compared to the rifampicin groups, demonstrated a stimulatory effect on PO activity.

The high dose of rifampicin inhibited CAT activity, while the low dose resulted in significant activation. The addition of *L. nobilis* leaf extract to the low-dose rifampicin group reduced the activation level. Conversely, adding *L. nobilis* leaf extract to the high-dose rifampicin group stimulated CAT activity. Notably, *L. nobilis* leaf extract alone led to a significant increase in CAT activity. These findings indicate that *L. nobilis* leaf extract plays a protective role against oxidative damage by stimulating CAT activity.

This study demonstrates that rifampicin significantly enhances the climbing ability of *D. melanogaster*, with the greatest improvement observed at a dose of 12.5 mg. The *L. nobilis* extract, when combined with rifampicin, may contribute to oxidative stress, as evidenced by increased MDA levels and inhibited PO activity. The extract alone was found to result in the lowest climbing performance, indicating a potential negative impact on motor function. GC-MS analysis identified key phytochemical components of *L. nobilis* extract, including Pinene, Sabinene, and Terpinyl acetate, and showed that their varying binding affinities could influence protein activity. Acute toxicity tests indicated that these components generally have low toxicity, though Pinene and Sabinene exhibited neurotoxic effects. The efficacy of rifampicin in improving motor performance is dose-dependent, and the effects of *L. nobilis* extract on oxidative stress and motor performance suggest both therapeutic and adverse outcomes.

Author Contributions
Conceptualization: ECA; Investigation: FGS, ECA, EİT.; Material and Methodology: FGS, ECA.; E.T.E.; Visualization: S.Ö.; Writing-Original Draft: ECA, FGS EİT, Writing-review & Editing: ECA.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Amagon, K. I., Agyo, D., Amagon, L., & Wanche, E. M. (2023). Toxicity and in vitro antioxidant potential of Curcuma longa Linn and Zingiber officinale Rosc rhizomes in Drosophila melanogaster. Journal of Pharmacy & Bioresources, 20(3), 125-133.

Aytar, Erdi Can. (2024). Antioxidant and antimicrobial properties of Stachys maritima via quantum dots and molecular docking. Chemistry & Biodiversity: e202401057.

Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 2018;46: W257–63. https://doi.org/10.1093/NAR/GKY318.

Barathi S, Sabapathi N, Kandasamy S, Lee J. Present status of insecticide impacts and eco-friendly approaches for remediation-a review. Environ Res 2024; 240:117432. https://doi.org/10.1016/J.ENVRES.2023.117432.

Biovia, D. S. (2015). Discovery studio modeling environment. San Diego, CA: Dassault Systemes.

Bouzıdı, Oulfa, et al. (2019). Chemical Composition and Insecticidal Activity of Laurus nobilis Essential Oil on Culiseta longiareolata (Diptera: Culicidae) larvae.

Bozcuk AN. Testing the protein error hypothesis of ageing in Drosophila. Exp Gerontol 1976; 11:103–6. https://doi.org/10.1016/0531-5565(76)90019-X.

Chintalchere JM, Dar MA, Shaha C, Pandit RS. Impact of essential oils on Musca domestica larvae: oxidative stress and antioxidant responses. Int J Trop Insect Sci 2021; 41:821–30. https://doi.org/10.1007/S42690-020-00272-Y/METRICS.

Draper HH, Hadley M. [43] Malondialdehyde determination as index of lipid Peroxidation. Methods Enzymol 1990; 186:421–31. https://doi.org/10.1016/0076-6879(90)86135-I.

Hung SY, Boucias DG. Phenoloxidase Activity in Hemolymph of Naive andBeauveria bassiana-InfectedSpodoptera exiguaLarvae. J Invertebr Pathol 1996; 67:35–40. https://doi.org/10.1006/JIPA.1996.0006.

Hosamani, R. (2009). Neuroprotective efficacy of Bacopa monnieri against rotenone induced oxidative stress and neurotoxicity in Drosophila melanogaster. Neurotoxicology, 30(6), 977-985.

Jang M, Asnin L, Nile SH, Keum YS, Kim HY, Park SW. Ultrasound-assisted extraction of quercetin from onion solid wastes. Int J Food Sci Technol 2013; 48:246–52. https://doi.org/10.1111/J.1365-2621.2012.03180.X.

Li Z, Li W, Mu Q, Zhu Y, Qin W, Shi X, et al. Rifampicin synergizes the toxicity of insecticides against the green peach aphid, Myzus persicae. Ecotoxicol Environ Saf 2024; 276:116291. https://doi.org/10.1016/J.ECOENV.2024.116291.

Luck, H. (1965) Catalase. In: Bergmeyer, H.U., Ed., Method of Enzymatic Analysis, Academic Press, New York and London, 885-894. http://dx.doi.org/10.1016/B978-0-12-395630-9.50158-4

Mailloux RJ. Mitochondrial Antioxidants and the Maintenance of Cellular Hydrogen Peroxide Levels. Oxid Med Cell Longev 2018;2018. https://doi.org/10.1155/2018/7857251.

Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv 2015; 5:27986–8006. https://doi.org/10.1039/C4RA13315C.

Pam D, Etuh AM, Oyeniran OI, Mdekere IW. Toxicity of Mangifera Indica aqueous stem bark extract evaluated in drosophila melanogaster used as model organism. Ann Pharm Fr 2021; 79:539–46. https://doi.org/10.1016/J.PHARMA.2021.02.005.

Paparella A, Nawade B, Shaltiel-Harpaz L, Ibdah M. A Review of the Botany, Volatile Composition, Biochemical and Molecular Aspects, and Traditional Uses of Laurus nobilis. Plants 2022, Vol 11, Page 1209 2022;11:1209. https://doi.org/10.3390/PLANTS11091209.

Park, J. H., Jung, J. W., Ahn, Y. J., & Kwon, H. W. (2012). Neuroprotective properties of phytochemicals against paraquat-induced oxidative stress and neurotoxicity in Drosophila melanogaster. Pesticide biochemistry and physiology, 104(2), 118-125.

Pendleton RG, Parvez F, Sayed M, Hillman R. Effects of pharmacological agents upon a transgenic model of Parkinson's disease in Drosophila melanogaster. J Pharmacol Exp Ther 2002; 300:91–6. https://doi.org/10.1124/JPET.300.1.91.

Rolta R, Salaria D, Sharma PP, Sharma B, Kumar V, Rathi B, et al. Phytocompounds of Rheum emodi, Thymus serpyllum, and Artemisia annua Inhibit Spike Protein of SARS-CoV-2 Binding to ACE2 Receptor: In Silico Approach. Curr Pharmacol Rep 2021; 7:135–49. https://doi.org/10.1007/S40495-021-00259-4/FIGURES/5.

Rolta R, Yadav R, Salaria D, Trivedi S, Imran M, Sourirajan A, et al. In silico screening of hundred phytocompounds of ten medicinal plants as potential inhibitors of nucleocapsid phosphoprotein of COVID-19: an approach to prevent virus assembly. J Biomol Struct Dyn 2021;39:7017–34. https://doi.org/10.1080/07391102.2020.1804457.

Salaria D, Rolta R, Sharma N, Dev K, Sourirajan A, Kumar V. In silico and In vitro evaluation of the anti-inflammatory and antioxidant potential of Cymbopogon citratus from North-western Himalayas. BioRxiv 2020:2020.05.31.124982. https://doi.org/10.1101/2020.05.31.124982.

Santos AR dos, Nascimento RFSC do, Cruz JD da da Silva Xavier A, Ramos A de S, Ferreira JLP, et al. Evaluation of essential oils and diluents against Chrysomya megacephala, an important mechanical vector. Journal of Natural Pesticide Research 2023; 3:100024. https://doi.org/10.1016/J.NAPERE.2023.100024.

Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biol 2017; 11:613–9. https://doi.org/10.1016/J.REDOX.2016.12.035.

Simões M, Huss A, Brouwer M, Krop E, Janssen N, Vermeulen R. Residential proximity to crops and agricultural pesticide use and cause-specific mortality: A prospective census-based cohort study in the Netherlands. Science of The Total Environment 2022; 817:152932. https://doi.org/10.1016/J.SCITOTENV.2022.152932.

Soh LS, Veera Singham G. Bacterial symbionts influence host susceptibility to fenitrothion and imidacloprid in the obligate hematophagous bed bug, Cimex hemipterus. Sci Rep 2022;12. https://doi.org/10.1038/S41598-022-09015-0.

Tang T, Zhang Y, Cai T, Deng X, Liu C, Li J, et al. Antibiotics increased host insecticide susceptibility via collapsed bacterial symbionts reducing detoxification metabolism in the brown planthopper, Nilaparvata lugens. J Pest Sci (2004) 2021; 94:757–67. https://doi.org/10.1007/S10340-020-01294-8/METRICS.

Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010; 31:455–61. https://doi.org/10.1002/JCC.21334.

Valéria Soares de Araújo Pinho, F., Felipe da Silva, G., Echeverria Macedo, G., Raquel Muller, K., Kemmerich Martins, I., Lausmann Ternes, A. P., ... & Posser, T. (2014). Phytochemical constituents and toxicity of Duguetia furfuracea hydroalcoholic extract in Drosophila melanogaster. *Evidence-Based Complementary and Alternative Medicine*, *2014*(1), 838101.

Purification of 2-Phenyl Ethyl Alcohol (PEA) Compound Naturally Found in Rose Water (*Rosa damascena* Mill.) by Flash Chromatography Technique

Osman Bodur*[1], Taner Erkaymaz[1]

Abstract: Rose water is the hydrosols obtained by distillation of the petals of the oil rose plant (*Rosa damascena* Mill.) using the distillation method. In these hydrosols, 2-phenyl ethyl alcohol (PEA) is the major compound. PEA compound is widely used in cosmetics, cleaning and food industries, especially in perfumery. PEA compound can be synthesized synthetically in laboratory environments. However, each synthesis step and subsequent purification processes increase the cost. In this study, as an alternative to synthetic methods, purification of PEA compound, which is naturally present in rose water, was carried out using flash chromatography method. It was studied in the flash chromatography device at wavelengths of UV1 λ: 254 nm UV2 λ: 265 nm UV3 λ: 274 nm UV4 λ: 320 nm. The pure product obtained was analyzed by GC-MS and it was determined that the PEA compound was 96% pure. With this technique, naturally occurring odor components in plants can be obtained with high purity. In addition, since it is close to the purity of the chemical standards used in the laboratory, higher purity products can be obtained with some corrections and repetitions in the method parameters. In this way, the products obtained can be used in many fields as well as used as standard chemicals in the laboratory. Standard chemicals can be obtained naturally in this way.

Keywords: 2-Phenyl Ethyl Alcohol, Rose water, Flash Chromatography, Purification

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1. INTRODUCTION

Aromatic waters are saturated aqueous solutions of essential oils and some water-soluble essential oil compounds. Rose water from oil rose (*Rosa damascena* Mill.) is obtained from the hydrosol portion of the distillation of fresh rose petals and contains aromatic compounds in the form of suspended particles (Baydar et al., 2013; Ciccarelli et al., 2013; Gallori et al., 2001). Aromatic waters contain low amounts of essential oils. In fact, rose waters also contain low amounts of essential oil (below 0.1%) and the main component is 2-phenylethyl alcohol (PEA) (Erbas and Baydar, 2016; Kurkcuoglu and Baser, 2003; Verma et al., 2011).

PEA is an aromatic alcohol with a colorless and rose-like odor (Kliszcz et al., 2021). PEA compound is naturally found in plants such as rose, carnation, hyacinth, orange blossom, ylang-ylang, geranium (Etschmann et al., 2002). PEA compound is widely used in cosmetics, cleaning and food industries, especially in perfumery (Chreptowicz et al., 2016; Hua and Xu, 2011; Politano et al., 2013; Scognamiglio et al., 2012). PEA is widely preferred in the perfume industry, especially because of its rose-like odor. This leads to an increase in the need for PEA compounds (Scognamiglio et al., 2012). PEA compound can be synthesized synthetically in laboratory environments. However, each synthesis step and subsequent purification processes increase the cost. In this study, as an alternative to synthetic methods, purification of PEA compound, which is naturally present in rose water, was carried out using flash chromatography method (Figure 1).

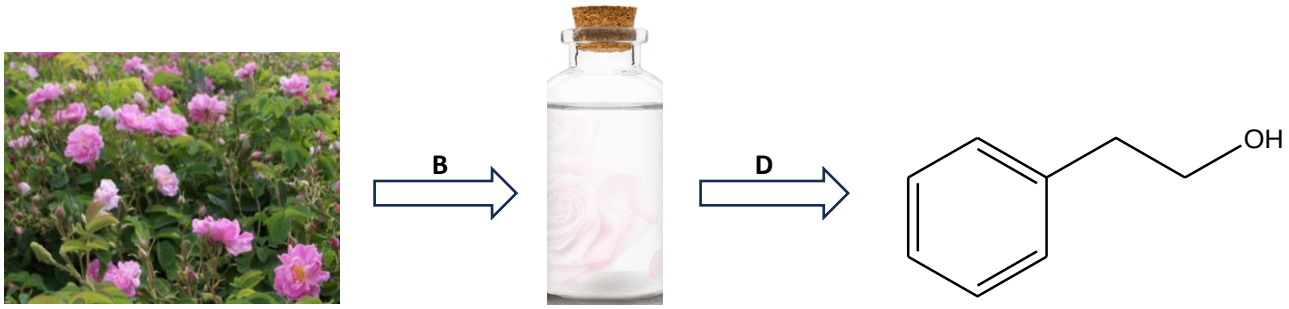

Figure 1. Schematic representation of the production of PEA (E) compound. A: *Rosa damascena* Mill. rose flowers B: Hydrodistillation C: Rose water D: Purification

2. MATERIAL AND METHOD

2.1. Rose Water Samples

Rose water samples were obtained from the store belonging to Manolya Dogal ve Aromatik Urunler Gida San. ve Tic. Ltd. Sti. located in the central district of Isparta province.

2.2. GC–MS Analysis

Analysis of the essential oil compounds in rose water samples was performed using a GC-MS instrument. 50 µL of the obtained the essential oil sample was taken and diluted with 10 mL of n-hexane solvent. After the mixture was homogenized by vortexing for 3 minutes, a 1/40 dilution was made with acetone in a 2 mL volumetric flask and injected into the GC-MS instrument.

Analyses were carried out with Thermo Scientific Trace 1300 GC gas chromatograph instrument, Thermo Scientific-ISQ7000 single quadrupole mass spectrometer detector (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) system. Chromatographic evaluations were made using Xcalibur software. TraceGOLD TG-624SilMS GC (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) column was used as the analytical column for chromatographic separation. The inlet temperature of the instrument was 250 °C. The injection volume was 2 µL. 1/5 split ratio was used. Helium gas was used as the carrier gas and the gas flow was 1.5 mL/min. The oven temperature was programmed from 35 °C (2 min.) to 100 °C at a rate of 2 °C /min., then from 100 °C (1 min.) to 250 °C at a rate of 5 °C/min. The detector temperature was 280 °C.

2.3. Identification of Compounds

Essential oil compounds were identified by computer search using their mass spectra either with known components (Adams, 1989), or by comparison of received chemical substances mass-spectrum which are in essential oils composition and according to mass-spectrum library (Wiley, 2007).

2.4. Flash Chromatography Analysis

In flash chromatography, Buchi brand device and FP ECOFLEX brand 40 g C18 column were used as column. Fractions were combined to apply flash chromatography, dissolved with DMSO and loaded onto the flash chromatography column. Solvent flow rate was set as 45 mL per min for appropriate separation. 50% water-50% ethanol was used as the starting solvent. The solvent ratio was adjusted to increase ethanol by 1% every 3 minutes. Flash chromatography was applied at UV1 λ: 254 nm, UV2 λ: 265 nm, UV3 λ: 274 nm, UV4 λ: 320 nm wavelengths. Flash chromatography was terminated in 60% water-40% methanol solvent system.

3. RESULTS

3.1. GC–MS analysis chromatograms

Commercial rose water was analyzed for essential oil components by GC-MS. Then, purification of PEA compound was carried out by rosewater flash chromatography. The sample obtained after the process was analyzed for essential oil

components by GC-MS. The chromatograms obtained before (Figure 2) and after (Figure 3) purification are given respectively.

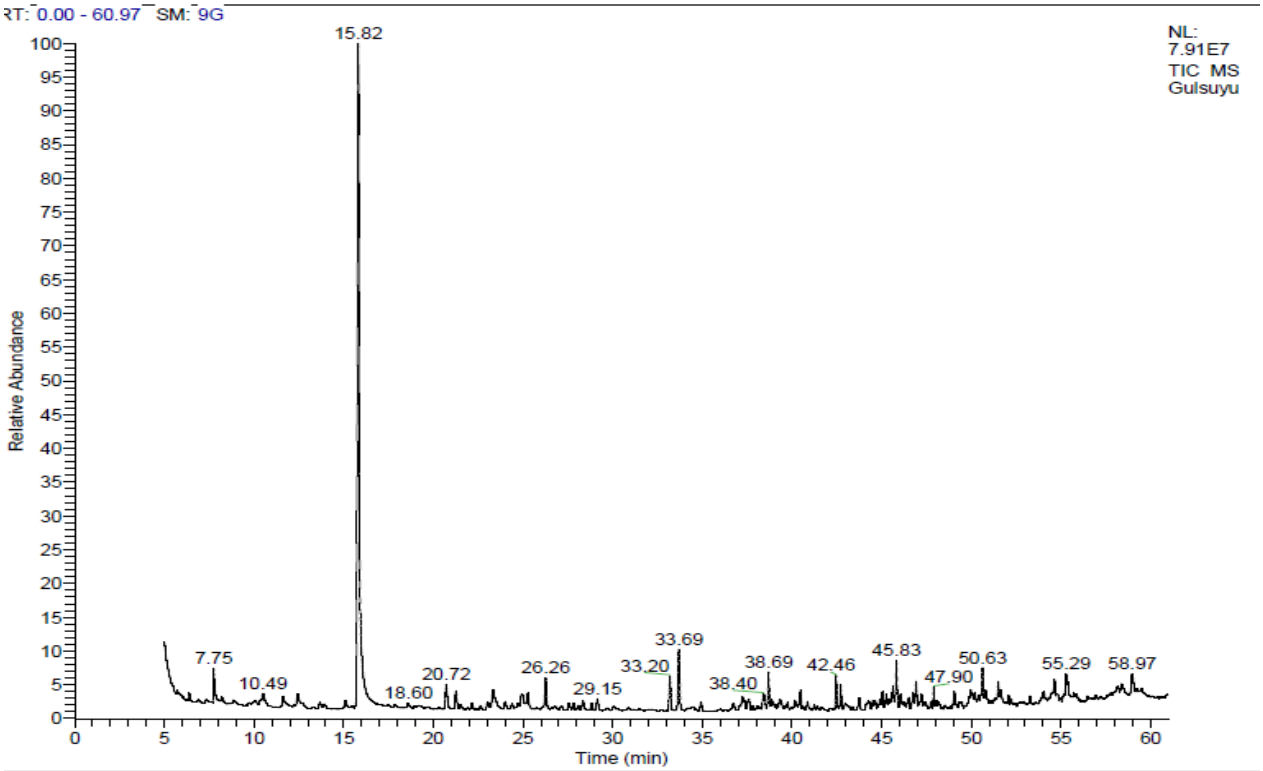

Figure 2. GC-MS Chromatogram of commercial rose water

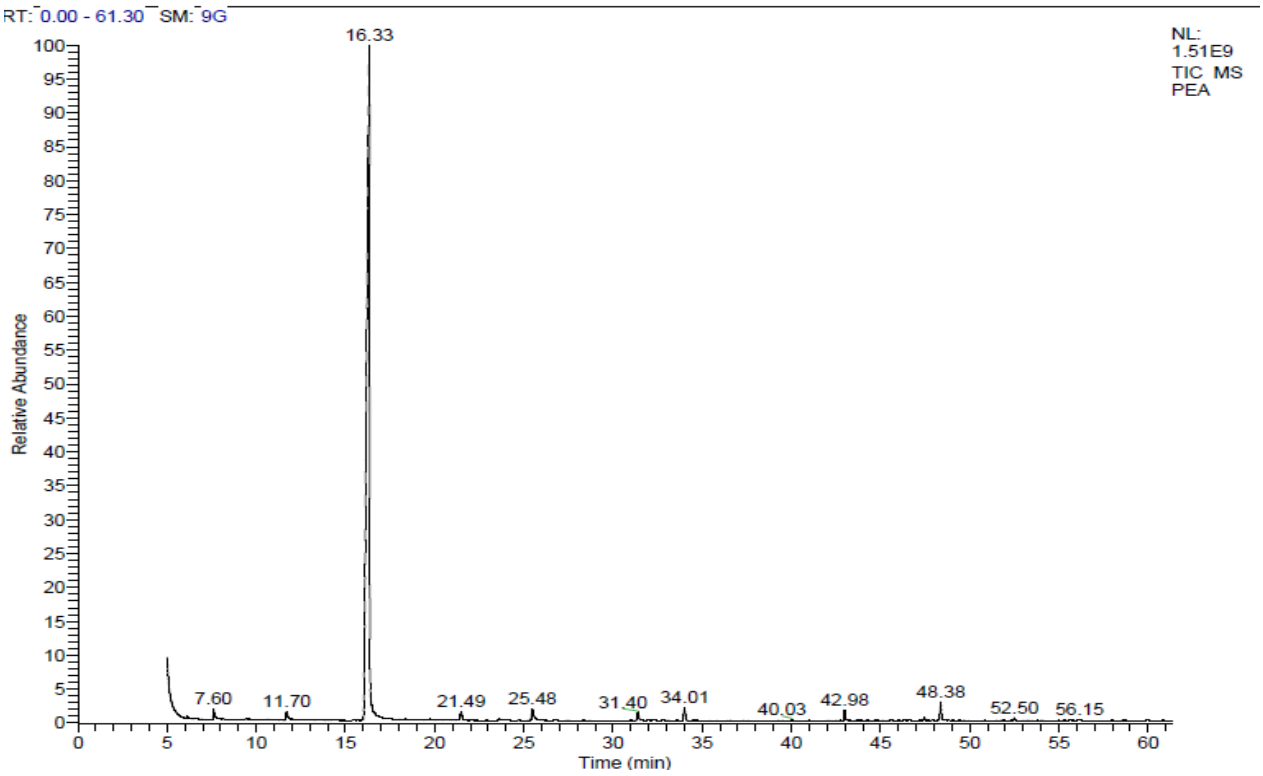

Figure 3. GC-MS Chromatogram of purified rose water

These peaks in the chromatograms were identified by library matching in the GC-MS instrument and the essential oil compounds of the oils are given in Table 1. According to the number of peaks in the chromatograms, while a total of 27 essential oil compounds were obtained in commercial rose water, 8 essential oil compounds were obtained after purification by flash chromatography. According to the results obtained, while PEA, the main compound of rose water, was 65.76% in commercial rose water, this value was determined as 95.90% after purification. In addition, 4,7-Dimethyl undecane (1.04%), m-Cymene (0.23%), p-Cymene (0.44%), Farrnesane (0.64%), Prehnitol (0.14%), Azulene (0.26%), 4-Terpineol (0.22%), α-Fenchyl alcohol (0.19%), n-Dodecane (1.36%), E-Citral (0.52%), n-Tetradecane (0.99%), 2.7,10-Trimethyl dodecane (2.32%), n-Pentadecane (4.49%), 1-Hexadecane (1.77%), 1-Heptadecane (2.92%), 1-Nonadecene (1.25%), n-Nonadecane (1.88%), n-Eicosane (0.84%) and n-Heneicosane (1.25%) compounds detected in commercial rose water remained below the detection limit after the purification process.

Table 1. The essential oil compositions of rose water samples.

No	Compound	Molecular Formula	CAS No	A (%)	B (%)	Identified Methods
1	Benzyl alcohol	C_7H_8O	100-516	0.90	0.67	GC-MS
2	Phenyl ethyl alcohol (PEA)	$C_8H_{10}O$	60-12-8	65.76	95.90	GC-MS
3	Azulene	$C_{10}H_8$	275-51-4	0.26	tr	GC-MS
4	Eugenol	$C_{10}H_{12}O_2$	97-53-0	0.21	0.50	GC-MS
5	m-Cymene	$C_{10}H_{14}$	535-77-3	0.23	tr	GC-MS
6	p-Cymene	$C_{10}H_{14}$	99-87-6	0.44	tr	GC-MS
7	Prehnitol	$C_{10}H_{14}$	488-23-3	0.14	tr	GC-MS
8	E-Citral	$C_{10}H_{16}O$	5392-40-5	0.52	tr	GC-MS
9	4-Terpineol	$C_{10}H_{18}O$	562-74-3	0.22	tr	GC-MS
10	α-Fenchyl alcohol	$C_{10}H_{18}O$	1632-73-1	0.19	tr	GC-MS
11	Nerol	$C_{10}H_{18}O$	106-25-2	0.63	0.10	GC-MS
12	Geraniol	$C_{10}H_{18}O$	106-24-1	1.25	0.12	GC-MS
13	α-Citronellol	$C_{10}H_{20}O$	7540-51-4	2.66	1.01	GC-MS
14	1-Dodecene	$C_{12}H_{24}$	112-41-4	1.98	0.56	GC-MS
15	n-Dodecane	$C_{12}H_{26}$	112-40-3	1.36	tr	GC-MS
16	4,7-Dimethyl undecane	$C_{13}H_{28}$	17301-32-5	1.04	tr	GC-MS
17	1-Tetradecene	$C_{14}H_{28}$	1120-36-1	2.51	0.76	GC-MS
18	n-Tetradecane	$C_{14}H_{30}$	629-59-4	0.99	tr	GC-MS
19	Farrnesane	$C_{15}H_{24}$	502-61-4	0.64	tr	GC-MS
20	2,7,10-Trimethyl dodecane	$C_{15}H_{32}$	74645-98-0	2.32	tr	GC-MS
21	n-Pentadecane	$C_{15}H_{32}$	629-62-9	4.49	tr	GC-MS
22	n-Hexadecane	$C_{16}H_{34}$	544-76-3	1.77	tr	GC-MS
23	1-Heptadecene	$C_{17}H_{34}$	6765-39-5	2.92	tr	GC-MS
24	1-Nonadecene	$C_{19}H_{38}$	18435-45-5	1.25	tr	GC-MS
25	n-Nonadecane	$C_{19}H_{40}$	629-92-5	1.88	tr	GC-MS
26	n-Eicosane	$C_{20}H_{42}$	112-95-8	0.84	tr	GC-MS
27	n-Heneicosane	$C_{21}H_{44}$	629-94-7	1.25	tr	GC-MS
	Others			1.36	0.38	GC-MS
	Identified components			27	8	

tr: trace (< 0.1), A: Commercial rose water B: Purified rose water

3.2. Flash chromatography process

In the purification of rose water by flash chromatography, compounds were collected in a total of 16 tubes (Figure 4). Each tube was then analyzed by GC-MS. When analyzed at UV-Vis wavelengths, it was seen that the PEA compound was between tubes 6-12 (purple) (Figure 5). Therefore, these tubes were combined and analyzed by GC-MS.

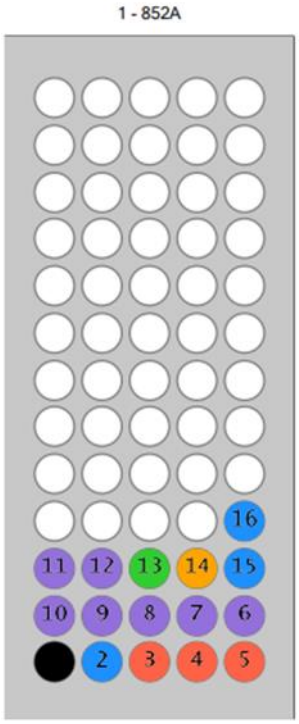

Figure 4. Tubes of compounds obtained by flash chromatography

Column: FP ECOFLEX C18 40g
Flow Rate: 45 mL/min
Equilibration: 3.0 min
Run Length: 15.0 min
Instrument Type: C-815
Mode: Flash
Sample type: Liquid

Solvent A: Ethanol
Solvent B: Water
Solvent C: Empty
Solvent D: Empty
Slope Detection: Off

UV Threshold: 0.05 AU
UV Sensitivity: Low
UV1 λ: 254 nm
UV2 λ: 265 nm
UV3 λ: 274 nm
UV4 λ: 320 nm
UV scan start λ: 254 nm (M)
UV scan end λ: 400 nm (M)

ELSD Threshold: 20 mV
ELSD Sensitivity: Low
Collection: Collect Peaks
Per-Vial Volume: 20 mL
Non-Peak Volume: 20 mL

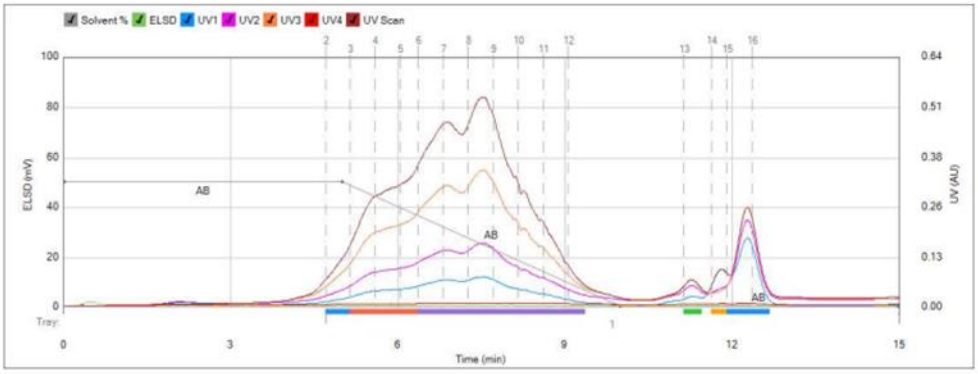

Figure 5. UV-vis chromatogram obtained by flash chromatography

4. DISCUSSION AND CONCLUSIONS

Commercially available rose water was analyzed by GC-MS to determine the amount of PEA in it and it was found to be 65.76%. Then, the PEA compound was purified by flash chromatography method. While the PEA ratio was 65.76%, this value was increased to 95.90% after the purification process. All necessary analyses and procedures were performed at Suleyman Demirel University Natural Products and Research Center (SUDUM). In this study, although flash chromatography method was preferred for the purification of PEA compound to obtain fast and high purity, it should be noted that the costs may be high considering the device used. Therefore, further research can be done in purification techniques or synthesis studies.

Acknowledgements

The authors are grateful to Manolya Dogal ve Aromatik Urunler Gida San. ve Tic. Ltd. Sti. for their support in providing rose water samples.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: T.E. O.B.; Investigation: O.B.; Material and Methodology: O.B., T.E.; Supervision: O.B., T.E.; Visualization: O.B.; Writing-Original Draft: O.B.; Writing-review & Editing: T.E.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Adams, R.P. (1989). Identification of essential oils by ion trap mass spectroscopy. Academic Press, New York, USA.

Baydar, H., Kuleasan, H., Kara, N., Secilmis-Canbay, H., Kineci, S. (2013). The effects of pasteurization, ultraviolet radiation and chemiclal preservatives on microbial spoilage and scent composition of rose water. Journal of Essential Oil-Bearing Plants, 16(2), 151–160.

Chreptowicz, K., Wielechowska, M., Główczyk-Zubek, J., Rybak, E., Mierzejewska, J. (2016). Production of natural 2-phenylethanol: from biotransformation to purified product. Food Bioprod Process, 100, 275–281.

Ciccarelli,D., Noccioli, C., Pistelli, L. (2013). Chemical composition of essential oils and aromatic waters from different Italian Anthemis maritima populations. Chemistry and Biodiversity, 10(9), 1667–1682.

Erbas, S., Baydar, H. (2016). Variation in scent compounds of oilbearing rose (Rosa damascena Mill.) Produced by headspace solid phase microextraction, hydrodistillation and solvent extraction. Records of Natural Products, 10(5), 555–565.

Etschmann, M. M. W., Bluemke, W., Sell, D., Schrader, J. (2002). Biotechnological production of 2-phenylethanol. Appl Microbiol Biotechnol, 59(1), 1–8.

Gallori, S., Flamini, G., Bilia, A.R., Morelli, I., Landini, A., Vincieri, F. F. (2001). Chemical composition of some traditional herbal drug preparations: essential oil and aromatic water of costmary (Balsamita suaveolens Pers.). Journal of Agricultural and Food Chemistry, 49(12), 5907–5910.

Hua, D.,, Xu, P. (2011). Recent advances in biotechnological production of 2-phenylethanol. Biotechnol Adv., 29(6), 654–660.

Kliszcz, A., Danel, A., Puła, J., Barabasz-Krasny, B., Mozdzen, K. (2021). Fleeting Beauty—The World of Plant Fragrances and Their Application. Molecules, 26(9), 2473.

Kurkcuoglu, M., Baser, H. C. (2003). Studies on Turkish rose concrete, absolute, and hydrosol. Chemistry ofNatural Compounds, 39(5), 457–464.

Politano, V. T., Diener, R. M., Christian, M. S., Hawkins, D. R., Ritacco, G., Api, A. M. (2013). The Pharmacokinetics of Phenylethyl Alcohol (PEA): Safety Evaluation Comparisons in Rats, Rabbits, and Humans. International Journal of Toxicology, 32(1), 39-47.

Scognamiglio, J., Jones, L., Letizia, C. S., Api, A. M. (2012). Fragrance material review on phenylethyl alcohol. Food and Chemical Toxicology, 50, 224–S239.

Scognamiglio, J., Jones, L., Letizia, C. S., Api, A. M. (2012). Fragrance material review on phenylethyl alcohol. Food Chem. Toxicol, 50, 224–S239.

Verma, R. S., Padalia, R. C., Chauhan, A., Singh, A., Yadav, A. K. (2011). Volatile constituents of essential oil and rose water of damask rose (Rosa damascena mill.) cultivars fromnorth indian hills. Natural Product Research, 25(17), 1577–1584.

Wiley, (2007). Registry, 8th ed. with NIST 05 MS Spectra, Revision 2005 D.06.00. s.l. Agilent Technologies.

Determination of Iron-Related Siderophore Production by Bacteria from Environmental Samples

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Abstract: Iron is essential for microorganisms in photosynthesis, oxygen release, respiration, the TCA cycle, gene regulation, nitrate synthesis, nitrogen fixation, ATP synthesis, and DNA synthesis. Due to the tendency of iron(III) to form insoluble ferric complexes and bind to high-affinity proteins in hosts, bacteria find it challenging to access iron. Bacteria synthesize siderophores, low molecular weight (<1000 Da) metal chelators with high iron affinity to overcome this. Siderophores bind iron(III) and transport it into bacterial cells, where specific enzymes reduce Fe^{3+} to Fe^{2+}, releasing the iron. Siderophores are detected using the CAS (chrome azurol S) universal assay, which relies on iron competition between the CAS ferric complex and the siderophore. A positive reaction is indicated by a color change from blue to orange. Siderophores are categorized into three main groups based on their chemical structures: carboxylate, catecholate, and hydroxamate. These classifications are determined by the functional groups that give them their binding affinity and selectivity for iron(III). Approximately 500 diverse siderophores have been identified, highlighting their biological significance and evolutionary adaptations. In our study, microorganisms were isolated from environmental samples and subjected to the CAS assay to detect siderophore production, indicated by orange zones. The methods including Arnow, Csaky, and Shenker were used to detect catecholate, hydroxamate, and carboxylate types, respectively. Techniques for siderophore detection and characterization can foster innovative applications in environmental and industrial microbiology. In conclusion, siderophore studies are pivotal in understanding microbial ecology, disease mechanisms, and biotechnological advancements.

Keywords: Siderophore, metal-chelate, bacteria, ıron uptake

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1. INTRODUCTION

Iron is the fourth most abundant element in the Earth's crust (Huber, 2005; Gamit and Tank, 2014). Siderophores facilitate the dissolution of iron elements in complexes other than bacteria (Kraemer, 2004). From a chemical perspective, siderophores are metal-chelating compounds released by organisms in environments where iron is deficient (Miethke and Marahiel, 2007). Notably, most aerobic and facultative anaerobic microorganisms can synthesize siderophores, with hundreds of different siderophore species being characterized to date (Saha et al., 2016). In addition to the binder Fe^{3+}, siderophores are soluble with Al^{3+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+}, as well as other metals, and can form metal-siderophore chelates (Dimkpa et al., 2009; Rajkumar et al., 2010). It has been demonstrated that both Gram-negative and Gram-positive bacteria can synthesize siderophores in iron-free media. The siderophore first binds tightly to iron, then forms a complex and moves through the cell membrane into the cell. Gram-negative bacteria differ from Gram-positive bacteria in siderophore transport (Ahmed and Holmstrom 2014). After iron release, siderophores are recovered by excretion through the system. The structure of siderophores varies from species to species. According to the characteristic functional groups, siderophores are classified into four types: hydroxamate, catecholate, hydroxycarboxylate, hydroxycarboxylate, and mixed types, the most common hydroxamate type (Khan et al. 2018). Approximately 500 different siderophores have been identified (Boukhalfa et al. 2003).

Siderophores can be classified into three main categories based on the duration of oxygen ligand donation for Fe^{3+} coordination. These categories include catecholates (or phenolates), hydroxamates (or carboxylates), and mixed types (Miethke and M. et al., 2007; Wencewicz and T.A. et al., 2009). Notable examples of siderophores containing catecholate (or phenolate) groups include enterobactin (produced by *Streptomyces* spp.), vibriobactin (produced by *Vibrio cholera*) and pyochelin (produced by *Pseudomonas aeruginosa*). Fungi produce siderophores, mainly hydroxamates (Van der Helm and D. et al., 2020; Holinsworth and B. et al., 2009), but very few are known to produce carboxylate (Holinsworth and B. et al., 2009; Thieken and A. et al., 1992) and phenolate (Jellison and J. et al., 1991; Capon and R. J. et al., 2007) compounds. The yeast Saccharomyces cerevisiae is not known to produce siderophores. However, it has been demonstrated to possess a siderophore-iron uptake system (Philpott and C. C. et al., 2008) and is capable of utilizing siderophores produced by other microorganisms (Lesuisse and E. et al., 2001; Haas and H. H. et al., 2008). While iron

deficiency is the primary regulator of siderophore synthesis, external factors such as pH, temperature, carbon source and other metals also exert a significant influence (Winkelmann, G. 2007).

The biosynthesis of siderophores in microorganisms is induced by intracellular iron deficiency, after which these small, high-iron affinity molecules are secreted into the environment to be scavenged by the cell for iron (Andrews and S.C. et al., 2003; Wandersman and C. et al., 2004; Salvail and H. et al., 2012). There are two pathways involved in the synthesis of siderophores: NRPS (Challis and G. L. et al., 2005; Oves-Costales and D. et al., 2009), independent of NRPS (Challis and G. L.et al., 2005; Oves-Costales and D. et al., 2009) and dependent on non-ribosomal peptide synthetases (NRPS) (Gehring and A.M. et al., 1997; Keating and T. A et al., 2000). Non-ribosomal peptide synthetases are large multi-enzyme complexes responsible for the synthesis of several biologically important peptidic products without an RNA template (Grünewald and J. et al., 2006).

In the context of agricultural practices, the inoculation of soil with Pseudomonas putida, which produces pseudobactin, has been demonstrated to enhance the growth and yield of a diverse range of plants (Kloepper et al., 1980). Furthermore, the excessive accumulation of heavy metals is toxic to the majority of plants and results in soil contamination, which in turn leads to a reduction in soil microbial activity and soil fertility, as well as yield losses (McGrath et al., 1995). In this concern, hydroxamate-type siderophores present in soil play an important role in immobilizing the metals. Many bacteria suppress the growth of deleterious microorganisms by production of siderophore, antibiotics, and cyanide (Husen, 2003). Siderophores are themselves growth inhibitors of various phytopathogenic fungi. In the treatment of thalassemia and certain other anemias, periodic whole-blood transfusions are required (Hershko et al., 2002). Since there is no specific physiological mechanism for the excretion of iron in man, continued transfusion therapy leads to a steady buildup of iron. Such disease can be efficiently treated with siderophore-based drugs and siderophore can act as a principal model (Pietrangelo, 2002).

2. MATERIAL AND METHOD

2.1. Bacterial isolation and growth conditions

For the isolation of siderophore-producing bacteria, 1 g of soil samples were weighed. 9 ml of NaCI was added to the FTS, thereby forming a soil suspension. A series of dilutions were made. Diluted suspensions (100 µL) were spread on Chrome azurol S (CAS) agar plates. Positive colonies were selected and repeatedly transferred to Luria-Bertani (LB) agar plates until pure cultures were obtained. The compositions of LB solid media were as follows: 10.0 g tryptone, 5.0 g yeast extract, 10.0 g NaCl, deionized water 1000 mL, 1.8% agar. Plates were incubated at 30 °C for 48 hours.

2.2. Iron chelating chrome azurol S test

The iron-chelating chromazurol S (CAS) test, as described by Schwyn and Neilands (1987), was conducted in both solid and liquid media. Selected microorganisms were cultivated on chromazurol S (CAS) agar plates, which undergo a color change from blue to yellow/orange upon binding to Fe and revert to blue upon release. To prepare the medium, 60.5 mg of CAS was dissolved in 50 mL of deionized water (dH_2O), followed by the addition of 10 mL of an iron solution containing 1 mM $FeCl_3$ and 10 mM HCl. HDTMA (72.9 mg) was dissolved in 40 mL of dH_2O before being combined with the CAS/iron solution, resulting in a total volume of 100 mL. Separately, 900 mL of each growth medium was prepared with the right amount of components for 1000 mL, as this would be the final volume of the medium without the addition of iron, but after combining with the CAS/iron solution. LB medium was used for the bacteria to utilize carbon sources and grow. For the solid medium, 1.8% agar was added to the liquid medium. The medium was sterilized at 121 °C for 30 minutes. The sterilized CAS semi-solid medium was cooled to approximately 60°C and poured into petri dishes. Color changes near the colonies on the plates were observed. The color around the colony on the plate changed from blue to orange, indicating siderophore production.

2.3. Determination of siderophore type

Isolates were cultivated in an iron-deficient liquid medium (MM9) (Payne, 1994). Tests were conducted on the supernatants of isolates cultured in MM9 medium. The final composition of the modified M9 medium (MM9 medium) was as follows: The solution was composed of 0.3 $g.L^{-1}$ KH_2PO_4, 0.5 $g.L^{-1}$ NaCl, 1.0 $g.L^{-1}$ NH_4Cl, 6.0 $g.L^{-1}$ NaOH, and 30.24 $g.L^{-1}$ PIPES. The solution was then subjected to autoclaving and subsequently supplemented with 30 mL of 10% (m/v) casamino acids (3% of 8-hydroxyquinoline in chloroform), 2.0 $g.L^{-1}$ fructose, 1 mL of 1M $MgCl_2$, and 1 mL of 100 mM $CaCl_2$. The solutions were prepared separately and sterilized. The type of siderophore was determined through the

implementation of specific assays. The detection of different siderophore types was conducted using the Arnow, Csaky, and Shenker tests, which employed the following specific methodologies:

2.3.1. Arnow test

An aliquot of 1.0 mL of the culture supernatant was combined with 0.1 mL of 5 mol.L^{-1} HCl. Subsequently, 1 mL of the nitrite-molybdate reagent (comprising 10.00 g $NaNO_2$ and 10.00 g Na_2MoO_4, dissolved in 100 mL deionized water) was added. If catechol was present in the supernatant, the nitrite in the solution underwent dissociation to form a yellow NO ligand. Subsequently, 0.5 mL of 2 mol.L^{-1} NaOH was added. If catecholates are found to contain siderophores, the solution will exhibit a red coloration (Arnow, 1937). The measurement was taken at a wavelength of 515 nm.

2.3.2.Csaky test

An aliquot of 1.0 mL of the culture supernatant was combined with one milliliter of 1 N H_2SO_4 and heated to boiling for 6 h. Subsequently, 3 mL of sodium acetate solution (3.50 g.L^{-1}) was added and thoroughly mixed. Subsequently, 0.5 mL of a sulfanilic acid solution (comprising 1.0 g of sulfanilic acid dissolved in 100 mL of 30% acetic acid) and 0.2 mL of an iodine solution (comprising 1.30 g of iodine dissolved in 100 mL of acetic acid) were added. The solution was then permitted to stand for 5 min at room temperature. Subsequently, 0.2 mL of sodium thiosulfate (2.00 g sodium thiosulfate dissolved in 100 mL deionized water) and 0.1 mL of α-naphthylamine solution (3.00 g α-naphthylamine in 1000 mL 30% acetic acid) were added, and the color was allowed to develop for 20–30 minutes. The presence of hydroxamate siderophores was confirmed by the observation of a pink solution color, as described by Csaky (Csaky, 1948). The measurement was taken at a wavelength of 526 nm.

2.3.3.Shenker test

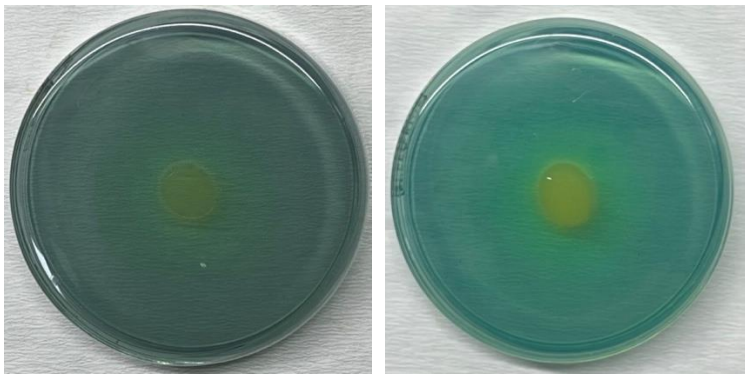
An aliquot of 1.0 mL of the culture supernatant was added to a tube, along with 1.0 mL of 250 µmol.L^{-1} $CuSO_4$ and 2.0 mL of an acetic acid buffer with a pH 4. The tube was then incubated at room temperature for 30 min. In the event that a hydroxycarboxylate siderophore is present in the supernatant, it will form a compound with copper. Through full wavelength scanning, a maximum absorption peak in the range of 190-280 nm was identified, thereby confirming the presence of a carboxylate siderophore in the supernatant (Shenker, 1992).

3. RESULTS

3.1. Results of chromeazurol S agar test

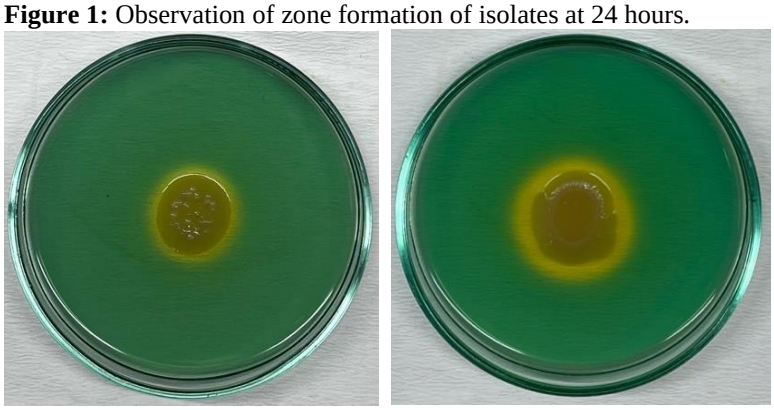
The results demonstrated that all isolates produced siderophores. The zone images of the various isolates are presented in Figure 1. Isolate 174 and isolate 61 demonstrated the greatest intensity of siderophore production. The zone of formation in yellow is more pronounced than that of isolate 61. As illustrated in Figure 2, All bacteria that produced siderophores formed a yellow zone around them. The production of siderophores was confirmed. Additionally, siderophore production was evaluated in a liquid medium. All isolates were found to produce siderophores, as expected. These results are illustrated in Figure 3. All isolates were found to produce siderophores, albeit at varying concentrations. It can be interpreted depending on the intensity of the shingles and yellow color.

The Arnow, Csaky, and Shenker tests were employed to ascertain the types of siderophores present. All isolates were found to be capable of producing catecholate, hydroxamate, and carboxylate-type siderophores. Isolate 174 exhibited a yellow coloration in the Arnow test. The results of these tests are illustrated in Figures 4 and 5. No pink color change was observed in the Csaky test. The spectrophotometric results obtained in the Shenker test indicate that isolate 61 produces carboxylate-type siderophores, with a maximum peak between 190-280. However, no peak formation was recorded for isolate 174. These results are shown in Figure 6.



Isolate 61 Isolate 174

Figure 1: Observation of zone formation of isolates at 24 hours.



Isolate 61 Isolate 174

Figure 2 : Observation of zone formation of isolates on day 5.

3.2. Chromeazurol S (CAS) liquid test results

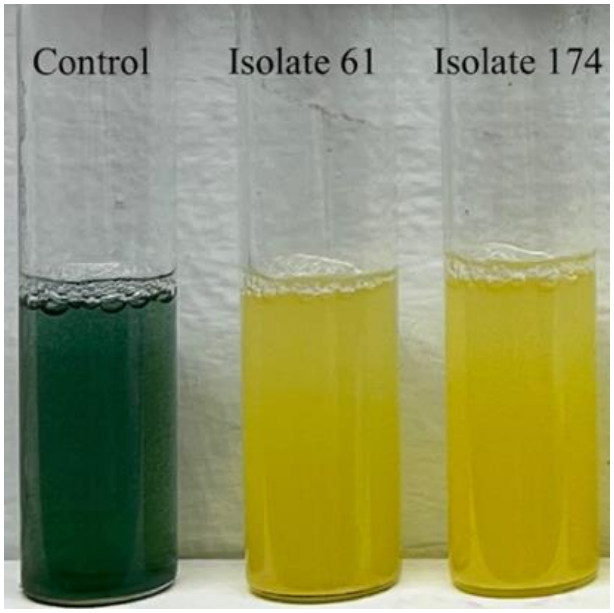


Figure 3 : Observation of color change of isolates on day 5.

3.3. Identification of siderophore types

Arnow test, Csaky test and Shenker test were used to determine siderophore types. In the arnow test, a yellow color change occurs after the addition of nitrite-molybdate. It is considered positive. Isolate 61 was evaluated as negative. Isolate 174 turned yellow at 30 minutes. It can be said to produce catecholate type siderophores at low concentration.

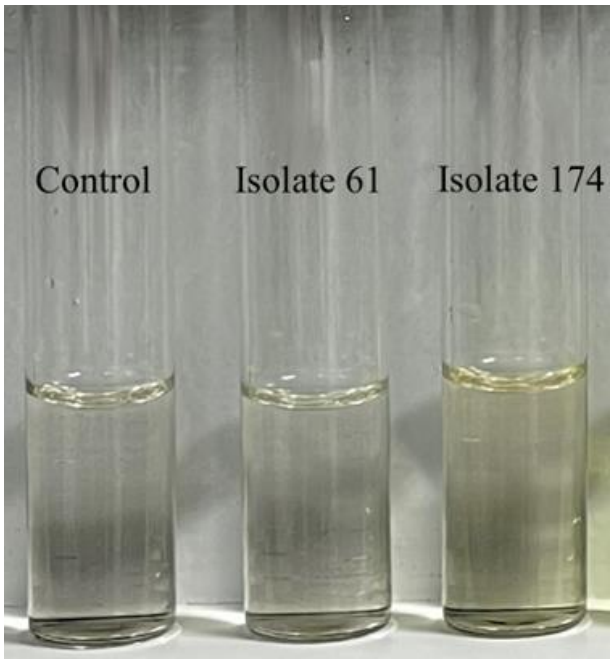


Figure 4: Arnow's test results for the detection of catecholate-type siderophores in isolates.

A pink color change in the Csaky test is considered positive. According to the Csaky test results, pink-white color formation was observed in isolate 61. isolate 174 has a white color formation. Both are negative results. It is interpreted as not producing hydroxamate type siderophore.

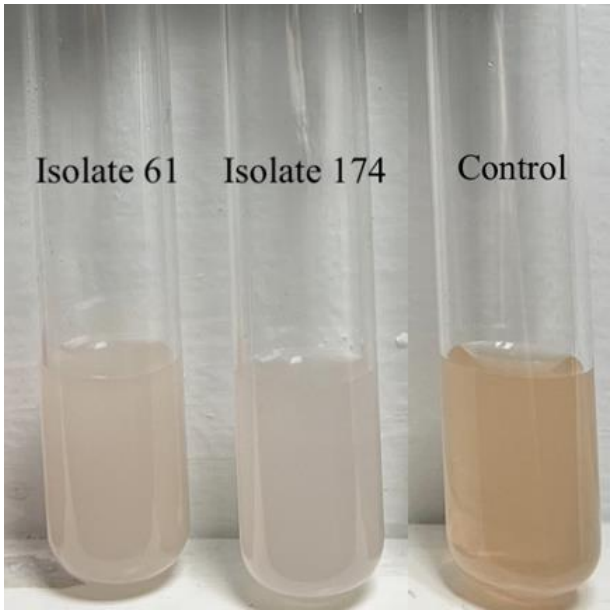
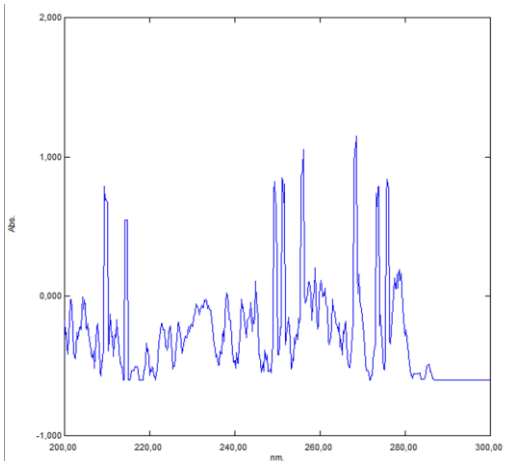
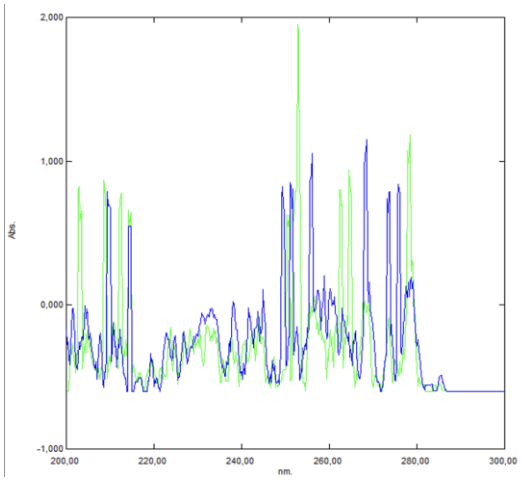


Figure 5: Results of hydroxamate-type siderophore activity, using cell-free culture supernatants.

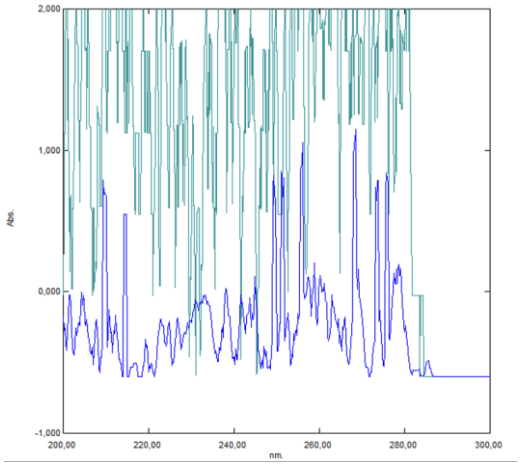
The maximum peak between 190-280 nm is a positive result for Shenker test. Maximum peak was observed in isolate 61. Positive result. Mixed peak formations were observed in isolate 174. There is no maximum peak formation. Negative result.



Control



- Isolate 61



- Isolate 174

Figure 6 : Spectrophotometric results of Shenker's test for the detection of carboxylate-type siderophores in isolates.

4. DISCUSSION AND CONCLUSIONS

Siderophores are of vital importance to the survival of microorganisms and their ability to adapt to their environment. Furthermore, they are a significant contributing factor in the development of pathogenicity and antibiotic resistance. The critical biological functions of siderophores have made them a significant area of focus in both microbiological research and medical applications. The diverse range of siderophore types enables organisms to adapt to varying environmental conditions and can exert a considerable influence on the virulence of pathogenic microorganisms. It is therefore important to gain a deeper understanding of siderophores if new approaches to the treatment of infectious diseases are to be developed. Further research is directed towards the development of effective methods for the utilization of siderophores in bioremediation and biocontrol, to enhance their application in the environment. To enhance the utility of siderophores in environmental applications, it is imperative to conduct comprehensive research on the variability of siderophores and their structural and functional attributes in the context of microbial communities.

REFERENCES

Ahmed, E., and Holmström, S. J., (2014). Siderophores in environmental research: roles and applications. *Microbial biotechnology*, *7*(3), 196-208. https://doi.org/10.1111/1751-7915.12117

Andrews, S. C., Robinson, A. K., & Rodríguez-Quiñones, F. (2003). Bacterial iron homeostasis. *FEMS microbiology reviews*, *27*(2-3), 215-237. https://doi.org/10.1016/S0168-6445(03)00055-X

Arnow, L. E. (1937). Colorimetric determination of the components of 3, 4-dihydroxyphenylalanine-tyrosine mixtures. *J. biol. Chem*, *118*(2), 531-537. https://doi.org/10.1016/S0021-9258(18)74509-2

Boukhalfa, H., Lack, J. G., Reilly, S. D., Hersman, L. E., and Neu, M. P., (2003). Siderophore production and facilitated uptake of iron plutonium in *P. putida* (No. LA-UR-03-0913). Los Alamos National Lab. (LANL), Los Alamos, NM (United States). https://doi.org/10.1063/1.1594658

Capon, R. J., Stewart, M., Ratnayake, R., Lacey, E., & Gill, J. H. (2007). Citromycetins and bilains A–C: new aromatic polyketides and diketopiperazines from Australian marine-derived and terrestrial Penicillium spp. *Journal of Natural Products*, *70*(11), 1746-1752. https://doi.org/10.1021/np0702483

Challis, G. L. (2005). A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. *Chembiochem*, *6*(4), 601-611. https://doi.org/10.1002/cbic.200400283

Csáky, T. Z., Hassel, O., Rosenberg, T., Lång, S., Turunen, E., & Tuhkanen, A. (1948). On the estimation of bound hydroxylamine in biological materials. *Acta chemica scandinavica*, *2*, 450-454. 10.3891/acta.chem.scand.02-0450

Dimkpa, C. O., Svatoš, A., Dabrowska, P., Schmidt, A., Boland, W., & Kothe, E. (2008). Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in Streptomyces spp. *Chemosphere*, *74*(1), 19-25. https://doi.org/10.1016/j.chemosphere.2008.09.079

Gamit, D. A., and Tank, S. K., (2014). Effect of siderophore producing microorganism on plant growth of Cajanus cajan (Pigeon pea). *Int J Res Pure Appl Microbiol*, *4*(1), 20.

Gehring, A. M., Bradley, K. A., & Walsh, C. T. (1997). Enterobactin biosynthesis in Escherichia coli: isochorismate lyase (EntB) is a bifunctional enzyme that is phosphopantetheinylated by EntD and then acylated by EntE using ATP and 2, 3-dihydroxybenzoate. *Biochemistry*, *36*(28), 8495-8503. https://doi.org/10.1021/bi970453p

Grünewald, J., & Marahiel, M. A. (2006). Chemoenzymatic and template-directed synthesis of bioactive macrocyclic peptides. *Microbiology and molecular biology reviews*, *70*(1), 121-146. https://doi.org/10.1128/mmbr.70.1.121-146.2006

Haas, H., Eisendle, M., & Turgeon, B. G. (2008). Siderophores in fungal physiology and virulence. *Annu. Rev. Phytopathol.*, *46*(1), 149-187. https://doi.org/10.1146/annurev.phyto.45.062806.094338
Hershko, C., Link, G., & Konijn, A. M. (2002). Cardioprotective effect of iron chelators. *Iron Chelation Therapy*, 77-89. https://doi.org/10.1007/978-1-4615-0593-8_5

Holinsworth, B., & Martin, J. D. (2009). Siderophore production by marine-derived fungi. *BioMetals*, *22*, 625-632. https://doi.org/10.1007/s10534-009-9239-y

Huber, D. L., (2005). Synthesis, properties, and applications of iron nanoparticles. *Small*, *1*(5), 482-501. https://doi.org/10.1002/smll.200500006

Husen, E. (2003). Screening of soil bacteria for plant growth promotion activities in vitro. *Indonesian Journal of Agricultural Science*, *4*(1), 27-31.

Jellison, J., Chandhoke, V., Goodell, B., & Fekete, F. A. (1991). The isolation and immunolocalization of iron-binding compounds produced by Gloeophyllum trabeum. *Applied microbiology and biotechnology*, *35*, 805-809. https://doi.org/10.1007/BF00169899

Keating, T. A., Marshall, C. G., & Walsh, C. T. (2000). Reconstitution and characterization of the Vibrio cholerae vibriobactin synthetase from VibB, VibE, VibF, and VibH. *Biochemistry*, *39*(50), 15522-15530. https://doi.org/10.1021/bi0016523

Khan, A., Singh, P., & Srivastava, A. (2018). Synthesis, nature and utility of universal iron chelator–Siderophore: A review. *Microbiological research*, *212*, 103-111. https://doi.org/10.1016/j.micres.2017.10.012

Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, *286*(5776), 885-886. https://doi.org/10.1038/286885a0

Kraemer, S. M., (2004). Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic sciences*, *66*, 3-18. https://doi.org/10.1007/s00027-003-0690-5

Lesuisse, E., Blaiseau, P. L., Dancis, A., & Camadro, J. M. (2001). Siderophore uptake and use by the yeast Saccharomyces cerevisiae. *Microbiology*, *147*(2), 289-298. https://doi.org/10.1099/00221287-147-2-289
McGrath, S. P., Chaudri, A. M., & Giller, K. E. (1995). Long-term effects of metals in sewage sludge on soils, microorganisms and plants. *Journal of industrial microbiology*, *14*, 94-104. https://doi.org/10.1007/BF01569890
Miethke, M., & Marahiel, M. A., (2007). Siderophore-based iron acquisition and pathogen control. Microbiol Rev, *71*(3), 413-451. https://doi.org/10.1128/mmbr.00012-07

Oves-Costales, D., Kadi, N., & Challis, G. L. (2009). The long-overlooked enzymology of a nonribosomal peptide synthetase-independent pathway for virulence-conferring siderophore biosynthesis. *Chemical communications*, (43), 6530-6541. https://doi.org/10.1039/B913092F

Payne, S. M. (1994). Detection, isolation and characterization of siderophores. *Methods in enzymology*, *235*, 329-344. https://doi.org/10.1016/0076-6879(94)35151-1

Philpott, C. C., & Protchenko, O. (2008). Response to iron deprivation in Saccharomyces cerevisiae. *Eukaryotic cell*, *7*(1), 20-27. https://doi.org/10.1128/ec.00354-07
Pietrangelo, A. (2002). Mechanism of iron toxicity. *Iron Chelation Therapy*, 19-43. https://doi.org/10.1007/978-1-4615-0593-8_2

Rajkumar, M., Ae, N., Prasad, M. N. V., & Freitas, H. (2010). Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in biotechnology*, *28*(3), 142-149. https://doi.org/10.1016/j.tibtech.2009.12.002

Saha, M., Sarkar, S., Sarkar, B., Sharma, B. K., Bhattacharjee, S., & Tribedi, P. (2016). Microbial siderophores and their potential applications: a review. *Environmental Science and Pollution Research*, *23*, 3984-3999. https://doi.org/10.1007/s11356-015-4294-0

Salvail, H., & Massé, E. (2012). Regulating iron storage and metabolism with RNA: an overview of posttranscriptional controls of intracellular iron homeostasis. *Wiley Interdisciplinary Reviews: RNA*, *3*(1), 26-36. https://doi.org/10.1002/wrna.102

Schwyn B, Neilands JB. (1987). Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 160:47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Shenker, M., Oliver, I., Helmann, M., Hadar, Y., & Chen, Y. (1992). Utilization by tomatoes of iron mediated by a siderophore produced by Rhizopus arrhizus. *Journal of plant Nutrition*, *15*(10), 2173-2182. https://doi.org/10.1080/01904169209364466

Thieken, A., & Winkelmann, G. (1992). Rhizoferrin: a complexone type siderophore of the mocorales and entomophthorales (Zygomycetes). *FEMS microbiology letters*, *94*(1-2), 37-41. https://doi.org/10.1111/j.1574-6968.1992.tb05285.x

Van der Helm, D., & Winkelmann, G. (2020). Hydroxamates and polycarboxylates as iron transport agents (siderophores) in fungi. In *Metal ions in fungi* (pp. 39-98). CRC Press. https://doi.org/10.1201/9781003067221

Wandersman, C., & Delepelaire, P. (2004). Bacterial iron sources: from siderophores to hemophores. *Annu. Rev. Microbiol.*, *58*(1), 611-647. https://doi.org/10.1146/annurev.micro.58.030603.123811

Wencewicz, T. A., Möllmann, U., Long, T. E., & Miller, M. J. (2009). Is drug release necessary for antimicrobial activity of siderophore-drug conjugates? Syntheses and biological studies of the naturally occurring salmycin "Trojan Horse" antibiotics and synthetic desferridanoxamine-antibiotic conjugates. *Biometals*, *22*, 633-648. 10.1007/s10534-009-9218-3

Winkelmann, G. (2007). Ecology of siderophores with special reference to the fungi. *Biometals*, *20*, 379-392. https://doi.org/10.1007/s10534-006-9076-1

Evaluation of Başkent University Kahramankazan Vocational School 2023-2024 Academic Year 3+1 Workplace Practice Education Model

Tuncer Demirel*[1], Sinan Can Altuntaş[1], Erkan Yildiz[1]

Abstract: The aim of this study is to share the data obtained as a result of the questionnaires applied to companies and students within the scope of the 3+1 workplace practice education model, which is included in the curriculum of Başkent University Kahramankazan Vocational School and implemented in the spring semester of the 2023-2024 academic year. The 3+1 workplace practice training model was carried out for 14 weeks. Survey data were obtained from 133 of the 136 students who participated in the application. The survey participation rate was 98%. Of the students participating in the survey, 23.3% were female (N=31) and 76.7% were male (N=102). The application was carried out in 76 different workplaces. 59.4% (N=79) of the students suggested the workplaces where they wanted to practice by themselves, while 40.6% (N=54) were found by the instructors. Job offers were made to 48.1% (N=64) of the students who participated in the application. While 57.8% (N=37) of the students accepted the job offer, 42.2% (N=27) did not accept the job offer. The reasons why students did not accept job offers were determined as 10.5% (N=14) because of the DGS exam, 6% (N=8) because they did not want to work, and 3.8% (N=5) because they did not like the conditions of the workplace. 51.9% (N=69) of the students were not offered a job. Among the reasons why companies did not make job offers, the biggest reason was found to be the lack of personnel needs. According to the students' perceptions, the application was evaluated as helping them to learn planned and organized work, teamwork and the practical equivalents of theoretical knowledge in business life, and that the application helped them to better prepare for business life and increase their chances of finding a job. According to the perceptions of the managers of the companies that accepted students, students helped the companies by taking responsibility for the work done in the companies and by working as a team, and it was also found that the students contributed to the development of the companies by offering new ideas and suggestions. As a result of the study, it was seen that the 3+1 workplace practice education model contributed to students to gain experience and adapt to business life more easily.

Keywords: Vocational Education, Vocational School, 3+1 Workplace Practice Education Model, Questionnaire Study, Statistics.

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1. GİRİŞ

Meslek yüksekokulları, belirli meslek ve iş alanlarında çalışacak nitelikli insan gücü yetiştirmek için kurulmuş yükseköğretim kurumlarıdır. Meslek yüksekokulları, öğrencilere teorik ve uygulamalı eğitim vererek, onları iş hayatına hazırlamaktadır. Meslek yüksekokulları, geleceğini kuracak ve inşa edecek olan gençlere, bilgi, beceri, davranış ve iş birliği içinde çalışma alışkanlığı kazandırmaktadır. Meslek yüksekokulları, iş dünyasında ihtiyaç duyulan nitelikli insan gücünü yetiştirerek, ülke ekonomisine katkı sağlamada son derece önemli bir konuma sahiptir. Meslek yüksekokulları, ülkemizde ihtiyaç duyulan nitelikli mesleki eğitimi vermek ve sorumluluk sahibi, üretken ve yetkin nitelikli eleman yetiştirmeyi amaçlamaktadır. Meslek yüksekokullarında eğitim-öğretim, dört yarıyıl boyunca teorik ve uygulamalı olarak sürdürülmektedir. Üç yarıyıl boyunca okulda teorik dersler görülüp bir yarıyılı da işletme, kurum ve kuruluşlarda uygulamalı eğitim olarak gerçekleştirerek öğrenciler mesleki hayatlarına hazırlanmaktadır (Altuntaş vd., 2021).

Verilen bilgilerin doğrultusunda, 3+1 ve 3+3 eğitim modelini uygulayan meslek yüksekokullarının sayısına bakıldığında bu sayının oldukça az olduğu gözlemlenmektedir. Belirtilen modeller içinde 3+1 uygulamasının ise sayıca daha fazla tercih edildiği saptanmıştır. Yüksek Öğretim Kurulu Başkanlığı mesleki eğitimin ülkemiz açısından önemine istinaden yaptığı toplantılarda ve 17 Haziran 2021 tarihli 31514 Sayılı Resmi Gazetede yayımladığı ‘’Yükseköğretimde Uygulamalı Eğitimler Çerçeve Yönetmeliği’’ ile üniversitelere 3+1 modeline geçmelerini tavsiye etmektedir. 3+1 modelinde ilk üç dönemi okulda tamamlayan öğrenciler gerekli kredi ortalamasını tutturmaları halinde dördüncü dönemde 14 hafta süreyle iş yerlerinde, o iş yerlerinin kurallarına tabi olarak çalışmakta ve süre sonunda uygulamayı tamamlamaktadırlar (YÖK, 2017).

Bu çalışmada, Başkent Üniversitesi Kahramankazan Meslek Yüksekokulu tarafından 2023-2024 akademik yılı bahar döneminde 11 farklı önlisans programı tarafından gerçekleştirilen 3+1 iş yeri uygulaması modelinden elde edilen sonuçların paylaşılması amaçlanmıştır. Ayrıca çalışma sonucunda dikkat çekilmek istenilen en önemli nokta; gerçekleştirilen iş yeri uygulaması eğitimi sonucunda uygulamaya katılan öğrencilerin mezun olduktan sonra istihdam edilebilme imkânlarında artış görülmesidir.

2. MATERYAL VE METOT

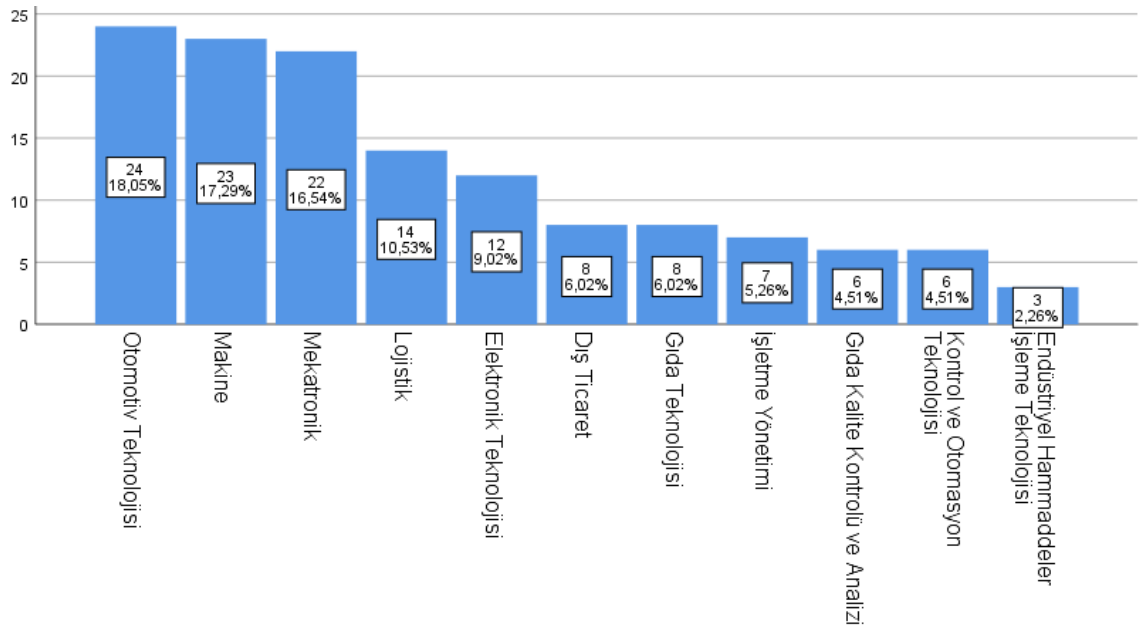
2023/2024 akademik yılı bahar döneminde 3+1 iş yeri uygulaması eğitimine katılan öğrenciler ile öğrenci kabul eden firmalardan anket tekniği ile veriler toplanmıştır. Toplanan veriler SPSS Statistics programı ile analiz edilmiştir. Bu veriler ışığında çalışma nicel metot kullanılarak oluşturulmuştur (Yıldız, 2020).

3+1 iş yeri uygulamasıyla ilgili öğrenci algılarını ölçebilmek için öğrencilere 17 adet soru yöneltilmiştir. Bu soruların 4 adedi üniversiteye kayıt öncesi faaliyetler, 1 adedi uygulama esnası öğretim elemanı desteği, 6 adedi uygulama esnası firma desteği ve 6 adedi de modele ilişkin öğrenci algılarını ölçmeye yöneliktir. Öğrencilerin yargılara 5'li Likert Ölçeği (1=Kesinlikle Katılmıyorum, 2=Katılmıyorum, 3=Ne Katılıyorum Ne Katılmıyorum, 4=Katılıyorum, 5=Kesinlikle Katılıyorum) kullanılarak cevap verilmesi istenmiştir.

3+1 iş yeri uygulamasıyla ilgili firma algılarını ölçebilmek için firmalara 9 adet soru yöneltilmiştir. İş yeri yetkilisinin kimlik, görev ve iletişim bilgileri 3 soruyla anketin birinci bölümünde tespit edilmiştir. İkinci bölümde 6 soruyla; firmanın özellikleri, öğrenciden beklentiler, öğrencilere sağlanan haklar ve istihdam planları belirlenmiştir. Firmaların yargılara 5'li Likert Ölçeği (1=Kesinlikle Katılmıyorum, 2=Katılmıyorum, 3=Ne Katılıyorum Ne Katılmıyorum, 4=Katılıyorum, 5=Kesinlikle Katılıyorum) kullanılarak cevap verilmesi istenmiştir.

3. BULGULAR

2023-2024 Akademik Yılı Bahar döneminde gerçekleştirilen iş başında eğitim uygulamasına yönelik sonuçlara ilişkin bilgiler aşağıdaki tablo ve grafiklerde sunulmuştur. Uygulamaya katılan 136 öğrencinin 133'ünden anket verileri elde edilmiştir. Ankete katılım oranı %98'dir. Ankete katılan öğrencilerin %23,3'ü kız (N=31), %76,7'si erkek (N=102) öğrencidir. Uygulamaya katılan öğrencilerin programlara göre dağılımı Grafik 1'de gösterilmiştir.



Grafik 1. Program Bazlı Öğrenci Sayıları

Uygulama 76 farklı iş yerinde gerçekleştirilmiştir. Bu firmalar içerisinde TUSAŞ, Man Türkiye A.Ş., Türk Traktör ve Kazan Soda gibi çok büyük miktarlarda eleman istihdam eden iş yerleri mevcuttur. Öğrencilerin 3+1 uygulamasını yaptığı

firma listesi Ek'te sunulmuştur. Öğrencilerin %59,4'ü (N=79) uygulama yapmak istedikleri iş yerlerini kendileri önermiş, %40,6'sına (N=54) ise öğretim elemanları tarafından yer bulunmuştur.
Öğrencilere sunulan ücret bilgileri Tablo 1'de istihdam bilgileri de Tablo 2'de sunulmuştur.

Tablo 1. Ücret Bilgileri

Ücret Alan Öğrenci Sayısı	Ücret Almayan Öğrenci Sayısı	Minimum Ödenen Ücret Miktarı	Maksimum Ödenen Ücret Miktarı	Ücret Ortalaması
107	26	3.400	17.002	6.192

Uygulamaya katılan öğrencilerin %80,5'ine (N=107) firmalar tarafından 3.400 TL ile 17.002 TL (asgari ücret) aralığında değişen miktarlarda ücret ödemesi yapılmıştır. Ortalama olarak alınan ücret miktarı ise 6.192 TL'dir. Öğrencilerin %19,5'ine (N=26) firmalar tarafından ödeme yapılmamıştır. Ücret dışında öğrencilerin %62,4'üne (N=83); bayram ikramiyesi, hediye çeki, yiyecek içecek parası ve yol parası vb. yardımlar yapılmıştır.

Tablo 2. İstihdam Bilgileri

Uygulama Sonucu İş Teklifi Alan ve Teklifi Kabul Eden Öğrenci Sayısı	Uygulama Sonucu İş Teklifi Alan ve Teklifi Kabul Etmeyen Öğrenci Sayısı	İş Teklifi Almayan Öğrenci Sayısı
37	27	69

Uygulamaya katılan öğrencilerin %48,1'ine (N=64) firmalar tarafından iş teklifi yapılmıştır. Öğrencilerin %57,8'i (N=37) iş teklifini kabul ederken, %42,2'si (N=27) ise iş teklifini kabul etmemiştir. Öğrencilerin iş tekliflerini kabul etmeme nedenleri; %10,5'i (N=14) DGS sınavı, %6'sı (N=8) çalışmak istememeleri, %3,8'i (N=5) de iş yeri şartlarını beğenmemeleri olarak tespit edilmiştir. Öğrencilerin %51,9'una (N=69) ise iş teklifi yapılmamıştır. Firmaların iş teklifi yapmama nedenleri arasında en büyük neden personel ihtiyaçlarının olmaması olarak tespit edilmiştir.

3+1 uygulaması bahar yarıyılında 14 hafta süreyle uygulanmıştır. Uygulamaya katılan firmaların %95,4'ü (N=104) 14 haftalık sürenin öğrenciyi tanımak ve personel ihtiyacı olması durumunda iş teklifi yapabilmek için yeterli bir süre olarak değerlendirmiştir. 14 haftalık süreyi yeterli bulmayan firmalar, ortalama olarak 18 haftalık bir sürenin daha uygun olacağı yönünde görüş belirtmişlerdir.

3+1 uygulamasıyla ilgili öğrenci algılarını ölçebilmek için öğrencilere 17 adet soru yöneltilmiştir. Bu soruların 4 adedi üniversiteye kayıt öncesi faaliyetler, 1 adedi uygulama esnası öğretim elemanı desteği, 6 adedi uygulama esnası firma desteği ve 6 adedi de modele ilişkin öğrenci algılarını ölçmeye yöneliktir. Öğrencilerin yargılara 5'li Likert Ölçeği (1=Kesinlikle Katılmıyorum, 2=Katılmıyorum, 3=Ne Katılıyorum Ne Katılmıyorum, 4=Katılıyorum, 5=Kesinlikle Katılıyorum) kullanılarak cevap verilmesi istenmiştir.

Üniversite kayıt öncesi faaliyetlere yönelik algılara Tablo 3'te, uygulama esnası firma desteğine yönelik algılara Tablo 4'te ve modele ilişkin öğrenci algılarına da Tablo 5'te yer verilmiştir.

Tablo 3. Üniversite Kayıt Öncesi Faaliyetlere Yönelik Sonuçlar

İfade	Ortalama
Kahramankazan Meslek Yüksekokulu web sayfasında 3+1 eğitim modeliyle ilgili bilgileri inceledim	**3,98**
Kahramankazan Meslek Yüksekokulunda uygulanmakta olan 3+1 eğitim modeli okulu tercih etmemde belirleyici olmuştur	3,55
Kahramankazan Meslek Yüksekokulunda uygulanmakta olan 3+1 eğitim modelini çevremdeki insanlardan duydum ve etkilendim	2,97
Üniversite tanıtım günlerinde 3+1 eğitim modeli hakkında bilgilendirildim	3,68
Üniversite Kayıt Öncesi Faaliyetler	**3,55**

Uygulama öncesi faaliyetlere yönelik öğrenci algılarının ortalaması 3,55 olarak gerçekleşmiştir. En yüksek ortalama 3,98 ile "Kahramankazan Meslek Yüksekokulu web sayfasında 3+1 eğitim modeliyle ilgili bilgileri inceledim"; en düşük ortalama 2,97 ile "Kahramankazan Meslek Yüksekokulunda uygulanmakta olan 3+1 eğitim modelini çevremdeki insanlardan duydum ve etkilendim" ifadelerinde gerçekleşmiştir. Hesaplanan ortalamalara göre öğrenci adaylarının 3+1 modeli hakkında yeterli seviyede bilgilendirildikleri anlaşılmıştır. 3+1 modeli 6 yıldır uygulanmakta olduğu ve pandemi dönemi uygulama yapma imkânı olmadığı için modele yönelik bilinirliğin zaman içinde oluşacağı değerlendirilmektedir.

Uygulama esnası öğretim elemanlarının desteğiyle ilgili sorulan "İş yeri uygulaması süresince yaşadığım problemlere program koordinatörü olan ilgili öğretim elemanımız çözüm üretmiştir" sorunun ortalaması 3,94 olarak hesaplanmıştır. Bu bulguya dayanarak ilgili öğretim elemanlarının uygulama süresince gerekli desteği öğrencilerine sağladıkları söylenebilir.

Tablo 4. Uygulama Esnası Firma Desteği Sonuçları

İfade	Ortalama
İş yeri uygulaması süresince iş yerinden gerekli rehberliği, ilgiyi, desteği ve alakayı gördüm	4,07
İş yeri kendi personelinden beni ayırt etmemiştir	4,00
İş yeri kendi personeline sağlamış olduğu servis, yemek vb. gibi imkânları bana da sağlamıştır	**4,30**
İş yeri uygulaması dersi süresince iş yerinin sağlamış olduğu rehberlik, teknik yetkinlik ve uygulamalı eğitim hizmeti yeterlidir	3,88
İş yeri uygulaması yapmış olduğum işletmede organize edilen sosyal aktivitelere ve faaliyetlere dâhil edilmedim (ters kodlanmıştır)	3,78
İş yeri uygulaması yapmış olduğum işletmede diğer personeller ortak kullanım alanlarında (yemekhane, ofis, mutfak, servis gibi) objektif (tarafsız) olmuşlardır	4,03
Uygulama Esnası Firma Desteği	**4,01**

Uygulama esnası firma desteğine yönelik öğrenci algılarının ortalaması 4,01 olarak gerçekleşmiştir. En yüksek ortalama 4,30 ile "İş yeri kendi personeline sağlamış olduğu servis, yemek vb. imkanları bana da sağlamıştır" ifadesinde gözlemlenmiştir. Tespit edilen ortalamalar ışığında uygulama esnasında firmalarında öğrencilere gerekli desteği sağladıkları anlaşılmıştır. Uygulamanın 14 hafta süreli zaman kısıtı olmasına rağmen firmaların; öğrencilere sağladıkları destekler, ücretlerin zamanında ödenmesi ve en önemlisi kendi personelinden öğrencileri ayrı görmemelerinin örnek seviyede bir davranış olduğu değerlendirilmektedir.

Bu bulgulara dayanarak önümüzdeki dönemde, 3+1 modeli uygulamasıyla üniversite-sektör iş birliklerinin daha da anlam kazanacağı ve böylece nitelikli işgücünü hazırlamak ve istihdam etmenin daha bilimsel olarak teşekkül edeceği değerlendirilmektedir.

Tablo 5. Modele İlişkin Öğrenci Algı Sonuçları

İfade	Ortalama
İş yeri uygulaması dersi; okulda öğretilen teorik bilgilerin pratik uygulamalara aktarılabilmesi adına yararlı ve faydalı bir eğitim sistemidir	4,05
İş yeri uygulaması dersi vasıtası ile okulda öğrenilen teorik bilgileri iş hayatında uygulayabilme şansım olmuştur	3,91
İş yeri uygulaması dersi yerine okulda teorik derslerin devam etmesi görüşündeyim (ters kodlanmıştır)	3,39
İş yeri uygulaması dersini gereksiz bir faaliyet olarak görmekteyim (ters kodlanmıştır)	3,95
İş yeri uygulaması yaptığınız işletmeyi iş yeri uygulaması yapacak olan arkadaşıma tavsiye ederim	3,83
İş yeri uygulaması planlı ve düzenli çalışma, ekip ve takım çalışmasına dâhil olma gibi hususlarda etkili rol oynamıştır	**4,11**
Modele İlişkin Öğrenci Algıları	**3,87**

3+1 iş yeri uygulaması eğitimi modeline yönelik öğrenci algılarının ortalaması 3,87 olarak gerçekleşmiştir. En yüksek ortalama 4,11 ile "İş yeri uygulaması planlı ve düzenli çalışma, ekip ve takım çalışmasına dâhil olma gibi hususlarda etkin rol oynamıştır" ifadesinde gözlemlenmiştir. Modele ilişkin öğrenci algıları doğrultusunda model uygulamasının öğrenci beklentilerine cevap veren bir sistem olduğu ifade edilebilir. Özellikle öğrencilerin teorik bilgilerini iş hayatında uygulama şansı elde etmiş olmaları ve uygulama yaptığı firmalardan iş teklifi almaları üniversite eğitimi sonrasındaki belirsizliklere ışık tutabilecek derecede önemli bir bulgu olarak değerlendirilmektedir.

Uygulama yapılan iş yerinde, iş yerinden kaynaklı sosyal problem yaşanma durumuna yönelik sorulan açık uçlu soruya öğrenciler herhangi bir problem yaşamadıklarını beyan etmişlerdir.

İş yeri uygulamasıyla ilgili ilave olarak ifade etmek istedikleri hususlara yönelik olarak sorulan açık soruya öğrenciler; tecrübe kazandıkları çok faydalı bir uygulama olduğu, okulda öğrendikleri teorik bilgileri uygulamada kullanabildiklerini, disiplinli ve programlı çalışma esaslarına alıştıkları, iş hayatına hazırlık açısından ciddi katkılar edindiklerini ifade etmişlerdir.

3+1 uygulamasıyla ilgili firma algılarını ölçebilmek için firmalara 9 adet soru yöneltilmiştir. Firmaların yargılara 5'li Likert Ölçeği (1=Kesinlikle Katılmıyorum, 2=Katılmıyorum, 3=Ne Katılıyorum Ne Katılmıyorum, 4=Katılıyorum, 5=Kesinlikle Katılıyorum) kullanılarak cevap verilmesi istenmiştir. Firma algılarına yönelik sonuçlara Tablo 6'da yer verilmiştir.

Tablo 6. Firma Algıları Sonuçları

İfade	Ortalama
İş yeri uygulaması dersi süresince iş yerimize gelen öğrenciler işletmede verimli ve işletmeye katkı sağlayacak şekilde çalışmışlardır	4,47
İş yeri uygulaması dersi; okulda öğretilen teorik bilgilerin pratik uygulamalara aktarılabilmesi adına yararlı ve faydalı bir eğitim sistemi modelidir	4,42
İş yeri uygulaması dersi vasıtası ile iş yerimize yönlendirilen öğrenciler okulda öğrendikleri teorik bilgileri iş hayatında uygulayabilme şansları olmuştur	4,29
İş yeri uygulaması dersi yerine okulda teorik derslerin devam etmesi görüşündeyim (ters kodlanmıştır)	3,24
İş yeri uygulaması dersini gereksiz ve verimsiz bir faaliyet olarak görmekteyim (ters kodlanmıştır)	4,05
İş yeri uygulaması dersi süresince iş yerimizin sağlamış olduğu rehberlik, teknik ve uygulamalı eğitim hizmetinin yeterli olduğunu düşünüyorum	4,38
İş yeri uygulaması dersi süresince üniversitedeki koordinatör öğretim elemanının sağlamış olduğu rehberlik ve iletişim hizmeti yeterlidir	4,05
İş yeri uygulaması dersi Üniversite-Sanayi ve İş Dünyası ortaklığı adına faydalı bir eğitim sistemidir	**4,49**
İş yeri uygulaması dersi kapsamında gelecek dönemlerde de üniversitenize destek ve katkı vermek isteriz	4,08
Firma Algıları	**4,16**

3+1 modeline yönelik firma algılarının ortalaması 4,16 olarak gerçekleşmiştir. En yüksek ortalama 4,49 ile "İş yeri uygulaması dersi Üniversite-Sanayi ve İş Dünyası ortaklığı adına güzel bir eğitim sistemidir" ifadesinde gözlemlenmiştir.

Bu sonuçlarla birlikte, 4,47 ortalama ile gerçekleşen "İş yeri uygulaması süresince iş yerimize gelen öğrenciler işletmede verimli şekilde ve işletmeye katkı sağlayarak çalışmışlardır"; 4,42 ortalama gerçekleşen "İş yeri uygulaması dersi; okulda öğretilen teorik bilgilerin pratik uygulamalara aktarılabilmesi adına yararlı ve faydalı bir eğitim sistem modelidir"; 4,29 ortalama ile gerçekleşen "İş yeri uygulaması dersi vasıtası ile iş yerimize yönlendirilen öğrenciler okulda öğrendikleri teorik bilgileri iş hayatında uygulayabilme şansları olmuştur" ve 4,05 ortalama ile gerçekleşen "İş yeri uygulaması dersini gereksiz ve verimsiz bir faaliyet olarak görmekteyim (ters kodlanmıştır)" yargılarının da çok önemli sonuçlar olduğu düşünülmektedir.

Firma algılarına yönelik ortalamaların oldukça yüksek olarak tespit edilmiş olması nedeniyle 3+1 model uygulamasının sektör tarafından da desteklenen bir uygulama olduğu değerlendirilmektedir. Dolayısıyla firmaların gelecek dönemde de uygulamaya destek verecekleri yönünde bir öngörüde bulunulabilir. Firmalara yöneltilen son sorunun gerçekleşen 4,08 ortalaması da öne sürülen öngörüye dayanak olarak belirtilebilir.

İş yeri uygulaması için firmaya gelen öğrencilerden kaynaklı sosyal problemlerle (işe uzak durma, iş yerinin düzenini bozucu tavırlar sergileme, öğrenmekten kaçınma, uyumsuzluk, performans düşüklüğü vb.) ilgili sorulan açık uçlu soruya bütün firmalar sorun yaşanmadığını belirtmişlerdir.

İş yeri uygulaması dersi konusunda ilave olarak belirtilmek istenen olumlu/olumsuz düşüncelere yönelik açık uçlu soruya; uygulamanın hem firma hem de öğrenci için çok faydalı bir uygulama olduğu, teorik bilginin pratiğe aktarılması için mükemmel bir fırsat olduğu ile öğrencilerin iş hayatına daha iyi hazırlanabilecekleri, öğrenciyi yakından tanıma imkânlarının olmasının büyük bir avantaj olduğu, uygulamadan memnun olduklarını, uygulama süresinin arttırılabileceğini ifade etmişlerdir.

4. TARTIŞMA VE SONUÇLAR

Üniversiteler, endüstri ve diğer sektörler arasındaki iş birlikleri, nitelikli öğrenci yetiştirme hedefleriyle her geçen gün daha kritik bir öneme sahip olmaktadır. Staj programları ve benzeri uygulamalar, sektör beklentilerinin karşılanma düzeyini değerlendirmeye yardımcı olmaktadır. Bu değerlendirmeler, yeni programların oluşturulması, ders içeriklerinin güncellenmesi ve teori ile pratik arasındaki dengeyi sağlama gibi akademik faaliyetlere yön veren önemli veriler sunmaktadır (Demirel ve Altuntaş, 2023).

3+1 iş yeri uygulaması, öğrencilere teorik bilgilerini pratikte uygulama fırsatı sunar, böylece iş hayatına daha iyi hazırlanmalarını sağlar. Bu program, öğrencilere iş yerindeki kurallar, amir-ast ilişkileri, ekip çalışması ve örgüt kültürü gibi konularda deneyim kazanma imkanı verir. Bu deneyimler, öğrencilerin iş hayatına uyum sağlamalarını kolaylaştırır.

3+1 iş yeri uygulaması, her öğrenci ve firma için büyük faydalar sağlayan bir faaliyet olarak kabul edilmektedir. Bu program sayesinde, öğrenciler teorik bilgilerini pratikte uygulama şansı bulurken, iş hayatına daha hazır bir şekilde adım atarlar. Aynı zamanda, firmalar da bu uygulama ile potansiyel yeni yetenekleri keşfedebilir ve işgücü ihtiyaçlarını karşılayabilirler.

Yıllar içinde elde edilen verilerle, 3+1 iş yeri uygulaması sisteminin geliştirilmesi için çeşitli tespitler yapılabilmektedir. Bu bulgular, sistemin daha etkin ve verimli hale getirilmesine katkı sağlayabilir.

Ek: Firma Listesi (3 sayfa)

Teşekkür
Yazarlar ankete katılım sağlayan tüm öğrencilere ve firmalara teşekkür etmektedir.

Çıkar Çatışması
Yazarların beyan edecekleri herhangi bir çıkar çatışması yoktur.

KAYNAKLAR

Altuntaş, S.C., Yıldız E., Şahin F.İ., (2021). 3+1 İş Başında Eğitim Uygulaması Modelinin Kahramankazan Meslek Yüksekokulu Örneğinde Değerlendirilmesi. ©2021 Published in 2nd International Symposium on Education and Change December 2021 ISEC2021 Turkey. https://doi.org/10.33793/acperpro.04.03.16.

Demirel, T., Altuntaş, S.C., (2023). Başkent Üniversitesi Kahramankazan Meslek Yüksekokulunda Uygulanan 3+1 İş Yeri Uygulaması Eğitim Modelinin 2022-2023 Akademik Yılı Kapsamında Değerlendirilmesi. 11th International Vocational Schools Symposium 05-07 October 2023, Düzce/Türkiye ISBN: 978-605-71407-6-0.

Yıldız, E. (2020). SmartPLS İle Yapısal Eşitlik Modellemesi Reflektif Ve Formatif Yapılar. Seçkin Yayıncılık, Ankara.

YÖK, (2017). Yükseköğretimde Uygulamalı Eğitimler Çerçeve Yönetmeliği. https://www.resmigazete.gov.tr/17.06.2021 (erişim tarihi: 24.06.2024).

Ekler

3+1 İş Başında Eğitim Uygulaması Yapılan Firma Listesi*

Firma Adı	Uygulamaya Giden Öğrenci Sayısı
MAN Türkiye A.Ş.	16
TAI TUSAŞ - Türk Havacılık ve Uzay Sanayii	15
Türk Traktör ve Ziraat Makinaları A.Ş.	12
Aygersan	6
FAF Vana Sanayi	4
Grand Mercure Otel	4
Ak Otomotiv Tas. Tur. Gid. İns. İth. İhr. San. Tic. Ltd. Şti.	2
Cumhurbaşkanlığı	2
Doğruer Grup Gümrük Müşavirliği	2
İnfinia	2
Karel Elektronik	2
Kazan Soda Elektrik Üretim A.Ş.	2
Metro Gross Market	2
Tavuk Dünyası Gıda San. ve Tic. A.Ş.	2
Türk Standartları Enstitüsü	2
ABC Grup otomobil	1
Ahşapsan Ambalaj Palet Orman Ürünleri ve Geri Kazanım San. Tic. Ltd. Şti.	1
Alfarock Delici	1
Alp Özler Lojistik A. Ş.	1
Alyıldız	1
Arkas Otomotiv Servis ve Ticaret	1
Artı Servis	1
Astor Enerji	1
Atatürk Orman Çiftliği	1
Ayhan Güngör Motorlu Araçlar	1
Aymer Makine San. Ltd. Şti.	1
Ay-Tur Turizm San. ve Tic. A.Ş. Turunc Resort Otel	1
Barko Elektronik	1
Baykoçlar Petrol İnş. Taah. Oto.	1

Firma Adı	Uygulamaya Giden Öğrenci Sayısı
BBS Otomotiv	1
Belekler Dayanıklı Tüketim Mal. İnş. Ltd. Şti.	1
Dak Oto Klinik Tamir Bakım	1
Detay Ford	1
Digikon Otomasyon	1
Durmaksan Makine	1
Ekol Lojistik A. Ş.	1
Ersan Galvaniz	1
Euro Power	1
Eyüp Belediyesi Yemekhanesi	1
Ezer Brothers Dizayn	1
Fıratko Makine İmalat Mühendislik Ltd. Şti.	1
FNSS	1
Gürbüzoğlu Elektrik Sanayi ve Ticaret Ltd. Şti.	1
Harput 33 Yemek San. ve Tic. Ltd. Şti	1
Havelsan Teknoloji Radar	1
İveo Elektronik Savunma	1
Katermak Makine	1
KFB Grup Hazır Proje (Gaziantep)	1
Koluman Motorlu Araçlar Tic. ve San. A. Ş.	1
Korgrup Demirçelik İnş. ve Orm. Ürünleri Ltd. Şti.	1
Lazersan Elektronik	1
M8 Spektral Savunma Sanayi A. Ş.	1
MAK Savunma	1
Mengerler Tic. Türk. A. Ş.	1
Mersin Yayla Bakliyat	1
Metin Işık Oto Tamir	1
Mg Mutlu Garaj Motorlu Araçlar	1
MİM Mühendislik İnşaat Çelik End. San. Tic. A. Ş.	1
MMT Mühendislik Mümessillik Tic. Ltd. Şti.	1
Mutlu Garaj	1

Firma Adı	Uygulamaya Giden Öğrenci Sayısı
Nurol Makine ve Sanayi A. Ş.	1
Orta Anadolu İhracatçı Birlikleri	1
Palmix Yapı Kimyasalları A. Ş.	1
Seyir Savunma A. Ş.	1
SGK Eskişehir Sosyal Güvenlik İl Müdürlüğü	1
TEI TUSAŞ Motor Sanayii A. Ş.	1
Teknosys Yazılım Elektronik	1
TMI Teknolojik	1
Tramecon Asansör	1
Turnak Gümrük Müşavirliği Ltd. Şti.	1
Ulaş Gümrük Müşaviliği Ltd. Şti.	1
Unmaş Unlu Mamüller San. ve Tic. A.Ş. (UNO)	1
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The Application of Artificial Intelligence in Chemistry: Transforming Discovery and Innovation

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Abstract: Artificial Intelligence (AI) is revolutionizing numerous fields, and chemistry is no exception. The integration of AI in chemistry has the potential to accelerate discovery and optimization processes, and lead to the development of novel compounds and materials. This article provides an overview of the application of AI in chemistry, highlighting key areas, main advantages, and future prospects. Here we explore how AI is transforming chemical research and what this means for the future of the field.

Keywords: Artificial Intelligence, chemistry, innovation.

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1. INTRODUCTION

The advent of artificial intelligence has triggered a paradigm shift across various scientific disciplines, including chemistry. Traditionally, chemical research has relied heavily on empirical approaches and trial-and-error methods, often making the “discovery process time-consuming and resource-intensive. However, the incorporation of AI, particularly machine learning (ML) and deep learning (DL) algorithms, has begun to address these challenges, offering new avenues for innovation and efficiency.AI's ability to process vast datasets, recognize patterns, and predict outcomes has made it an indispensable tool in modern chemistry. This article examines the current state of AI in chemistry, providing examples of its application in drug discovery, materials science, reaction optimization, and molecular design. It also discusses the advantages of using AI, such as reducing research costs and accelerating innovation, and offers a prognosis for the future development of AI-driven chemistry.

1.1. Areas and Examples

AI has revolutionized three primary areas in chemistry: drug discovery, materials science, and reaction optimization. In drug discovery, AI tools like DeepMind's AlphaFold predict protein structures, speeding up the identification of drug targets and molecule design. Materials science benefits from AI through predictive models that accelerate the discovery of new materials, such as metallic glasses with unique properties. For reaction optimization, AI techniques like Bayesian optimization and neural networks predict the best experimental conditions, reducing the time and resources needed for chemical research. These applications highlight how AI enhances precision and efficiency across diverse chemical disciplines. We looked into these three main areas with examples (Figure 1)

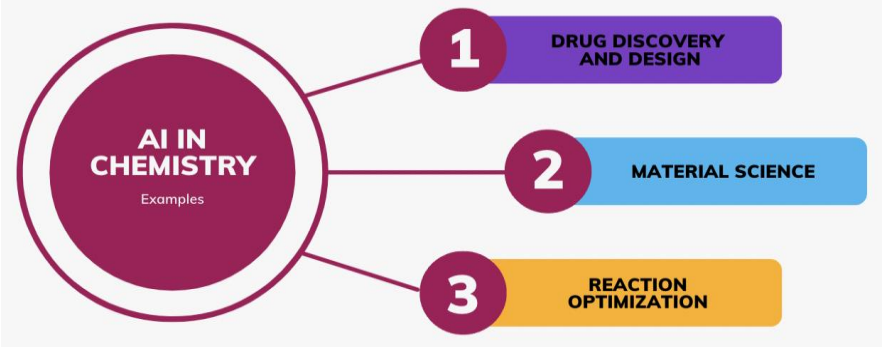

Figure. 1. Examples of AI in Chemistry

1.2. AI in Drug Discovery

One of the most promising applications of AI in chemistry is in the field of drug discovery. Traditionally, drug development is a lengthy and costly process, often taking over a decade and costing billions of dollars. AI has the potential to significantly reduce both the time and cost associated with this process.

Example: DeepMind's AlphaFold

DeepMind's AlphaFold is a prime example of AI's transformative impact on chemistry. AlphaFold, an AI-based protein structure prediction tool, has achieved remarkable success in predicting the 3D structures of proteins from their amino acid sequences. Accurate protein structure prediction is crucial in drug discovery, as it allows researchers to understand the biological mechanisms at play and design drugs that can effectively target specific proteins.

According to a 2020 study published in Nature, AlphaFold demonstrated a median global distance test (GDT) score of 92.4, significantly outperforming other computational methods in predicting protein structures. This breakthrough has been hailed as a "solution" to a 50-year-old problem in biology, underscoring the potential of AI to accelerate drug discovery.

Example: AI-Driven Drug Design

Beyond protein structure prediction, AI is also being used to design new drugs. Generative models, such as Generative Adversarial Networks (GANs) and Variational Autoencoders (VAEs), can generate novel molecular structures with desired properties. For instance, a 2019 study by Zhavoronkov et al. demonstrated the use of GANs in designing novel inhibitors for the enzyme DDR1, which is implicated in cancer. The AI model was able to generate potential drug candidates in a matter of weeks, a process that would typically take years using traditional methods.

1.3. AI in Materials Science

AI's application in materials science is another area where it is making significant contributions. The discovery and optimization of new materials are critical for various industries, including energy, electronics, and manufacturing. However, similar to drug discovery, the traditional trial-and-error approach in materials science is often slow and resource-intensive.

Example: Materials Genome Initiative

The Materials Genome Initiative (MGI), launched by the U.S. government in 2011, aims to accelerate the discovery, development, and deployment of advanced materials. AI plays a crucial role in this initiative by enabling the analysis of large datasets to predict the properties of new materials. For example, AI models can predict the thermal conductivity, electrical properties, and mechanical strength of materials based on their atomic structure.

A 2021 study published in Science Advances highlighted the use of AI in predicting the properties of metallic glasses, a class of materials with unique properties such as high strength and elasticity. The study demonstrated that an AI model could predict the glass-forming ability of metallic alloys with an accuracy of over 90%, significantly reducing the time required to identify new materials.

Example: AI in Catalyst Design

Catalysts are essential for a wide range of chemical processes, including energy conversion, environmental protection, and chemical manufacturing. AI is increasingly being used to design and optimize catalysts with enhanced performance. For example, researchers have developed AI models that can predict the activity and selectivity of catalysts based on their atomic structure.

A notable example is the work of Jorgensen et al., who used a machine learning algorithm to design a novel catalyst for the electrochemical reduction of CO2, a process that converts carbon dioxide into valuable chemicals. The AI-designed catalyst exhibited higher activity and selectivity than previously known catalysts, demonstrating the potential of AI to drive innovation in catalysis.

1.4. AI in Reaction Optimization

Reaction optimization is a critical aspect of chemical research, as it determines the efficiency, yield, and sustainability of chemical processes. Traditional reaction optimization often involves extensive experimentation, which can be time-consuming and costly. AI offers a more efficient approach by predicting the optimal conditions for a given reaction based on historical data.

Example: Bayesian Optimization in Organic Synthesis

Bayesian optimization is a powerful AI technique that has been successfully applied to reaction optimization in organic synthesis. This method involves building a probabilistic model of the reaction landscape and using it to identify the optimal conditions with minimal experimentation.

In a 2018 study published in Nature, researchers applied Bayesian optimization to the Suzuki-Miyaura coupling reaction, a widely used reaction in organic synthesis. The AI model was able to identify the optimal reaction conditions with 85% fewer experiments than traditional methods, significantly reducing the time and resources required for optimization.

Example: AI-Driven Reaction Prediction

In addition to optimization, AI is also being used to predict the outcomes of chemical reactions. Reaction prediction models, such as those based on neural networks, can predict the products of a reaction based on the reactants and conditions. These models are trained on large datasets of known reactions, allowing them to generalize to new, unseen reactions.

A 2020 study published in Chemical Science demonstrated the use of a neural network-based model for reaction prediction. The model achieved an accuracy of 90% in predicting the products of organic reactions, outperforming traditional rule-based approaches. This ability to accurately predict reaction outcomes can greatly accelerate the discovery of new chemical reactions and pathways.

2. DISCUSSION

2.1. Advantages of AI in Chemistry

The integration of AI in chemistry offers several significant advantages that have the potential to transform the field.

i) Accelerated Discovery

AI's ability to rapidly analyze large datasets and predict outcomes can significantly speed up the discovery process in chemistry. For example, the use of AI in drug discovery and materials science has already led to the identification of novel compounds and materials in a fraction of the time required by traditional methods.

ii) Cost Reduction

By reducing the need for extensive experimentation and trial-and-error approaches, AI can lower the costs associated with chemical research and development. This is particularly important in industries such as pharmaceuticals, where the high cost of drug development is a major barrier to innovation. For example, Segler et al. (2018) demonstrated that AI-based synthesis planning reduces resource expenditure by efficiently predicting synthetic routes, cutting the time and cost of reaction planning. Similarly, Stokes et al. (2020) highlighted that AI-accelerated antibiotic discovery can dramatically lower development costs by shortening the research cycle and reducing laboratory testing.

iii) Enhanced Predictive Power

AI models can identify patterns and correlations in complex chemical data that may be difficult or impossible for humans to detect. This enhanced predictive power allows for more accurate predictions of chemical properties, reaction outcomes, and material performance.

iv) Improved Sustainability

AI can contribute to the development of more sustainable chemical processes by optimizing reaction conditions, reducing waste, and identifying more environmentally friendly alternatives. For example, AI-driven catalyst design can lead to more efficient energy conversion processes, reducing the environmental impact of chemical manufacturing.

2.2. Challenges and Limitations

Despite its advantages, the application of AI in chemistry is not without challenges. One of the main limitations is the quality and quantity of available data. AI models require large, high-quality datasets for training, and in many areas of chemistry, such data may be scarce or difficult to obtain.

Additionally, the interpretability of AI models remains a challenge. While AI can make accurate predictions, understanding the underlying reasons for these predictions is often difficult. This "**black box**" nature of AI can be a barrier to its adoption in fields where interpretability is critical.

Another challenge is the integration of AI with existing chemical knowledge and workflows. AI models must be able to complement and enhance traditional approaches rather than replace them entirely. This requires collaboration between chemists and AI experts to ensure that AI tools are used effectively.

3. PROGNOSIS FOR THE FUTURE

The future of AI in chemistry looks promising, with the potential for even greater advancements in the coming years. As AI technology continues to evolve, we can expect to see more sophisticated models that can handle increasingly complex chemical problems.

3.1. AI and Quantum Chemistry

One of the most exciting areas of future development is the integration of AI with quantum chemistry. Quantum chemistry involves the use of quantum mechanical principles to predict the properties of molecules and materials. However, quantum chemical calculations are computationally expensive and time-consuming, limiting their application to small systems.

AI has the potential to accelerate quantum chemistry by providing approximate solutions to quantum mechanical problems with much lower computational cost. For example, AI models can be trained to predict the results of quantum chemical calculations based on a limited set of training data. This could enable the application of quantum chemistry to larger and more complex systems, opening up new possibilities for molecular design and materials discovery.

3.2. AI-Driven Autonomous Laboratories

Another exciting development is the concept of AI-driven autonomous laboratories. These are laboratories where AI algorithms control robotic systems to perform experiments, analyze data, and make decisions in real-time. Autonomous laboratories have the potential to dramatically accelerate the pace of chemical research by performing thousands of experiments in parallel without human intervention.

A notable example is the work of Burger et al., who developed an autonomous laboratory for the optimization of organic reactions. The AI-driven system was able to identify optimal reaction conditions in a fraction of the time required by traditional methods. As AI technology continues to advance, we can expect to see more widespread adoption of autonomous laboratories in chemical research.

3.3. AI and Personalized Medicine

In the field of drug discovery, AI has the potential to revolutionize personalized medicine. Personalized medicine involves tailoring treatments to individual patients based on their genetic makeup, lifestyle, and other factors. AI can analyze large datasets of patient information to identify patterns and predict the most effective treatments for specific individuals.

For example, AI models can be used to predict how different patients will respond to a particular drug based on their genetic profiles. This could lead to the development of more effective and personalized treatments, improving patient outcomes and reducing the risk of adverse effects.

4. DISCUSSION AND CONCLUSIONS

The application of artificial intelligence in chemistry is offering new opportunities for discovery, innovation, and efficiency. From drug discovery and materials science to reaction optimization and catalyst design, AI is enabling chemists to tackle complex problems more effectively and at a lower cost.

While challenges remain, the future of AI in chemistry is bright. As AI technology continues to advance, we can expect to see even greater integration of AI into chemical research, leading to new breakthroughs and innovations that were previously unimaginable. The ongoing collaboration between chemists and AI experts will be crucial in realizing the full potential of AI in this exciting and rapidly evolving field.

REFERENCES

Senior, A. W., Evans, R., Jumper, J., et al. (2020). "Improved protein structure prediction using potentials from deep learning." Nature, 577(7792), 706-710.

Zhavoronkov, A., Ivanenkov, Y. A., Aliper, A., et al. (2019). "Deep learning enables rapid identification of potent DDR1 kinase inhibitors." Nature Biotechnology, 37, 1038-1040.

Ding, H., Fujii, K., & Chen, L. (2021). "AI-accelerated discovery of metallic glasses." Science Advances, 7(6), eaaz4883.

Jørgensen, M., Peterson, A. A., & Studt, F. (2020). "Machine learning-based CO2 reduction catalyst discovery." Nature Communications, 11, 4485.

Granda, J. M., Donina, L., Dragone, V., Long, D. L., & Cronin, L. (2018). "Controlling an organic synthesis robot with machine learning to optimize and discover new reactions." Nature, 559(7714), 377-381.

Schwaller, P., Laino, T., Gaudin, T., et al. (2020). "Molecular transformer: A model for uncertainty-calibrated chemical reaction prediction." Chemical Science, 11(6), 2035-2044.

Burger, B., Maffettone, P. M., Gusev, V. V., et al. (2020). "A mobile robotic chemist." Nature, 583(7815), 237-241.

Segler, M. H. S., Preuss, M., & Waller, M. P. (2018). "Planning chemical syntheses with deep neural networks and symbolic AI." Nature, 555(7698), 604-610.

Stokes, J. M., Yang, K., Swanson, K., et al. (2020). "A deep learning approach to antibiotic discovery." Cell, 180(4), 688-702.

Determination of Cytotoxic and Anti-Carcinogenic activity of Tannic Acid on Ishikawa and HTB-114 Cell Lines

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Abstract: Tannic acid is a polyphenolic compound found naturally in plants. It is found in high amounts in many different plants including tea, coffee, grapes and some tree barks. It is used medicinally for its antioxidant, antimicrobial and anti-inflammatory properties. In some pharmaceutical formulations, tannic acid also has wound-healing and digestive properties. This biological compound stands out as a remarkable substance both for its biological functions in plants and for its various industrial and medical applications. The cytotoxic and anticancer properties of tannic acid have become the focus of scientific research in recent years. Laboratory studies have shown that tannic acid can inhibit the growth and spread of some cancer cells. In this regard, the effect of tannic acid on cytotoxic and apoptotic activity, cell migration and colony formation in Ishikawa and HTB-114 cell lines were investigated in this study. For this purpose, the cytotoxic activity of tannic acid on cell lines was determined by MTT test. HEK293 cell line was used as control group. The half maximal effective concentration (EC50) of tannic acid on cell lines was found to be 39.4 µM for Ishikawa and 62.7 µm for HTB-114. The apoptotic effects of tannic acid were determined by staining the cells with Annexin V/PI by using Flow Cytometry. It was determined that tannic acid showed apoptotic activity in Ishikawa and HTB-114 cell lines and apoptosis rates were found to be 27.4% and 33.2%, respectively. The effect of tannic acid on cell migration was determined by scratching assay. For this purpose, a scratch was made on the petri dish containing the cells and the movement of the cells was observed at 0, 24, 48, 72 hours. Microscope images were analyzed with the help of ImageJ software and wound area percentages were calculated. At the end of 72 hours, Ishikawa cell line showed 87.6% clearance in the wound area where tannic acid was applied, while it was 25.3% in the control group. Likewise, in the HTB-114 cell line, the wound area in the cells treated with tannic acid was 128.4% and cell morphology deterioration was observed, while the area in the control group was 43.4%. Tannic acid was found to inhibit colony formation in cell lines. For this purpose, cells treated with tannic acid were incubated for 7-10 days to form colonies. The formed cell colonies were stained with crystal violet and analyzed. Colony formation in tannic acid treated cells was 1.5% in Ishikawa cell line and 1.2% in HTB-114 cell line. All of these results suggest that tannic acid has the potential to be an alternative drug for endometrial cancer. Further studies will be needed to test this hypothesis.

Keywords: Tannic acid, Cytotoxicity, Apoptosis, Metastasis, Endometrial cancer cell lines

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1. INTRODUCTION

Cancer is a complex group of diseases defined by the uncontrolled growth and spread of abnormal cells. These cells can invade nearby tissues and organs, potentially causing life-threatening conditions. With over 100 different types of cancer, each presents unique characteristics, treatment challenges, and prognoses. Among the most common types are breast, lung, prostate, and colorectal cancers (Hassanpour & Dehghani, 2017). As the global incidence of cancer continues to rise, it remains a leading cause of death worldwide. Cancer treatment varies based on the type, stage, and location of the disease, as well as the patient's overall health. Traditional treatments include surgery, radiation therapy, chemotherapy, and, more recently, targeted therapies and immunotherapy. While these treatments can be effective in managing or curing cancer, they often come with significant side effects (Zugazagoitia et al., 2016).

The search for more effective and less toxic cancer therapies has sparked growing interest in the use of natural products. Plants, herbs, and other natural substances have long been used in traditional medicine to treat various conditions, including cancer (Dar et al., 2017). In recent years, scientific research has increasingly supported the potential of these natural products in cancer treatment. Many natural compounds have shown promise as anticancer agents due to their ability to inhibit cancer cell growth, trigger apoptosis, and prevent metastasis (Azmi et al., 2006). Some of the most studied natural compounds include curcumin, resveratrol, green tea polyphenols, and tannic acid (Chaachouay & Zidane, 2024). Tannic acid, a naturally occurring polyphenol found in various plants, fruits, and nuts, is known for its antioxidant,

antimicrobial, and astringent properties. Structurally, tannic acid consists of a central glucose molecule surrounded by several gallic acid units, making it a potent compound with diverse biological activities (Serrano et al., 2009). Historically, it has been used in traditional medicine for wound healing and as a treatment for various ailments due to its ability to bind to proteins and other organic compounds (Jing et al., 2022).

In recent years, tannic acid has garnered significant interest in oncology due to its potential anticancer properties (Jing et al., 2022). Research has demonstrated that tannic acid operates through multiple mechanisms to inhibit the growth and spread of cancer cells. A key aspect of its anticancer activity is its potent antioxidant effects (Gülçin et al., 2010). By neutralizing free radicals and reducing oxidative stress, tannic acid helps protect cells from DNA damage, a crucial factor in cancer development and progression (Khan & Hadi, 1998). Additionally, tannic acid can disrupt the cancer cell cycle by halting cells in specific phases, preventing further division and proliferation. This cell cycle arrest can lead to decreased tumor growth and increased sensitivity of cancer cells to conventional treatments like chemotherapy and radiation (Jing et al., 2022). Furthermore, tannic acid's anti-inflammatory properties add to its anticancer potential. Since chronic inflammation is a known risk factor for various cancers, tannic acid's ability to reduce inflammation may help lower cancer risk and slow disease progression (Yeo et al., 2020).

The Ishikawa cell line originates from a well-differentiated adenocarcinoma of the human endometrium and is widely used in research to study endometrial cancer and hormonal signalling pathways, particularly those involving estrogen and progesterone receptors. Because the Ishikawa cell line retains many features of normal endometrial cells, it serves as a valuable model for exploring the effects of hormones, drugs, and other factors on endometrial cancer (Nishida, 2002). The HTB-114 cell line, also known as SK-UT-1, is derived from a human uterine leiomyosarcoma, a rare and aggressive type of soft tissue sarcoma that develops from smooth muscle cells (Pistilli et al., 2015). These tumors typically arise in the uterus, though leiomyosarcomas can also form in other smooth muscle tissues. The HTB-114 cell line was established from a metastatic lesion of a leiomyosarcoma, contributing to its aggressive behaviour (Reichardt, 2012).

Laboratory studies have shown that tannic acid can inhibit the growth and spread of some cancer cells. In this regard, the effect of tannic acid on cytotoxic and apoptotic activity, cell migration and colony formation in Ishikawa and HTB-114 cell lines were investigated in this study.

2. METHOD

2.1. Cell lines and cytotoxic activity

Ishikawa (Human Endometrial Adenocarcinoma) and HTB-114 (Human Uterine Leiomyosarcoma, SK-UT-1) cell lines were utilized in the study, while the HEK293 (Human Embryonic Kidney cells) cell line served as the control. Cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and kept in an incubator set at 37°C with 5% CO2 and 95% humidity. To evaluate cytotoxicity, the MTT assay was employed (Arslan et al., 2020). About 2,000 cells were seeded into a 96-well plate and left to grow for 24 hours. Dimethyl sulfoxide (DMSO) served as the control in this experiment. After 24 hours of treatment, the medium was substituted with 100 µl of fresh medium and 10 µl of MTT reagent, followed by incubation at 37°C for approximately 3 hours. The formazan crystals that formed were then dissolved by adding 50 µl of DMSO. The absorbance at 590 nm was recorded using a microplate spectrophotometer. The EC50 value was derived from the cell viability data according to previously established methods (Tüfekçi et al., 2024).

2.2. Colony formation assay

A total of 1,000 cells were plated into 6-well plates and incubated at 37°C with 5% CO2 for 24 hours. After this period, the EC50 concentration of tannic acid was administered. Following 24 hours of treatment, the medium containing tannic acid was replaced with fresh medium. The medium was then refreshed every 2-3 days, and incubation was continued. After about 10-12 days, the medium was discarded, and the colonies were fixed using cold methanol. The fixed colonies were stained with 0.4 mg/ml crystal violet. The stained colonies were analysed using Image J software and compared to the control group (Mutlu et al., 2022).

2.3. Scratch-Wound Assay

Approximately 30,000 cells were plated into 6-well plates and incubated for 24 hours to allow the cell density to reach approximately 90%. After incubation, the medium was removed, and the wells were rinsed with phosphate-buffered saline (PBS). A sterile 200 µl pipette tip was then used to create a straight scratch across the surface of each well. Following the scratch, the cells were washed three times with PBS to clear away any dislodged debris. Fresh medium was added, and

the cells were treated with the EC50 concentration of tannic acid for 24 hours. Images of the scratch were captured at 0, 12, 24, and 48 hours. The results from the EC50-treated group were compared to the control group for analysis (Kart et al., 2024).

2.5. Determination of Apoptosis

To assess apoptosis, 30,000 cells were seeded into 6-well plates and incubated. The cells were then exposed to the EC50 concentration of tannic acid for 24 hours. Following treatment, the cells were rinsed with PBS, detached using 500 µl of Trypsin-EDTA, and collected into microcentrifuge tubes. The cells were centrifuged at 2,000 rpm for 5 minutes. Apoptosis was evaluated using the Annexin V-FITC/PI Apoptosis Detection Kit (Elabscience, USA). The percentages of live, early apoptotic, late apoptotic, and necrotic cells were then analysed through flow cytometry (Sahin et al., 2024).

2.6. Statistical analysis

Each study was performed in triplicate, and the results are presented as Mean ± Standard Deviation for each dataset. Differences between or within groups were analysed using GraphPad Prism 9.

3. RESULTS

The cytotoxic activity of tannic acid was assessed against Ishikawa, HTB-114, and non-tumorigenic HUVEC cell lines using the MTT assay. In this study, four different concentrations of tannic acid (ranging from 25 to 100 µM) were applied for 24 hours to evaluate its cytotoxic effects. The results indicate that tannic acid exhibited cytotoxic activity in both the Ishikawa and HTB-114 cell lines. The data demonstrated that the compound significantly inhibited cell proliferation in a dose- and time-dependent manner. The EC50 (half-maximal effective concentration) value of tannic acid was found to be 39.4 µM for the Ishikawa cell line and 62.7 µM for the HTB-114 cell line (Figure 1).

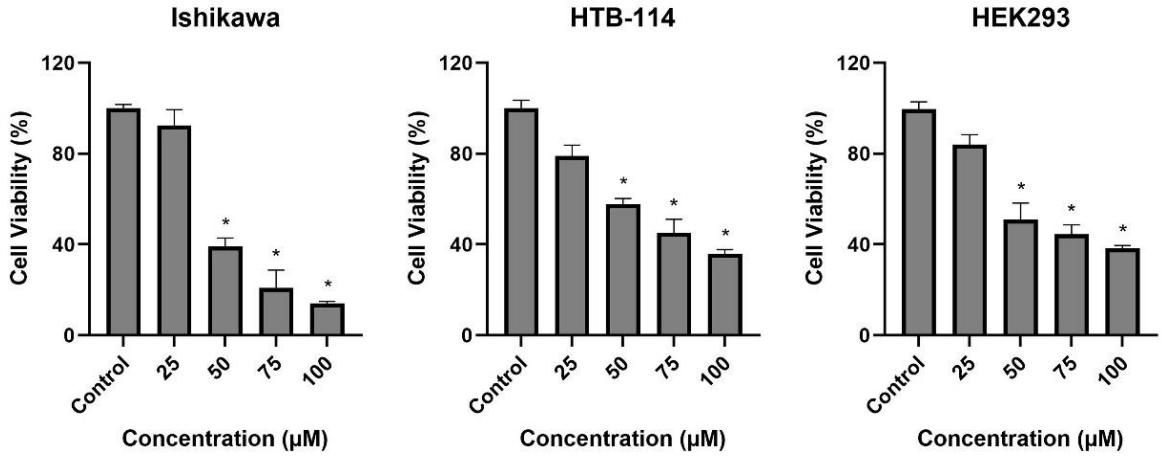

Figure 1: The graph shows the cytotoxic effects of tannic acid on the Ishikawa, HTB-114, and HEK293 cell lines. The standard deviation is indicated with Error bars. *P < 0.05 denotes statistically significant differences compared to the control group.

Tannic acid was found to inhibit colony formation in the Ishikawa and HTB-114 cell lines. For this purpose, cells treated with tannic acid were incubated for 7-10 days to form colonies. The formed cell colonies were stained with crystal violet and analysed. Colony formation in tannic acid treated cells was 1.5% in Ishikawa cell line and 1.2% in HTB-114 cell line compared to control group (Figure 2).

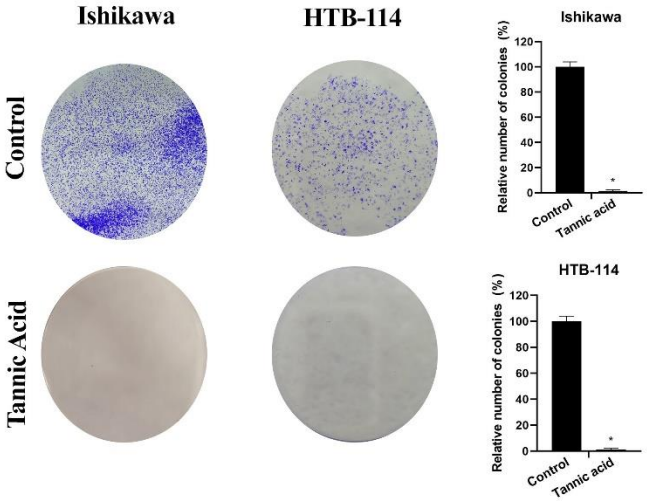

Figure 2: The colony formation assay results are shown, featuring images of Ishikawa and HTB-114 cells treated with tannic acid alongside control wells. Bar graphs represent the differences in colony numbers between tannic acid -treated and control groups for both cell lines, with *P < 0.05 indicating statistical significance.

Microscope images were analyzed with the help of ImageJ software and wound area percentages were calculated. At the end of 72 hours, Ishikawa cell line showed 87.6% clearance in the wound area where tannic acid was applied, while it was 25.3% in the control group. Likewise, in the HTB-114 cell line, the wound area in the cells treated with tannic acid was 128.4% and cell morphology deterioration was observed, while the area in the control group was 43.4% (Figure 3).

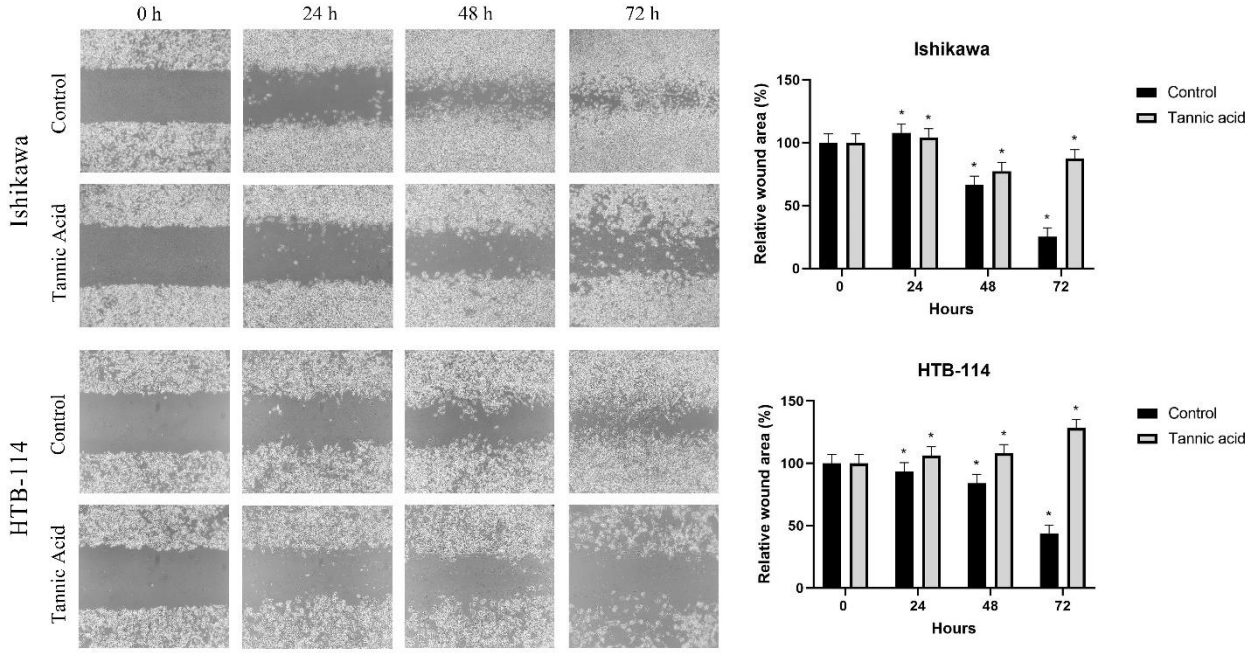

Figure 3: Representative images from the scratch-wound healing assay of Ishikawa and HTB-114 cell lines at 0, 24, 48, and 72 hours are presented. The relative wound area (%) of migrated cells was determined using densitometric analysis with ImageJ. *P < 0.05 indicates a significant difference compared to the control group.

The percentage of apoptotic cells was determined using a flow cytometer. Cells were treated with the EC50 dose of tannic acid, with paclitaxel applied as the positive control. It was determined that tannic acid showed apoptotic activity in Ishikawa cell lines and early and late apoptosis rates were found to be 27.4%. However, the apoptosis rate in the cell line was determined to be 1.59 % in the negative control and 27.47 % in the positive control (Figure 4).

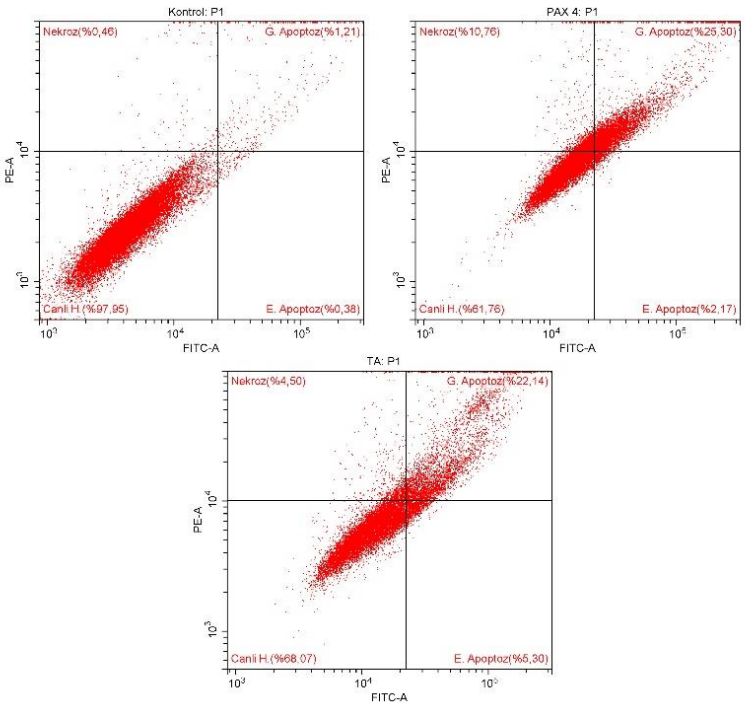

Figure 4: Flow cytometry scatter plots illustrate the results of the apoptosis assay conducted on the Ishikawa cell line. PAX refers to Paclitaxel (11.4 µM).

In HTB-114 cell lines, it was determined that tannic acid showed apoptotic activity and early and late apoptosis rates were found to be 33.2%. The apoptosis rate in the cell line was determined to be 4.40 % in the negative control and 12.92 % in the positive control (Figure 5).

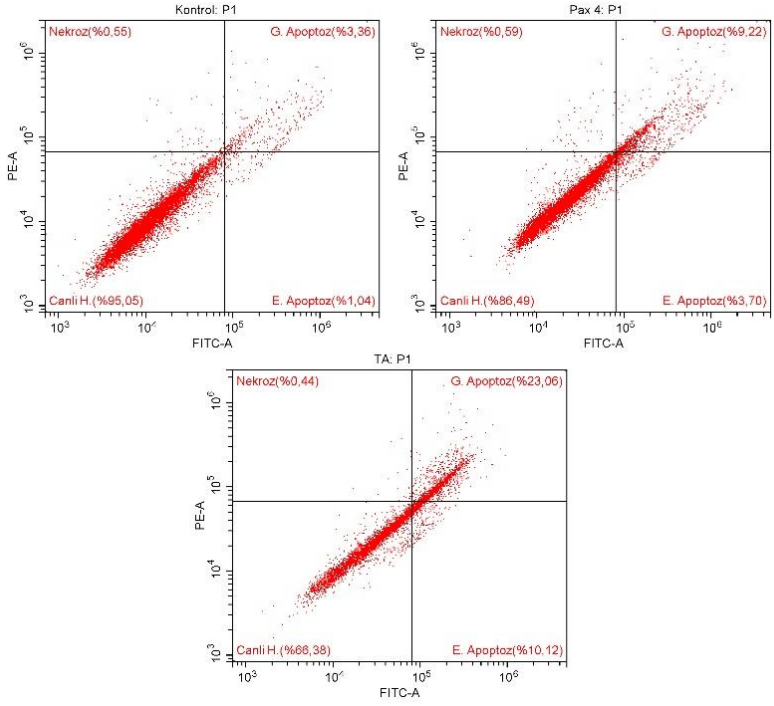

Figure 5: Flow cytometry scatter plots show the results of the apoptosis assay performed on the HTB-114 cell line. PAX: Paclitaxel (11.4 µM)

4. DISCUSSION AND CONCLUSIONS

This study investigated the anticancer activity of tannic acid on Ishikawa and HTB-114 cell lines. The cytotoxic activity of tannic acid was determined using the MTT assay with four different concentrations. The data demonstrated that the compound significantly inhibited cell proliferation in a dose- and time-dependent manner. The EC50 (half-maximal effective concentration) value of tannic acid was found to be 39.4 μM for the Ishikawa cell line and 62.7 μM for the HTB-114 cell line. Additionally, it was determined that tannic acid reduced proliferation by inhibiting colony formation in cells treated with the obtained EC50 value. According to the results analysed using Image J, colony formation in tannic acid-treated cells was 1.5% in the Ishikawa cell line and 1.2% in the HTB-114 cell line compared to the control group. Furthermore, it was identified that tannic acid delayed cell migration, as determined by the scratch-wound healing test. The scratched cells were exposed to the EC50 dose of tannic acid for 72 hours. At the end of 72 hours, the Ishikawa cell line showed 87.6% clearance in the wound area where tannic acid was applied, while it was 25.3% in the control group. Similarly, in the HTB-114 cell line, the wound area in the cells treated with tannic acid was 128.4%, and cell morphology deterioration was observed, while the area in the control group was 43.4%. Tannic acid demonstrated apoptotic activity in both Ishikawa and HTB-114 cell lines. The percentage of apoptotic cells was determined using a flow cytometer, revealing early and late apoptosis rates of 27.4% and 33.2%, respectively.

In a study conducted by Romero and colleagues (Romero et al., 2002), the effects of five different polyphenols found in red wine (rutin, morin, tannic acid, gallic acid, and quercetin) on cell proliferation were investigated in the LNCaP cell line at 24, 48, and 72 hours. Significant inhibition was observed with tannic acid at different concentrations at 24 hours (10 mmol/L), 48 hours (10 mmol/L), and 72 hours (5 mmol/L). Additionally, the apoptotic index of tannic acid was found to be significantly higher compared to the control group at concentrations of 5 and 10 mmol/L ($P<0.01$), and this effect remained consistent throughout the first 72 hours.

Tannic acid is a natural compound that significantly inhibits the proliferation, clonogenic potential, and metastatic abilities of non-small cell lung cancer (NSCLC) cells. In tests conducted on normal bronchial epithelial cell line (BEAS-2B), it was observed that TA maintained cell viability above 90% within the concentration range of 2.5 to 40 μM, indicating that it is non-toxic. Studies on A549 and H1299 cell lines demonstrated that TA suppressed cell growth in a dose- and incubation time-dependent manner. The IC50 values were determined to be 23.76 μM at 48 hours and 10.69 μM at 72 hours for A549 cells, and 21.58 μM at 48 hours and 7.136 μM at 72 hours for H1299 cells (Hatami et al., 2022). Tannic acid also inhibits the ability of cancer cells to form colonies. Long-term tests conducted over 14 days on A549 and H1299 cell lines showed that tannic acid reduced both the number and size of colonies in a dose-dependent manner. This finding is consistent with our study, suggesting that tannic acid suppresses the tumor-forming potential of cancer cells (Hatami et al., 2022). In migration and invasion tests, the anti-metastatic effects of tannic acid were prominently observed. The migratory ability of A549 and H1299 cells, assessed using Boyden chamber assays and wound healing experiments, was significantly reduced by tannic acid. Furthermore, transwell assays revealed that tannic acid inhibited the invasion capability of cancer cells (Hatami et al., 2022)

In another study, human pre-adipocytes and HER2+ breast cancer cells were cultured on cross-linked type I collagen beads with tannic acid (Jordan & Booth, 2018). This process led to the reshaping of the collagen matrix and the release of tannic acid through the adhesion, growth, and proliferation of the cells. The concentrations of tannic acid in the conditioned media were determined, and the induced apoptosis was quantitatively assessed. Following the release, the viability of HER2+ breast cancer and normal breast epithelial cells was measured, and the expression of caspase genes and proteins was evaluated. The results showed that HER2+ cells underwent caspase-mediated apoptosis after exposure to tannic acid and exhibited increased sensitivity to tannic acid. The study demonstrates that tannic acid has a therapeutic effect on breast cancer types and induces apoptosis through caspase pathways.

The potential of tannic acid as a breast cancer therapeutic agent is suggested in relation to its effects on breast cancer cells with overexpressed fatty acid synthase (FAS). A study conducted by Nie et al. (2016)demonstrated that tannic acid inhibits FAS activity and induces apoptosis in MDA-MB-231 and MCF-7 cells. The MTT assay performed on cell lines revealed that tannic acid decreased cell viability in a dose- and time-dependent manner. The IC50 values for MDA-MB-231 cells after 6, 12, 18, and 24 hours of treatment were 5.8, 5.0, 3.5, and 2.5 μM, respectively, while for MCF-7 cells, the values were >10, 8.0, 6.0, and 4.0 μM. The apoptotic activity of tannic acid was assessed using Hoechst 33258 staining and flow cytometry. In MDA-MB-231 cells, early apoptosis rates were found to be 17.25%, 20.60%, and 28.00%, while late apoptosis rates were 18.23%, 37.35%, and 48.21%. In MCF-7 cells, early apoptosis rates were measured at 6.70%, 21.48%, and 22.58%, with late apoptosis rates at 27.85%, 44.36%, and 54.05%. These results align with our study and demonstrate that tannic acid also induces dose-dependent apoptosis in these cell lines.

In conclusion, the study demonstrates that tannic acid exhibits significant anticancer activity against Ishikawa and HTB-114 cell lines by inhibiting cell proliferation in a dose- and time-dependent manner. The obtained EC50 values indicate that tannic acid effectively reduces cell growth and colony formation. Additionally, the compound delays cell migration and induces apoptotic activity, as evidenced by the increased rates of early and late apoptosis. These findings suggest that tannic acid has potential as a therapeutic agent in cancer treatment, warranting further investigation into its mechanisms and applications in oncology.

Acknowledgements
We would like to thank Amine Hafis ABDELSALAM for his contributions to the study.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: K.K.; Investigation: K.K. C.K..; Material and Methodology: K.K., C.K.; Supervision: Ş.A.; Visualization: K.K., C.K.; Writing-Original Draft: K.K; Writing-review & Editing: K.K., Ş.A.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Arslan, Ş., Kocabıyık, K., Mutlu, D., & Semiz, G. (2020). Assessment of Cytotoxic and Apoptotic Effects of Salvia syriaca L. in Colorectal Adenocarcinoma Cell Line (Caco-2). *Iranian Journal of Pharmaceutical Research*, *20*(3), 235–242.

Azmi, A. S., Bhat, S. H., Hanif, S., & Hadi, S. M. (2006). Plant polyphenols mobilize endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: A putative mechanism for anticancer properties. *FEBS Letters*, *580*(2), 533–538. https://doi.org/10.1016/j.febslet.2005.12.059

Chaachouay, N., & Zidane, L. (2024). Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates*, *3*(1), 184–207. https://doi.org/10.3390/ddc3010011

Dar, R. A., Shahnawaz, M., & Qazi, P. H. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, *6*(6), 349–351. https://doi.org/10.31254/phyto.2017.6608

Gülçin, İ., Huyut, Z., Elmastaş, M., & Aboul-Enein, H. Y. (2010). Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry*, *3*(1), 43–53. https://doi.org/10.1016/j.arabjc.2009.12.008

Hassanpour, S. H., & Dehghani, M. (2017). Review of cancer from perspective of molecular. *Journal of Cancer Research and Practice*, *4*(4), 127–129. https://doi.org/10.1016/J.JCRPR.2017.07.001

Hatami, E., B Nagesh, P. K., Sikander, M., Dhasmana, A., Chauhan, S. C., Jaggi, M., & Yallapu, M. M. (2022). Tannic Acid Exhibits Antiangiogenesis Activity in Nonsmall-Cell Lung Cancer Cells. *ACS Omega*, *7*(27), 23939–23949. https://doi.org/10.1021/acsomega.2c02727

Jing, W., Xiaolan, C., Yu, C., Feng, Q., & Haifeng, Y. (2022). Pharmacological effects and mechanisms of tannic acid. *Biomedicine & Pharmacotherapy*, *154*, 113561. https://doi.org/10.1016/j.biopha.2022.113561

Jordan, L. G., & Booth, B. W. (2018). HER2 $^{+}$ breast cancer cells undergo apoptosis upon exposure to tannic acid released from remodeled cross-linked collagen type I. *Journal of Biomedical Materials Research Part A*, *106*(1), 26–32. https://doi.org/10.1002/jbm.a.36205

Kart, N. B., Sulak, M., Mutlu, D., Kuzucu, V., Arslan, S., & Dogan, N. M. (2024). Evaluation of Anti-Biofilm and in Vitro Wound Healing Activity of Bacterial Cellulose Loaded with Nanoparticles and Borax. *Journal of Polymers and the Environment*. https://doi.org/10.1007/s10924-024-03308-3

Khan, N. S., & Hadi, S. M. (1998). Structural features of tannic acid important for DNA degradation in the presence of Cu(II). *Mutagenesis*, *13*(3), 271–274. https://doi.org/10.1093/mutage/13.3.271

Mutlu, D., Cakir, C., Ozturk, M., & Arslan, S. (2022). Anticancer and apoptotic effects of a polysaccharide extract isolated from Lactarius chrysorrheus Fr. in HepG2 and PANC-1 cell lines. *Archives of Biological Sciences*, *74*(4), 315–324. https://doi.org/10.2298/ABS220803030M

Nie, F., Liang, Y., Jiang, B., Li, X., Xun, H., He, W., Lau, H. T., & Ma, X. (2016). Apoptotic effect of tannic acid on fatty acid synthase over-expressed human breast cancer cells. *Tumor Biology*, *37*(2), 2137–2143. https://doi.org/10.1007/s13277-015-4020-z

Nishida, M. (2002). The Ishikawa Cells from Birth to the Present. *Human Cell*, *15*(3), 104–117. https://doi.org/10.1111/j.1749-0774.2002.tb00105.x

Pistilli, A., Rende, M., Crispoltoni, L., Montagnoli, C., & Stabile, A. M. (2015). LY294002 induces *in vitro* apoptosis and overexpression of p75NTR in human uterine leiomyosarcoma HTB 114 cells. *Growth Factors*, *33*(5–6), 376–383. https://doi.org/10.3109/08977194.2015.1118096

Reichardt, P. (2012). The treatment of uterine sarcomas. *Annals of Oncology*, *23*, x151–x157. https://doi.org/10.1093/annonc/mds359

Romero, I., Páez, A., Ferruelo, A., Luján, M., & Berenguer, A. (2002). Polyphenols in red wine inhibit the proliferation and induce apoptosis of LNCaP cells. *BJU International*, *89*(9), 950–954. https://doi.org/10.1046/j.1464-410X.2002.02790.x

Sahin, C., Mutlu, D., Erdem, A., Kilincarslan, R., & Arslan, S. (2024). New cyclometalated iridium(III) complexes bearing substituted 2-(1H-benzimidazol-2-yl)quinoline: Synthesis, characterization, electrochemical and anticancer studies. *Bioorganic Chemistry*, *151*, 107706. https://doi.org/10.1016/j.bioorg.2024.107706

Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A., & Saura-Calixto, F. (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition & Food Research*, *53*(S2). https://doi.org/10.1002/mnfr.200900039

Tüfekçi, A. R., Demirtaş, İ., Akşit, H., Arslan, Ş., Kocabıyık, K., Zeybek, S., Ozen, T., & Köksal, E. (2024). Two new compounds from endemic *Centaurea paphlagonica* (Bornm.) Wagenitz and their cytotoxic activities. *Chemical Biology & Drug Design*, *103*(1). https://doi.org/10.1111/cbdd.14409

Yeo, J., Lee, J., Yoon, S., & Kim, W. J. (2020). Tannic acid-based nanogel as an efficient anti-inflammatory agent. *Biomaterials Science*, *8*(4), 1148–1159. https://doi.org/10.1039/C9BM01384A

Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S., & Paz-Ares, L. (2016). Current Challenges in Cancer Treatment. *Clinical Therapeutics*, *38*(7), 1551–1566. https://doi.org/10.1016/J.CLINTHERA.2016.03.026

Assessment of Cytotoxic and Apoptotic Effects of Omeprazole on Ishikawa and HTB-114 Cell Lines, and Its Impact on Cell Migration and Colony Formation Capability

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Abstract: Omeprazole is a medicine belonging to the class of proton pump inhibitors (PPIs) and is used to treat conditions such as acid reflux, stomach ulcers and gastroesophageal reflux disease (GERD) by reducing the production of stomach acid. Omeprazole blocks the secretion of stomach acid by blocking proton pumps in the parietal cells of the stomach. This reduces acid damage in the stomach and intestines, helping to relieve symptoms and promote healing. Recent research has focused on the potential anticancer effects of omeprazole. These effects may occur through mechanisms by which omeprazole may affect cell growth, metastasis and the tumor microenvironment. Laboratory studies have shown that omeprazole may show growth inhibitory and apoptotic effects in some cancer cells. In particular, omeprazole is thought to affect the energy metabolism and acidic environment of tumor cells, making it more difficult for cancer cells to survive. Furthermore, some studies suggest that omeprazole may improve the efficacy of chemotherapy drugs and reduce drug resistance. In the light of these information, the cytotoxic and apoptotic, cell migration and colony formation properties of omeprazole towards Ishikawa and HTB-114 cell lines were determined in this study. For this purpose, the cytotoxic activity of omeprazole on cell lines was assessed using the MTT assay. HUVEC cell line was used as control group. The half-maximal effective concentration (EC_{50}) of omeprazole on cell lines was determined to be 177.9 µM for the Ishikawa cell line and 92.1 µM for the HTB-114 cell line. The apoptotic effects of omeprazole were assessed using Annexin V/PI staining via Flow Cytometry. Omeprazole induced apoptosis in Ishikawa and HTB-114 cell lines, with apoptosis rates of 12.22% and 6.9%, respectively. The impact of omeprazole on cell migration was evaluated using the scratching assay. A scratch was made in the petri dish containing the cells, and cell movement was monitored at 0, 24, 48, and 72 hours. Microscope images were analysed with ImageJ software to calculate wound area percentages. After 72 hours, the wound area in the Ishikawa cell line treated with omeprazole was 62.8% closed, compared to 36.3% in the control group. After 48 hours, the wound area in the HTB-114 cell line treated with omeprazole was 48.3%, whereas it was 19.7% in the control group. Omeprazole also inhibited colony formation in cell lines. Cells treated with omeprazole were incubated for 7-10 days to allow colonies to form. These colonies were stained with crystal violet and analysed. Colony formation in omeprazole-treated cells was 69.6% in the Ishikawa cell line and 17.8% in the HTB-114 cell line. According to these results, it is considered that omeprazole exhibits significant biological effects on specific cancer cell lines and may have potential as an anticancer drug.

Keywords: Omeprazole, Cytotoxicity, Apoptosis, Metastasis, Endometrial cancer cell lines

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1. INTRODUCTION

Omeprazole is a commonly used proton pump inhibitor (PPI) that works by decreasing the production of stomach acid. It is frequently prescribed for conditions such as gastroesophageal reflux disease (GERD), peptic ulcers, and Zollinger-Ellison syndrome. By inhibiting the hydrogen-potassium ATPase enzyme system in gastric parietal cells, omeprazole effectively reduces acid secretion, alleviating acid-related discomfort and promoting healing of the gastrointestinal mucosa(Minalyan et al., 2017).

One of the primary ways omeprazole demonstrates its anticancer effects is by modifying the tumor microenvironment. Tumors often have an acidic microenvironment that can support cancer cell survival, proliferation, and treatment resistance. By raising the pH in these acidic environments, omeprazole can inhibit cancer cell growth and enhance the efficacy of chemotherapy (Numico et al., 2017). Additionally, omeprazole's capacity to alter pH in the tumor microenvironment can lead to apoptosis, or programmed cell death, in cancer cells. This is particularly important because

evasion of apoptosis is a hallmark of cancer. By encouraging cell death in malignant tissues, omeprazole may potentially shrink tumors and improve patient outcomes (Bellone et al., 2013).

Omeprazole is believed to influence the energy metabolism and acidic conditions of tumor cells, making it more challenging for cancer cells to survive. Furthermore, some studies indicate that omeprazole may enhance the effectiveness of chemotherapy agents and reduce drug resistance. Given this context, this study aimed to evaluate the cytotoxic, apoptotic, cell migration, and colony formation effects of omeprazole on the Ishikawa and HTB-114 cell lines.

2. METHOD

2.1. Cell Culture and Cytotoxic Activity

Ishikawa (Human Endometrial Adenocarcinoma) and HTB-114 (Human Uterine Leiomyosarcoma, SK-UT-1) cell lines were used in the study. HUVEC (Human Umbilical Vein Endothelial Cells) cell line was used as a control. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and incubated at 37°C with 95% humidity and 5% CO2 (Arslan et al., 2020) Cytotoxicity was assessed using the MTT assay. Approximately $2x10^3$ cells were plated into a 96-well plate and allowed to incubate for 24 hours. Dimethyl sulfoxide (DMSO) was used as the control. After 24 hours of treatment, the medium was replaced with 100 µl of fresh medium and 10 µl of MTT solution, followed by incubation at 37°C for about 3 hours. The resulting formazan crystals were dissolved by adding 50 µl DMSO. Absorbance was measured at 590 nm using a microplate spectrophotometer. The EC_{50} value was calculated based on cell viability as previously described (Tüfekçi et al., 2024).

2.2. Colony Formation Assay

$1x10^3$ cells were seeded into 6-well plates and incubated at 37°C with 5% CO2 for 24 hours, after which the EC_{50} dose of omeprazole was applied. Following 24 hours of exposure, the medium containing the EC_{50} dose was replaced with fresh medium. The medium was subsequently changed every 2-3 days, and incubation was continued. After approximately 10-12 days, the medium was removed, and the colonies were fixed with cold methanol. The fixed cells were then stained with crystal violet at a concentration of 0.4 mg/ml. The stained colonies were analysed using the Image J software and compared with the control group(Mutlu et al., 2022)

2.3. Scratch-Wound Assay

$30x10^3$ cells were seeded into 6-well plates and incubated for 24 hours, allowing the cell density to reach 90%. The medium was then removed, and the wells were washed with phosphate-buffered saline (PBS). A sterile 200 µl pipette tip was used to make a straight scratch across each well. After scratching, the cells were washed three times with PBS to remove any dislodged cell debris. Fresh medium was added, and the cells were treated with the EC_{50} dose of omeprazole for 24 hours. Photographs of the scratch were taken at 0, 12, 24, and 48 hours. The EC_{50} treatment group was compared with the control group for evaluation (Kart et al., 2024)

2.4. Determination of Apoptosis

For the determination of apoptosis, $30x10^3$ cells were seeded into 6-well plates and incubated. The cells were then treated with the EC_{50} dose of omeprazole for 24 hours. After treatment, the cells were washed with PBS, detached using 500 µl Trypsin-EDTA, and collected into microcentrifuge tubes. The cells were then centrifuged at 2000 rpm for 5 minutes. Apoptosis was assessed using the Annexin V-FITC/PI Apoptosis Kit (Elabscience, USA), and the percentages of live, early apoptotic, late apoptotic, and necrotic cells were analysed by flow cytometry (Sahin et al., 2024)

2.5. Statistical analysis

Each study was conducted in triplicate. The results are presented as Mean ± Standard Deviation for each dataset. Differences between or within groups were analysed using GraphPad Prism 9.

3. RESULTS

The cytotoxic activity of omeprazole was evaluated against Ishikawa, HTB-114 and non-tumorigenic HUVEC cell lines using the MTT assay. In the study, seven different concentrations of omeprazole (7.8 to 500 µM) were applied for 24 hours to determine its cytotoxic activity. The results indicate that omeprazole exhibited cytotoxic activity in the Ishikawa and HTB-114 cell lines. The obtained data showed that the compound significantly inhibited cell proliferation in a dose-

and time-dependent manner. The half-maximal effective concentration (EC_{50}) of omeprazole was determined to be 177.9 µM for the Ishikawa cell line and 92.1 µM for the HTB-114 cell line. In contrast, less cytotoxic effect was observed on the non-tumorigenic HUVEC cell line.

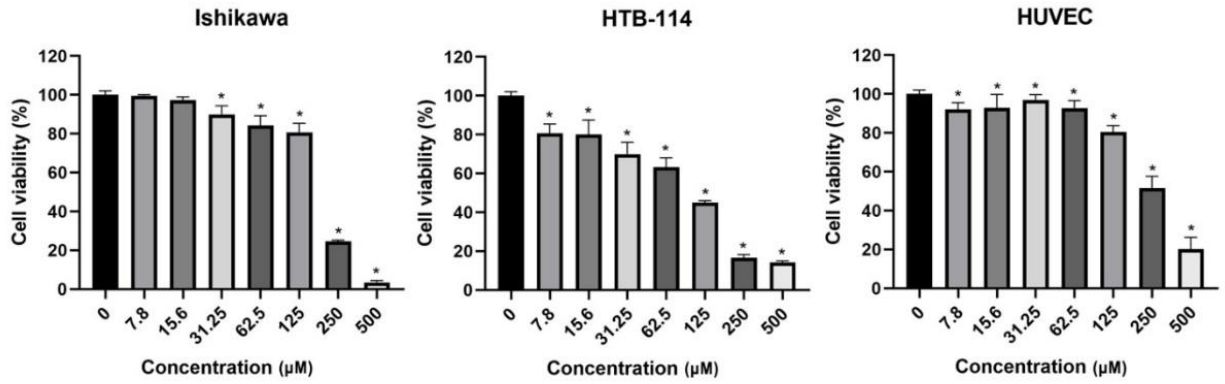

Figure 6: The cytotoxic effects of Omeprazole on Ishikawa, HTB-114 and HUVEC cell lines were shown in graphs. Error bars represent the standard deviation. *P < 0.05 indicates a statistically significant difference compared to the control group.

It was also observed that omeprazole inhibited colony formation in the Ishikawa and HTB-114 cell lines. In omeprazole-treated cells, relative colony formation was observed to be 69.6% in the Ishikawa cell line and 17.8% in the HTB-114 cell line, with representative images shown in Figure 2.

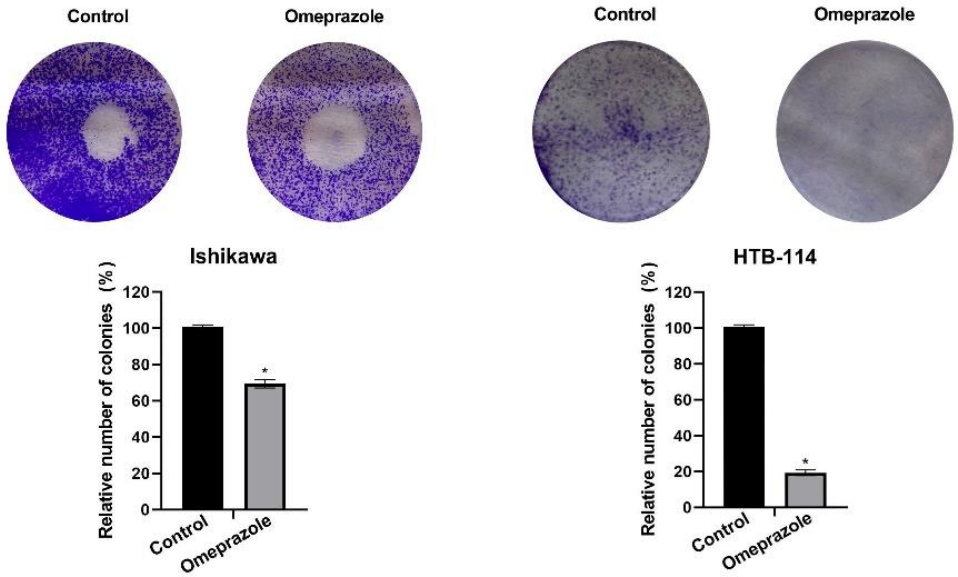

Figure 7: The results of the colony formation assays are presented. Representative images show the wells containing Ishikawa and HTB-114 cells treated with omeprazole and the control wells. Bar graphs illustrate the relative differences in colony numbers for Ishikawa and HTB-114 cells treated with omeprazole and the control group. *P < 0.05.

A wound-scratch assay was performed to determine the effect of omeprazole on cell migration. For this purpose, Ishikawa cells were exposed to the EC_{50} dose of omeprazole for 72 hours. Cell migration was observed and recorded using a microscope every 24 hours. It was found that omeprazole significantly inhibited cell migration in the Ishikawa cell line. After 72 hours, the wound area in the Ishikawa cell line treated with omeprazole was 62.8% closed, compared to 36.3% in the control group.

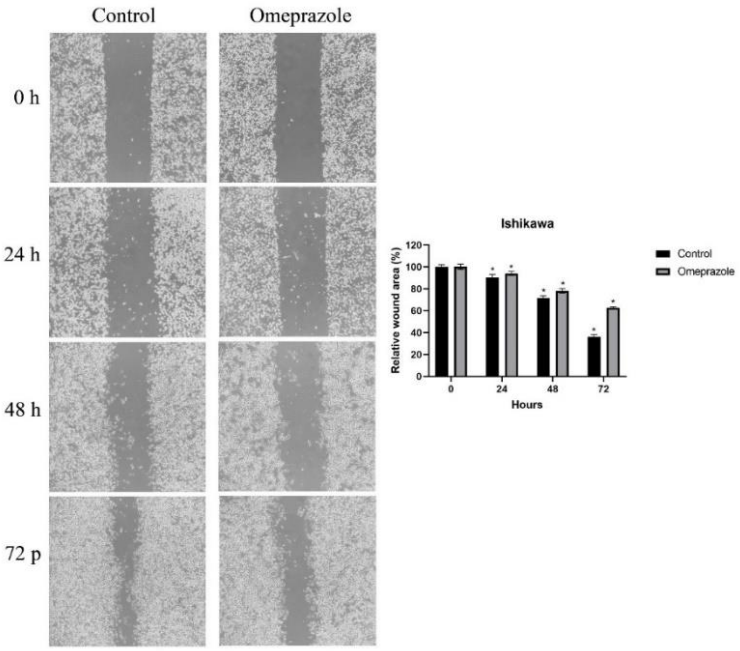

Figure 8: Representative images from the scratch wound healing assay of Ishikawa cell lines at 0, 24, 48 and 72 hours are shown. The relative wound area (%) of migrated cells was calculated using densitometric measurements with ImageJ. *P < 0.05 compared to the control group.

However, HTB-114 cells were exposed to the EC_{50} dose of omeprazole for 48 hours. It was observed that omeprazole also inhibited cell migration in the HTB-114 cell line. After 48 hours, the wound area in the HTB-114 cell line treated with omeprazole was 48.3%, whereas it was 19.7% in the control group.

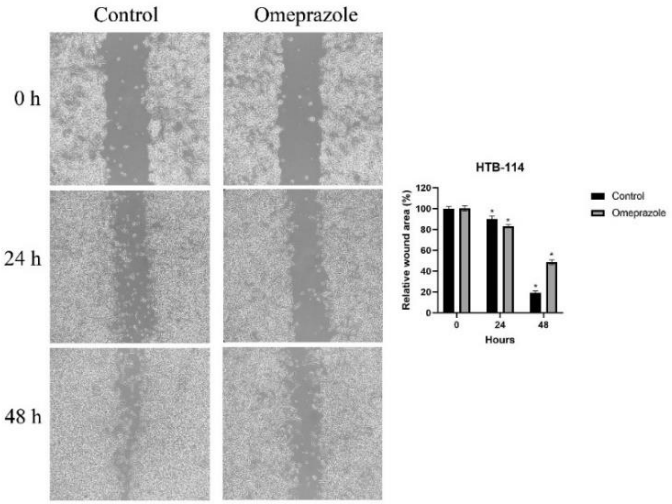

Figure 9: Representative images from the scratch wound healing assay of HTB-114 cell lines at 0, 24, and 48 hours are shown. The relative wound area (%) of migrated cells was calculated using densitometric measurements with ImageJ. *P < 0.05 compared to the control group.

The percentages of apoptotic cells were assessed using a flow cytometer. Cells were treated with the EC_{50} dose of omeprazole, paclitaxel was used as the positive control. The results indicated that the apoptosis rate in the Ishikawa cell line was 2.76% in the negative control, 38.55% in the positive control, and 12.22% with omeprazole.

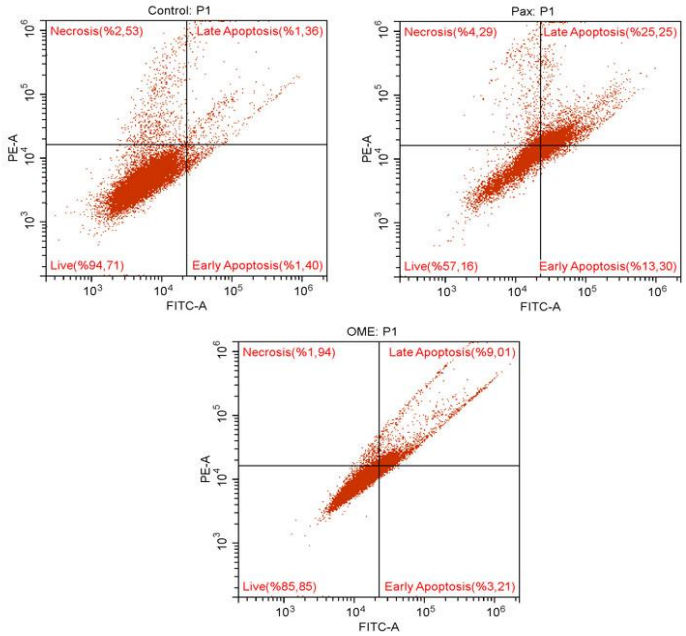

Figure 10: Flow cytometry scatter plots illustrate the results of the apoptosis assay conducted on the Ishikawa cell line. PAX refers to Paclitaxel (11.4 µM).

All the same, in the HTB-114 cell line, the apoptosis rates were 4.32% in the negative control, 16.04% in the positive control, and 5.95% with omeprazole (Figure 2). In conclusion, omeprazole demonstrated an apoptotic effect compared to the negative control.

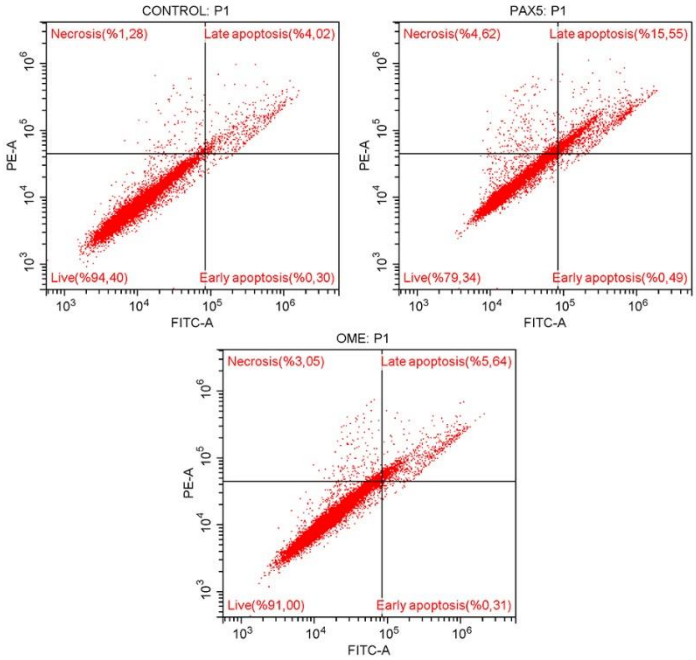

Figure 11: Flow cytometry scatter plots show the results of the apoptosis assay performed on the HTB-114 cell line. PAX: Paclitaxel (11.4 µM)

4. DISCUSSION AND CONCLUSIONS

In this study, the cytotoxic and apoptotic effects of omeprazole on Ishikawa and HTB-114 cell lines, as well as its effects on cell migration and colony formation, were investigated. The cytotoxic activity of omeprazole on the cell lines was determined using the MTT assay. It has been determined that omeprazole exhibits dose-dependent cytotoxic activity on Ishikawa and HTB-114 cell lines. The EC_{50} values of omeprazole in the Ishikawa and HTB-114 cell lines were found to be 177.9 µM and 92.1 µM, respectively. Subsequent studies conducted using the EC_{50} values revealed that omeprazole inhibited colony formation and reduced proliferation in both the Ishikawa and HTB-114 cell lines. Compared to the control group, colony formation in omeprazole-treated cells was evaluated as 69.6% in the Ishikawa cell line and 17.8% in the HTB-114 cell line. Additionally, a wound-scratch assay was performed to investigate the effect of omeprazole on migration. As a result, the wound area in the Ishikawa cell line was measured as 36.3% in the control group and 62.8% in omeprazole-treated cells. In the HTB-114 cell line, the wound area was measured as 19.7% in the control group and 48.3% in omeprazole-treated cells. These results indicate that omeprazole inhibits cell migration. Moreover, the apoptotic effects of omeprazole were also investigated in the study. The percentages of apoptotic cells in omeprazole-treated cell lines were evaluated using flow cytometry. The results indicated that the apoptosis rate in the HTB-114 cell line was 4.32% in the negative control, 16.04% in the positive control, and 5.95% with omeprazole. In the Ishikawa cell line, the apoptosis rates were 2.76% in the negative control, 38.55% in the positive control, and 12.22% with omeprazole.

The antitumor effects of omeprazole have been identified in various cancer cell lines in conducted studies. These cell lines include SKOV3-TR, Heya8-MDR, RMG-1, ES-2, neuroblastoma cells (SHSY5Y), glioblastoma (U-87), colon carcinoma cells (320WT and 320MUT), human microglia cells (THP), human gastric cancer cells (HGC-27), human colon cancer cells (HCT-116 and HCA-7), and Jurkat T lymphocytes. The effects of omeprazole on these cell lines have been observed at concentrations ranging from 10 to 106 µM (Paz et al., 2020) In a study conducted on RPMI 8226 and U266 myeloma cell lines, omeprazole was shown to slightly inhibit cell proliferation. This effect was observed most prominently at a high concentration of 200 µM, where cell proliferation was inhibited by approximately 10-30% (Canitano et al., 2016).

In a study conducted by Hou and colleagues (2018), the effects of omeprazole on the proliferation of Barrett's Esophagus cells (CP-A and CP-B) were investigated using the CCK-8 assay. The study demonstrated that omeprazole reduced cell viability in CP-A and CP-B cells in a dose-dependent manner. Omeprazole increased the number of cells in the G0/G1 phase while decreasing the number in the S phase, effectively arresting the cell cycle. Additionally, omeprazole increased the levels of P21 and P27 proteins and reduced the levels of cell cycle-related proteins (Cyclin D1, CDK2, CDK4).

The effects of omeprazole on pancreatic cancer cell lines (PancTu-1, MiaPaCa-2, ASPC-1, Panc89, Panc1, and Colo357) were investigated by Udelnow and colleagues (2011). Omeprazole was found to exhibit antiproliferative effects at non-toxic concentrations. It was also noted that omeprazole reversed the growth-promoting effects of 5-fluorouracil on the cells. Additionally, omeprazole was found to modulate the lysosomal transport pathway, leading to cell death. The IC_{50} values of omeprazole ranged from 9 to 42 mg/ml, depending on the cell line. These findings suggest that omeprazole has the potential to induce hormesis in chemotherapy and to overcome chemotherapy resistance mechanisms in pancreatic cancer.

In a study conducted by Jin and colleagues (2014), it was shown that both TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin, 10 nM) and omeprazole (200 and 300 µM) significantly reduced cell migration in the MDA-MB-231 cell line using a wound healing assay (scratch assay). The results were consistent with the inhibition of MDA-MB-231 cell invasion observed after omeprazole treatment. In another study conducted by Jin and colleagues (2020), it was demonstrated that omeprazole inhibited the proliferation, migration, and invasion of glioblastoma cells derived from patient 15-037 (both wild-type and AhR knockout) only in cells that expressed AhR. This effect was not observed in cells where AhR was suppressed. Omeprazole, dependent on AhR, also enhanced the suppression of invasion-promoting genes such as MMP9, CXCL12, and CXCR4. The combination of omeprazole and temozolomide further inhibited cell proliferation and migration. In SCID mice injected subcutaneously with 15-037 cells, omeprazole (100 mg/kg/injection) inhibited and delayed tumor growth. However, tumor growth resumed once treatment was discontinued. Omeprazole was well-tolerated but showed limited efficacy due to solubility issues.

In a study conducted with the HuH-6 Clone-5 cell line, it was shown via electron microscopy that the group treated with Baf-A1 exhibited significant morphological changes in hepatoblastoma cells compared to the untreated group. Morphological observations and apoptotic cell detection through flow cytometry revealed that after 48 hours of treatment, Baf-A1 inhibited hepatoblastoma cell proliferation by inducing apoptosis. In Baf-A1-treated cells, an increase in the number of apoptotic hepatoblastoma cells was observed in both the early apoptosis group (PI-negative, Annexin-V-

positive) and the late apoptosis group (PI-positive, Annexin-V-positive) compared to cells not treated with Baf-A1 (Morimura et al., 2008).

Proton pump inhibitors (PPIs) were shown to exhibit significant antiproliferative and pro-apoptotic effects, particularly in low pH environments, on tumor B cells and leukemic cells. PPIs are known to trigger cell death through early production of reactive oxygen species (ROS) and by causing damage to lysosomal and mitochondrial membranes. Additionally, studies have demonstrated that PPIs make cancer cells more sensitive to chemotherapeutic agents (De Milito et al., 2007)

In this study, it was determined that omeprazole exhibited cytotoxic activity on Ishikawa and HTB-114 cell lines. Additionally, it was found that omeprazole inhibited cell migration and reduced proliferation, thereby preventing colony formation in these cell lines. Omeprazole, a proton pump inhibitor, showed some apoptotic activity in the Ishikawa and HTB-114 cell lines compared to the negative control group. However, it can be said that it is not a strong apoptotic agent compared to the positive control. These findings suggest that the compound exhibits selective cytotoxic activity against cancer cells and could potentially serve as an anticancer agent. However, considering its lower cytotoxic effects on normal cell lines, further toxicity and safety studies are warranted.

Acknowledgements
We would like to thank Amine Hafis ABDELSALAM for his contributions to the study.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: K.K.; Investigation: K.K. A.I.; Material and Methodology: K.K., A.I.; Supervision: Ş.A.; Visualization: K.K., A.I.; Writing-Original Draft: K.K; Writing-review & Editing: K.K., Ş.A.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Arslan, Ş., Kocabıyık, K., Mutlu, D., & Semiz, G. (2020). Assessment of Cytotoxic and Apoptotic Effects of Salvia syriaca L. in Colorectal Adenocarcinoma Cell Line (Caco-2). *Iranian Journal of Pharmaceutical Research*, *20*(3), 235–242.

Bellone, M., Calcinotto, A., Filipazzi, P., De Milito, A., Fais, S., & Rivoltini, L. (2013). The acidity of the tumor microenvironment is a mechanism of immune escape that can be overcome by proton pump inhibitors. *OncoImmunology*, *2*(1), e22058. https://doi.org/10.4161/onci.22058

Canitano, A., Iessi, E., Spugnini, E. P., Federici, C., & Fais, S. (2016). Proton pump inhibitors induce a caspase-independent antitumor effect against human multiple myeloma. *Cancer Letters*, *376*(2), 278–283. https://doi.org/10.1016/J.CANLET.2016.04.015

De Milito, A., Iessi, E., Logozzi, M., Lozupone, F., Spada, M., Marino, M. L., Federici, C., Perdicchio, M., Matarrese, P., Lugini, L., Nilsson, A., & Fais, S. (2007). Proton Pump Inhibitors Induce Apoptosis of Human B-Cell Tumors through a Caspase-Independent Mechanism Involving Reactive Oxygen Species. *Cancer Research*, *67*(11), 5408–5417. https://doi.org/10.1158/0008-5472.CAN-06-4095

Hou, Y., Hu, Q., Huang, J., & Xiong, H. (2018). Omeprazole Inhibits Cell Proliferation and Induces G0/G1 Cell Cycle Arrest through Up-regulating miR-203a-3p Expression in Barrett's Esophagus Cells. *Frontiers in Pharmacology*, *8*. https://doi.org/10.3389/fphar.2017.00968

Jin, U.-H., Lee, S.-O., Pfent, C., & Safe, S. (2014). The aryl hydrocarbon receptor ligand omeprazole inhibits breast cancer cell invasion and metastasis. *BMC Cancer*, *14*(1), 498. https://doi.org/10.1186/1471-2407-14-498

Jin, U.-H., Michelhaugh, S. K., Polin, L. A., Shrestha, R., Mittal, S., & Safe, S. (2020). Omeprazole Inhibits Glioblastoma Cell Invasion and Tumor Growth. *Cancers*, *12*(8), 2097. https://doi.org/10.3390/cancers12082097

Kart, N. B., Sulak, M., Mutlu, D., Kuzucu, V., Arslan, S., & Dogan, N. M. (2024). Evaluation of Anti-Biofilm and in Vitro Wound Healing Activity of Bacterial Cellulose Loaded with Nanoparticles and Borax. *Journal of Polymers and the Environment*. https://doi.org/10.1007/s10924-024-03308-3

Minalyan, A., Benhammou, J. N., Artashesyan, A., Lewis, M., & Pisegna, J. R. (2017). Autoimmune atrophic gastritis: current perspectives. *Clinical and Experimental Gastroenterology*, *Volume 10*, 19–27. https://doi.org/10.2147/CEG.S109123

Morimura, T., Fujita, K., Akita, M., Nagashima, M., & Satomi, A. (2008). The proton pump inhibitor inhibits cell growth and induces apoptosis in human hepatoblastoma. *Pediatric Surgery International*, *24*(10), 1087–1094. https://doi.org/10.1007/s00383-008-2229-2

Mutlu, D., Cakir, C., Ozturk, M., & Arslan, S. (2022). Anticancer and apoptotic effects of a polysaccharide extract isolated from Lactarius chrysorrheus Fr. in HepG2 and PANC-1 cell lines. *Archives of Biological Sciences*, *74*(4), 315–324. https://doi.org/10.2298/ABS220803030M

Numico, G., Fusco, V., Franco, P., & Roila, F. (2017). Proton Pump Inhibitors in cancer patients: How useful they are? A review of the most common indications for their use. *Critical Reviews in Oncology/Hematology*, *111*, 144–151. https://doi.org/10.1016/j.critrevonc.2017.01.014

Paz, M. F. C. J., de Alencar, M. V. O. B., de Lima, R. M. P., Sobral, A. L. P., do Nascimento, G. T. M., dos Reis, C. A., Coêlho, M. do P. S. de S., do Nascimento, M. L. L. B., Gomes Júnior, A. L., Machado, K. da C., de Menezes, A.-A. P. M., de Lima, R. M. T., de Oliveira Filho, J. W. G., Dias, A. C. S., dos Reis, A. C., da Mata, A. M. O. F., Machado, S. A., Sousa, C. D. de C., da Silva, F. C. C., … Melo Cavalcante, A. A. de C. (2020). Pharmacological Effects and Toxicogenetic Impacts of Omeprazole: Genomic Instability and Cancer. *Oxidative Medicine and Cellular Longevity*, *2020*, 1–21. https://doi.org/10.1155/2020/3457890

Sahin, C., Mutlu, D., Erdem, A., Kilincarslan, R., & Arslan, S. (2024). New cyclometalated iridium(III) complexes bearing substituted 2-(1H-benzimidazol-2-yl)quinoline: Synthesis, characterization, electrochemical and anticancer studies. *Bioorganic Chemistry*, *151*, 107706. https://doi.org/10.1016/j.bioorg.2024.107706

Tüfekçi, A. R., Demirtaş, İ., Akşit, H., Arslan, Ş., Kocabıyık, K., Zeybek, S., Ozen, T., & Köksal, E. (2024). Two new compounds from endemic *Centaurea paphlagonica* (Bornm.) Wagenitz and their cytotoxic activities. *Chemical Biology & Drug Design*, *103*(1). https://doi.org/10.1111/cbdd.14409

Udelnow, A., Kreyes, A., Ellinger, S., Landfester, K., Walther, P., Klapperstueck, T., Wohlrab, J., Henne-Bruns, D., Knippschild, U., & Würl, P. (2011). Omeprazole Inhibits Proliferation and Modulates Autophagy in Pancreatic Cancer Cells. *PLoS ONE*, *6*(5), e20143. https://doi.org/10.1371/journal.pone.0020143

Purification of Naturally Found Carvacrol Compound in Oregano Thyme by Flash Chromatography Technique and Its Use as Natural Antibiotic by Encapsulation Method

Taner Erkaymaz *[1], Osman Bodur [1]

Abstract: Origanum species, which are widely used and marketed as spices, occupy a large place among plants collected from nature. The genus Origanum, belonging to the Labiatae family, consists of many species and subspecies with great interspecific and intraspecific diversity. More than half of the thyme exported from our country is obtained from cultivated thyme culture production. The thyme species Origanum onites and Origanum vulgare subsp. are grown around Isparta, Denizli and Izmir. Among the species traded in Turkey, Origanum onites L. is the most collected and exported species. The balls of Origanum onites L., which form the leaf and flower collection, are consumed as spices. The essential oil of Origanum onites L., which contains significant amounts of carvacrol and thymol, has antibacterial, antispasmodic, antiseptic, antimicrobial, cytotoxic, antioxidant and antifungal activity and the source of the therapeutic effect is shown to be carvacrol. The essential oil of the plant is used in food as well as in medicine and perfumery.
In this study, essential oil was obtained from Origanum onites plant by distillation of water vapor. The essential oil was analyzed by GC/MSD instrument. According to the results, the main component carvacrol was determined as 73.11%. In this study, high purity carvacrol should be used. So, purification of the naturally occurring carvacrol compound in thyme was carried out using flash chromatography method and obtained %97,48 purity carvacrol. It is necessary to enable carvacrol to behave like the active ingredient of an antibiotic drug. Encapsulation method was considered as a method that can penetrate the cell wall and increase its effectiveness. In order to increase the effectiveness of this compound, the Liposome Encapsulation method was used, which can protect the molecule and facilitate its passage through the cell wall.

Keywords: Carvacrol, Origanum onites, Flash Chromatography, Purification, Liposome Encapsulation, Antibiotic

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1. INTRODUCTION

Origanum species, which are widely used and marketed as spices, occupy a large place among plants collected from nature. The genus Origanum, belonging to the Labiatae family, consists of many species and subspecies with great interspecific and intraspecific diversity. More than half of the thyme exported from our country is obtained from cultivated thyme culture production (Mheen, 2006).

Figure 1. Origanum minutiflorum

Figure 2. Origanum vulgare

The thyme species Origanum onites and Origanum vulgare subsp. are grown around Isparta, Denizli and Izmir. Among the species traded in Turkey, Origanum onites L. is the most collected and exported species (Başer, 2002). The balls of Origanum onites L., which form the leaf and flower collection, are consumed as spices. The essential oil of Origanum onites L., which contains significant amounts of carvacrol has antibacterial, antispasmodic, antiseptic, antimicrobial, cytotoxic, antioxidant and antifungal activity and the source of the therapeutic effect is shown to be carvacrol. The essential oil of the plant is used in food as well as in medicine and perfumery (Özcan et al., 2001).

Figure 3. Origanum onites

Carvacrol is a compound compatible with the human body in terms of molecular structure and biological balance stability. Since it is a natural monoterpene, it is among the compounds that can easily pass through the stomach and intestinal wall. Despite this ease, it is not a very successful compound in passing through microorganisms and human cell walls. For this reason, the equivalent amount of antibacterial, antiseptic, antimicrobial, cytotoxic, antioxidant and antifungal activity is very low compared to the amount taken into the body (Özcan et al., 2001). It is necessary to ensure that carvacrol can behave like the active ingredient of an antibiotic drug.

2. MATERIAL AND METHOD

2.1. Origanum onites plant

In this study, Origanum onites thyme species was used as thyme oil.

2.2. GC–MS Analysis

A gas chromatography–mass spectrometry (GC-MS) instrument was utilised for the analysis of the essential volatile compounds present in origanum onites oil. A 50 µL sample of the essential oil was taken and diluted with 15 mL of n-hexane solvent. Following a three-minute vortexing process, a 1/40 dilution was prepared with acetone in a 2 mL vial and subsequently injected into the GC-MS instrument.

The analyses were carried out using a Thermo Scientific Trace 1300 GC gas chromatograph instrument and a Thermo Scientific-ISQ7000 single quadrupole mass spectrometer detector (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) system. The chromatographic evaluations were conducted using the Xcalibur software. The analytical column employed for chromatographic separation was the TraceGOLD TG-624SilMS GC (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). The inlet temperature of the instrument was set to 250 °C. An injection volume of 2 µL was employed. A split ratio of 1/5 was employed. Helium was employed as the carrier gas, with a flow rate of 1.5 mL/min. The oven temperature was programmed from 35 °C (2 min) to 100 °C at a rate of 2 °C/min, then from 100 °C (1 min) to 250 °C at a rate of 5 °C/min. The detector temperature was set at 280 °C.

2.3. Identification of Compounds

The identification of essential oil compounds was performed via computer search utilising their mass spectra, either in conjunction with known components (Adams, 1989) or through comparison of the mass spectra of received chemical substances, which were then cross-referenced against the mass spectrum library (Wiley, 2007).

2.4. Application of Flash Chromatography

In flash chromatography, Buchi brand device (BÜCHI Labortechnik AG., Flawil, Switzerland) and FP ECOFLEX brand 40 g C18 column were used as column. The fractions were combined and subjected to flash chromatography, after which they were dissolved with DMSO and loaded onto the flash chromatography column. The solvent flow rate was set at 50 mL per minute, which was deemed an appropriate rate for the separation process. A solvent system comprising 30% water and 70% ethanol was initially employed. The ratio of the two solvents was then adjusted in changing of 1% every 1 minute. The process was conducted using flash chromatography with the following wavelengths: UV1 λ: 254 nm, UV2 λ: 265 nm, UV3 λ: 274 nm, UV4 λ: 320 nm. The separation process was terminated using a solvent system comprising 80% water and 20% methanol.

2.5. Liposome Coating Encapsulation Method

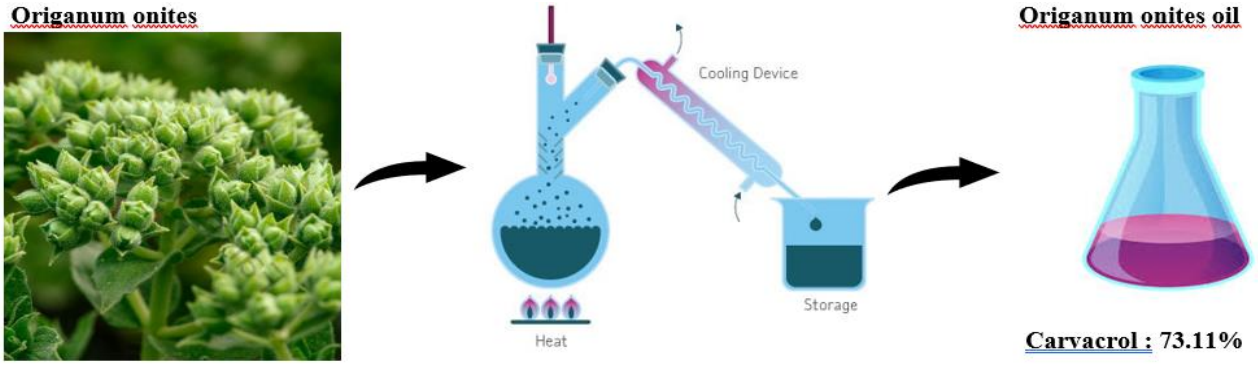
The Buchi encapsulator B-390 device (BÜCHI Labortechnik AG., Flawil, Switzerland) was used in the liposome coating encapsulation method. Encapsulation method was considered as a method that can overcome the cell wall and increase its effectiveness. In order to increase the effectiveness of this compound, Liposome Encapsulation method, which can protect the molecule and facilitate its passage through the cell wall, was used.

3. RESULTS

3.1. Obtaining Oregano Oil by Water Vapour Distillation and analysis by GC–MS

In this study, essential oil was obtained from Origanum onites plant by distillation of water vapour. The essential oil was analyzed by GC/MSD instrument. According to the results, the main component carvacrol was determined as 73.11%.

Figure 4. Obtaining Oregano Oil by Water Vapour Distillation



3.2. Flash chromatography

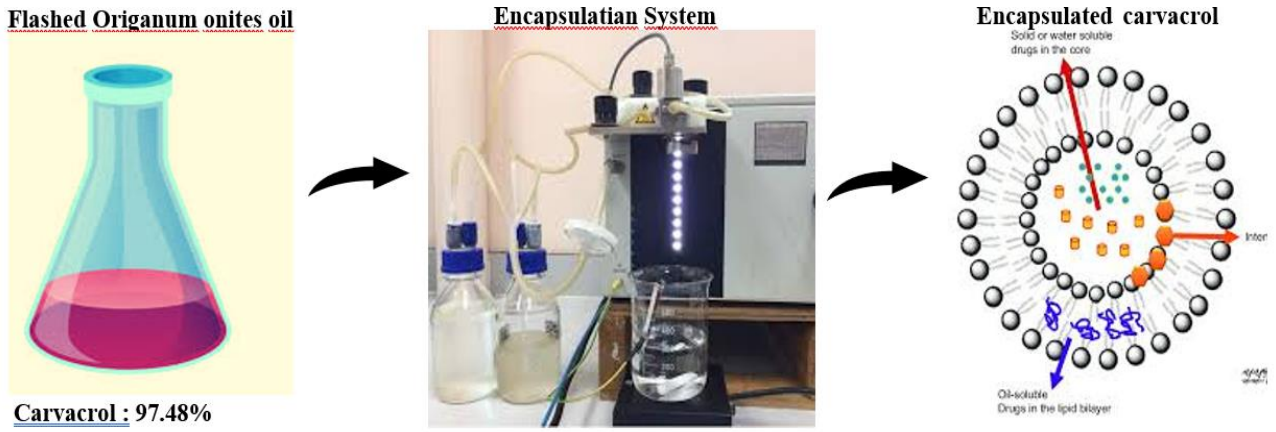
In the purification of carvacrol by flash chromatography, the compounds were collected in a total of 15 tubes. Each tube was then subjected to analysis by GC-MS. Upon analysis at UV-Vis wavelengths, it was observed that the carvacrol compound was present between tubes 7 and 11. Consequently, these tubes were combined and subjected to further analysis by GC-MS. The objective of this study was to obtain a highly purified carvacrol compound. To this end, the naturally occurring carvacrol in thyme was purified using the flash chromatography method, resulting in a carvacrol compound with a purity of 97.48% (in figüre 5).

Figure 5. Purifying Carvacrol Compound By Using Flash Chromatography

3.3. Liposome Coating Encapsulation

It is essential to facilitate the functionality of carvacrol as an active ingredient in an antibiotic drug. The encapsulation method was identified as a potential approach to penetrate the cell wall and enhance the compound's efficacy. To augment the potency of this compound, the liposome encapsulation method was employed showned in Figure 6, which can safeguard the molecule and facilitate its passage through the cell wall.

Figure 6. Encapsulation of Carvacrol Molecule



Liposomes are two-layered and spherical lipid particles composed of polar lipids used in pharmaceutical, personal care, chemical and food industries to encapsulate both hydrophobic and hydrophilic compounds. They are formed by dispersing polar lipids in a polar medium such as water (Gibis et al., 2012; Taylor et al., 2005). The main sources of polar lipids in nature are egg, soya and sunflower lecithin. These lecithins have been used as emulsifiers or structure modifying agents in foods for many years and are in the Generally Recognised as Safe (GRAS) category according to the US Food and Drug Administration (FDA) (Laye et al., 2008; Administration, 2006).

4. DISCUSSION AND CONCLUSIONS

The minimum purity rate of carvacrol, the active substance in oregano oil, has been increased to a minimum of 95%. It is hypothesised that this active substance is then transformed into a smart biomolecule through a liposomal encapsulation process. Liposomes, which are formed by the combination of lecithins to form a double-layered spherical structure, are important in terms of encapsulating functional components that can be soluble in both water and fat (Taylor et al., 2005). It has been shown that the encapsulation of functional components in liposomes increases the stability of the encapsulated substance, eliminates its interaction with the environment, and thus maintains its activity for a longer time in environments that would normally cause degradation (Chun et al., 2013).

The active substance carvacrol, which has been demonstrated to exhibit a range of biological activities including antibacterial, antispasmodic, antiseptic, antimicrobial, cytotoxic, antioxidant and antifungal properties, indicates that it may serve as a promising starting point for the development of a new therapeutic natural drug when encapsulated.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: T.E. O.B.; Investigation: O.B.; Material and Methodology: O.B., T.E.; Supervision: O.B., T.E.; Visualization: O.B.; Writing-Original Draft: O.B.; Writing-review & Editing: T.E.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Mheen, H. Van der, (2006). Selection and Production of Oregano Rich in Essential Oil and Carvacrol. Acta Hort. 709, ISHS 2006, 95-99.

Başer, K.H.C. (2002). The Turkish Origanum Species, In: Oregano, The Genera Origanum and Lippia, Ed.: S.E. Kintzios, Taylor and Francis, UK.

Özcan, M., J.C. Chalchat ve A. Akgül. (2001). Composition of The Essential Oils of Origanum majorana L. And Origanum vulgare subsp. hirtum Growing Wild in Turkey. Workshop on Agricultural and Quality Aspects of Medicinal and Aromatic Plants, May 29-June 01, 2001, Adana/Turkey, p. 175-184.

Wiley, (2007). Registry, 8th ed. with NIST 05 MS Spectra, Revision 2005 D.06.00. s.l. Agilent Technologies.

Taylor, T.M., P.M., Davidson, B.D., Bruce, Weiss, J., (2005). Liposomal nanocapsules in food science and agriculture. Critical Reviews in Food Science and Nutrition 45 (7-8): 587-605.

Gibis, M., Vogt, E., Weiss, J., (2012). Encapsulation of polyphenolic grape seed extract in polymercoated liposomes. Food & Function 3 (3): 246-254.

Laye, C., McClements, D.J., Weiss, J., (2008). Formation of biopolymer-coated liposomes by electrostatic deposition of chitosan. Journal of Food Science 73(5): N7-N15.

Administration, U.S.F.a.D., (2006). Guidance for industry: Guidance on the labeling of certain uses of lecithin derived from soy under section 403 (w) of the federal food, drug, and cosmetic act.

Chun, J.Y., Choi, M.J., Min, S.G., Weiss, J., (2013). Formation and stability of multiple-layered liposomes by layer-by-layer electrostatic deposition of biopolymers. Food Hydrocolloids 30 (1): 249-257.

Healthcare Systems And Teaching

Tiziana Ceka[1]

Abstract: In terms of health, there is still much to be done, even though there has been progress in recent years. According to WHO (2018), there was an improvement in the health status of populations between 2012 and 2015. However, there are inequalities because the longest healthy life span is recorded in countries whose economies are in better shape. Also, WHO emphasizes that although it has recorded improvements, Africa is starting from a very low base, and current levels remain lower than those of the rest of the world. The 2030 Agenda for Sustainable Development, adopted by the United Nations General Assembly in September 2015, highlights the issues of education, health and well-being. Among the seventeen sustainable development goals defined in this program (UN, 2015), we note goal 3 "Ensure healthy lives and promote well-being for all at all ages" and goal 4 "Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all." These two, which place particular emphasis on education, health and well-being, will undoubtedly help achieve the other sustainable development goals. And it is in this vision, evoking the essential role of education in society, UNESCO (2017) indicates that it will help achieve the sustainable development goals and is, therefore, a means of improving the health and well-being of populations. There is therefore an important link between education, health and well-being. Indeed, if health conditions the educational process (WHO, 1997), it must be indicated that quality education is one of the keys to better health and therefore well-being. The issues of education, health and well-being are extremely important and linked issues. Taking the case of education, we note with UNESCO (2018) that among all regions, it has the highest rates of exclusion from education. More than a fifth of children aged approximately 6 to 11 are not in school, followed by a third of children aged approximately 12 to 14.

Keywords: education, health, population, well-being, society

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1. INTRODUCTION

The health of students is a major component of their well-being. As such, it is necessary to develop knowledge and behaviors that are favorable to their health among young people. However, school is the most favorable place for this: on the one hand, students spend more than a third of their waking hours there; on the other hand, it is the most appropriate place for the egalitarian acquisition of knowledge and behaviors that are favorable to health, by addressing students from different family and socio-economic backgrounds.

French public health policy states that health education is an essential factor in student well-being, academic success and equity. The objective of this report is therefore to understand what is addressed to students at school in terms of health, how and by whom, and what they retain from it in relation to quality of life.

First, this report attempts to understand which health indicators are addressed at school. Indeed, some are often addressed (such as sex education, healthy living, prevention of dangerous games, etc.), while others are rarely addressed, although they are cited as important (by health professionals or official documents). These include, for example, gambling, oral health or prevention of antisocial behavior. On the educational level, however, action is more often directed towards a set of key factors that influence young people's choices rather than on a health problem per se. Thus, most actions aim to raise awareness of the development of personal and social skills that help structure identity, cope with difficulties and build a unique vision of the world, in order to adopt healthy behaviors that protect against risky behaviors.

Secondly, this report presents the different actors promoting health within the school, as well as their roles: school nurses, school psychologists, and teachers. The difficulties of dialogue that can sometimes arise between these different actors are highlighted, and the major role that teachers must play in the health education process (particularly SVT and PE teachers), but also the lack of resources and means that teachers sometimes face. Other actors intervene in health education: families must be involved in this learning, thanks to an in-depth dialogue with teaching staff. Finally, INPES and other organizations organize prevention campaigns through extremely varied means adapted to young people (YouTube channel, websites, Facebook page, etc.). This report also presents students' perceptions of health promotion at

school and its impact on their quality of life. It shows that, on the one hand, students tend to forget the one-off interventions carried out for them, in favor of longer-term interventions that seem more effective. On the other hand, young people demonstrate good knowledge of behaviors that promote well-being and health, and yet hardly comply with them. This report also provides recommendations drawn from these lessons, in particular, on the need to include parents in the health education circle and for greater collaboration between the different actors working for prevention within the school.

2. MATERIAL AND METHOD

We used databases listing English and French psychology and educational science articles: Cairn (health & school: 19,898 articles), Science direct, Psycinfo (school & health education: 2,045 articles in pdf) and Researchgate (researchers' portal) with the following keywords: in French: éducation à la santé, qualité de vie et santé, and in English: health education, health literacy, health promoting school, school health. We also searched for French and international reports dealing with health education and school interventions. In the end, the studies came mainly from France and Canada, but also from Germany, Australia, Scotland, Finland, Italy, Ireland, Kuwait, Malaysia, Morocco, Norway, New Zealand, the Netherlands, Portugal, the United Kingdom and Switzerland. Some are international. Given the very large number of publications, we focused our research on studies dealing more specifically with the link between health and quality of life of students, health education interventions on the one hand, and on the other hand, on the results of the HBSC 2010 survey. The HBSC (Health Behaviour in School-aged Children) survey is the only international database on health promotion among 11-15 year-olds (Boyce et al., 2008). It is conducted in collaboration with the WHO Regional Office for Europe, in which 41 countries and regions of Europe-WHO and North America now participate. It provides a snapshot of the health, school experiences, life contexts (family, schools, friends) and behaviours (health-promoting or unhealthy) of school-aged young people aged 11, 13 and 15 (Godeau et al., 2012).

2.1. Simulation

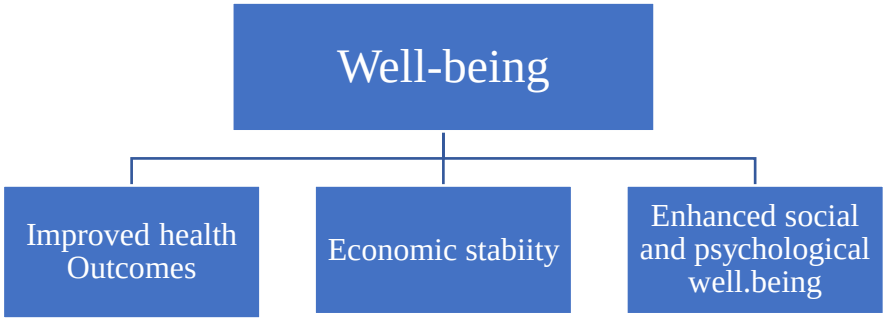

Figure 1. Three consequences that education brings to well-being

Table 1. (Times New Roman,10pt, iki yana yaslı/two sided)

Activities	Explanation
Improving the time of reading and writing medical documents	In this way, medical professionals better understand the branch and field to which they belong from people with more years of work and study who share their experience, from which important decisions are made
Engagement in seminars and trainings for health and well-being	This is very important for ordinary individuals, but of course also for student or employed medical staff, who can receive as much information as possible but also give and create a discussion.

3. RESULTS

It should be noted that nutrition and injury prevention are very often addressed in primary school (eating habits can indeed date back to early childhood), while drugs, AIDS and sexuality are given priority in secondary education, since risky behaviours in these areas are most often adopted in adolescence. On the other hand, pupils benefit from medical monitoring at school, with a very comprehensive initial assessment between the child's fifth and sixth year.

However, in terms of education, rather than focusing on health problems per se, it is currently more often proposed to act on a set of key factors that influence young people's choices and the issues that affect them. Some of these factors concern the development of personal and social skills that allow them to structure their identity, face the challenges of daily life, build their vision of the world and develop their power of action and thus "think for themselves and resist the forms of influence exerted on them by stereotypes, peer pressure, the power of the media but also immediate emotional reactions". They allow them to adopt healthy behaviors and protect themselves against risky behaviors. The necessary skills are, according to the World Health Organization: "the ability to make decisions and solve problems, creative reasoning and critical thinking, self-awareness and empathy, communication and interpersonal skills, the ability to cope with emotions and control stress".

The field of health promotion in schools encompasses the school environment, the implementation of health education programs, medical examinations and health checks at key ages in schooling and, for the schooling of students with special needs, the early detection of health problems or deficiencies in care that could hinder schooling, as well as the reception, listening, support and individualized monitoring of students. We will focus here more particularly on health education programs directly aimed at students, asking ourselves, on the one hand, what are the health indicators on which these interventions focus, who are the promotion actors and what resources are available to them. On the other hand, we think it would be interesting to look at the importance of the contexts in which young people evolve for their health and how students perceive the link between the promotion of their health and their quality of life.

It is difficult to cite an exhaustive list of "forgotten" or neglected health indicators. However, here are a few examples. In Canada, note the recent appearance of the theme of "Contacts with nature" whose necessity for well-being and health was also recently identified in Europe. In Australia, the focus is also on the prevention of antisocial behavior. The prevention of school dropout is proposed by Quebecers. Oral health, gambling and games of chance are cited in the summary of recommendations of the National Institute of Public Health in Quebec, but rarely found in scientific studies. This can be explained by the fact that they can be the subject of one-off interventions, chosen by the school on the basis of needs identified in the children welcomed.

Beyond health indicators, the teaching of psychology, interrupted since 2003, while it is present in many foreign countries, aims in particular, as in Switzerland for example, to "bring the student to reflect and work on himself and with others, by becoming aware of himself as an individual and a social person". The reintroduction of this teaching was also recently proposed to the Higher Council of French Programs, with as potential content: "experimental psychology, psychoanalysis, health, developmental psychology, methods of psychology, perception, memory, intelligence, the group, attention, learning, social manipulation, all depending on the series at a rate of 2 or 3 hours per week"

4. DISCUSSION AND CONCLUSIONS

The health education policy provides for the accountability of all stakeholders in the education system (inspection, management, teaching, education, guidance, social, health, technician, worker and service/TOS staff) as well as openness to new partners to implement actions or gather a certain number of resources, if necessary.

For public middle and high schools, the Health and Citizenship Education Committee (CESC) is set up, responsible for implementing health education in the establishment. This is a body for reflection, observation and monitoring in which parents are represented. This notion of citizenship refers to critical thinking, autonomy and accountability for health acts that it aims to develop in young people.

However, while health education is not absent from schools, a recent study conducted among 207 people working in 5 French colleges showed that 89% of professionals felt involved in health education, it is not a central object in the school's activity, nor a component of health promotion actions that aim to help people build a positive image of themselves and their health. A DEGESCO note indicates, in 2011, that 78% of the actors who lead health education actions are health and social service personnel and highlights the lack of motivation of the teaching team, time constraints and lack of time among the obstacles to the implementation of health education actions. In the scientific literature, there is more research on nurses (and/or school psychologists) and teachers, with the question of their representations of health (e.g.: biomedical approach vs. global education of the child), their training in health matters and their areas of expertise compared to other actors.

According to article 2 of decree no. 2012-762 of May 9, 2012, members of the nursing corps who are assigned to educational establishments participate in health prevention and education actions for pupils and students. They provide personalized support and monitoring of students throughout their schooling. Under the authority of the head of the

establishment, they are responsible for promoting and implementing health policy for all students: general health actions, mandatory assessments, prevention.

In their study conducted in the Lyon and Clermont-Ferrand academies, Berger et al. (2009) report that 94% of school nurses who returned the questionnaire said they carried out health education activities: 30% alone and 70% with a partner (internal or external to the establishment). They addressed one or more themes in 557 sessions that are divided thematically: sexuality (80%), addictive behavior (60%), nutrition (54%), smoking (52%) and alcohol (44%). This work was mainly carried out for 54% in middle school, 12% in high school and 15% in primary school. These sessions are offered in the form of educational sequences that are not very integrated into a project (34%). The most used system is the half-class sequence with educational tools, which may be simply information brought to their attention (prescriptive approach), allowing for debate in order to solicit group reflection. The objective, as formulated by the nurses in this study, is "to provide information to enable responsible choices". Berger et al. (2009), (p. 651) speak here of "illusion", because it is established that being informed is not enough to change behavior. This system also reinforces social inequalities by sending a verbal message, in the form of teaching, to the students who are having the most difficulty at this level and often the most at risk in terms of health behavior. We should also note here the risk of missing out on the socialization of their parents, whose health education is likely to be carried out by these children.

The motivations for health education interventions are very diverse. While 40 nurses out of the 188 interviewed (23%) explain that it is the ministerial texts (definition of the role and missions) that form the basis of their health education actions, others, a little more numerous (30%), say they respond to specific needs and requests (23), to the projects implemented (19; CESC, establishment or school projects), to particular situations determined by triggering events (15 drunken students, suicide cases, accidents).

Collective reflection for ES projects is directly linked to the work carried out or not in CESC (Health and Citizenship Education Committee).
It is also interesting to note that while 30% work alone, without external partnership, 62% express a feeling of professional isolation. Among the external partnerships mentioned, we find:
1. State services (police, gendarmerie, firefighters);
2. Health education associations;
3. Thematic associations, such as Family Planning Centers and the National Association for the Prevention of Alcoholism and Addictions).
The main obstacles to the implementation of health education activities are: lack of time
(23) and in particular the difficulty of having more time with students (12), negative or unenthusiastic reaction/lack of support from colleagues and management (17), being newly assigned to the position (15), lack of training (12), insufficient equipment (10).

Acknowledgements / Teşekkür
It should be written as short as possible and expressing the contribution made without giving the number.

Ethics Committee Approval / Etik Kurul Onayı
N/A

Peer-review / Akran Değerlendirmesi
Externally peer-reviewed.

Author Contributions / Yazar Katkıları
Conceptualization: H.S.; Investigation: H.S.; Material and Methodology: H.S., S.Ö.; Supervision: H.S., E.T.E.; Visualization: S.Ö.; Writing-Original Draft: H.S., D.A.; Writing-review & Editing: D.A., S.Ö.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest / Çıkar Çatışması
The authors have no conflicts of interest to declare.

Funding / Finansal Destek
The authors declared that this study has received no financial support.

REFERENCES

Gaborit, E. and N. Haschar-Noé (2014). A school "sanitization" enterprise. The example of the "Healthy School" approach in Quebec. Social Sciences and Health 2 (32), 33–56.

Gaillard, A., I. Urdapilleta, and . Sale-Wuillemin (2009, June). Social representation of food in children aged 6 to 11. In Communication at the 3rd international conference "Social Psychology and Communication", Tarbes, France. Available from: <http://psc2009.iuttarbes.fr/spip.php?article65>.

Garnier, C., L. Marinacci, and M. Quesnel (2007). Social representations of food, health and illness in young children. Revue Service social 1 (53), 109–122.

Godeau, E., F. Navarro, and C. Arnaud (2012). The health of middle school students in France / 2010. Paris.

Grondin, M.-A., A.-S. Berges, M.-C. D'Agrosa-Boiteux, V. Azais-Braesco, M. Tissier, J. Cassagnes, and L. Gerbaud (2009). Prevention of cardiovascular diseases and cancers from primary school onwards: "Être & savoir, l'école de la santé", evaluation of an intervention on 2,207 children. Revue d'Epidémiologie et de Santé Publique 1 (57), 52–61.

Guiet-Silvain, J., D. Jourdan, S. Parayre, C. Simar, F. Pizon, and D. Berger (2011). Health education in schools, a historical and international perspective. Education Crossroads 2 (32), 105–127.

Haldre, K., K. Rahu, M. Rahu, and H. Karro (2009). Individual and familial factors associated with teenage pregnancy: an interview study. European Journal of Public Health 3 (19), 266–270.

Hamel, M., L. Blanchet, and C. Martin (2001). We'll be much better! Determinants of health and well-being in school-aged children. Sainte Foy, Quebec.

Aaroe, L., T. Torsheim, and B. Wold (2001). Sense of coherence and school-related stress as predictors of subjective health complaints in early adolescence: Interactive, indirect or direct relationships? Social Science & Medicine 5 (53), 603–614.

Agostini, M., and J. Mallet (2010). Understanding resistance to health education in light of the Socratic model. Revue des sciences de l'Éducation 3 (36), 631–647.

Bantuelle, M., and R. Demeulemeester (2008). Best practice framework. Risky behaviors and health: taking action in schools.

Barnekow, V., G. Buijs, S. Clift, B. Bruun-Jensen, P. Paulus, D. Rivett, and I. Young (2006). Health-promoting schools: A resource for developing indicators. Technical report, International Planning Committee (IPC), European Network of Health Promoting Schools, WHO Regional Office for Europe, Copenhagen.

Berger, D., D. Flenghi, S. Marchand-Mallet, J.-C. Rochigneux, and A. Mougniotte (2012). Participatory approach to global health in middle and high schools: "diagnosanté" in Rhône-Alpes. Public Health 5 (24), 387–401.

Berger, D. and D. Jourdan (2006). EPS and prevention of addictive behaviors. What training in IUFM? Toulouse.

Berger, D., M. Nekaa, and P. Courty (2009). School nurses: representations and practices of health education. Public Health 6 (21), 641–657.

INPES (2008). By listening to music too loudly, we end up hearing it only halfway. Press kit, INPES. http://www.inpes.sante.fr/70000/dp/08/dp081023.pdf (last consulted on 20/07/2015).

Determination of *In Vitro* Antioxidant Activities, Total Lycopene and Carotenoid Contents of Tomato (*Lycopersicum esculentum)* products

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Abstract: The tomato, scientifically known as *Lycopersicum esculentum*, belongs to the Solanaceae family and is widely cultivated as a popular vegetable crop. The tomato is a fundamental component in daily cuisine in many countries and is considered the second most prevalent vegetable worldwide. With the increase in the use of tomato products, there has been a significant emphasis on researching their possible effects on human health and the bioactive components that are responsible for these advantageous benefits. Thus, this study aimed to determine the carotenoid concentration and their the primary constituent, lycopene, of dried tomatoes and tomato sauce commonly consumed in Turkey. For analysis of total lycopene and total carotenoid we used a fast and simple spectrophotometric method, at 472 and 475 nm, in hexane:acetone:ethanol extract. The total lycopene and carotenoid concentration was expressed as mg/g sample. Lipophilic total antioxidant capacity (TAC) in samples was measured by CUPRAC method. TAC was expressed as micromole trolox equivalents (µmol TR/g-sample) depending on the trolox standard curve. In tomato products the lycopene content had the folowing values: in dried tomato approximately 2.04 mg/g and in tomato sauce approximately 0.24 mg/g. Total carotenoid contents of dried tomato and tomato sauce were calculated to be 0.39 /g and 3.14 mg/g, respectively. Dried tomato and tomato sauce prepared from fresh tomatoe gave CUPRAC values of total antioxidant capacity of 19.1 and 3 µmol TR/g-sample, respectively. The findings revealed that dried tomatoes were better in terms of both total antioxidant capacity and total carotenoids when compared to tomato sauce. However, further research is needed to determine which of these two tomato products has the highest bioavailability when consumed in the diet.

Keywords: Lycopene, tomato products, tomato sauce, CUPRAC assay, total carotenoid

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1. INTRODUCTION

The tomato (*Lycopersicum esculentum*), a member of the Solanaceae family, is a highly popular vegetable crop. The tomato is a primary ingredient in everyday meals in numerous countries and ranks as the second most prominent vegetable globally (Pinela et al., 2016). The eating of raw tomatoes and their products is a significant source of antioxidants in human nutrition. Various tomato-based products, including juice, ketchup, tomato paste, soup, and sauce, are widely used in the human diet (Liu et al., 2015). Tomatoes are a rich source of antioxidants, including both hydrophilic and lipophilic varieties (Barros et al., 2012; Pinela et al.,2012).

Lycopene is a vivid red pigment and phytochemical that is present in tomatoes and other red fruits (Alda et al., 2009). Some fruits and vegetables that have a high lycopene content are gac, tomatoes, watermelon, pink grapefruit, pink guava, papaya, red bell pepper, seabuckthorn, wolfberry (also known as goji, a berry related to tomatoes), and rosehip (Alda et al., 2009). Lycopene plays a crucial role in the production of various carotenoids, such as beta carotene, which are responsible for the yellow, orange, or red coloration, as well as for the processes of photosynthesis and photoprotection, in plants, algae, and other photosynthetic organisms. Lycopene is a valuable food coloring agent because of its strong color and lack of toxicity (Gupta et al., 2015). From a structural perspective, it is a tetraterpene that is formed by combining eight isoprene units. It is made up entirely of carbon and hydrogen. Lycopene is a C40 polyisoprenoid compound containing 13 double bonds. It is the most prevalent carotenoid found in ripe tomatoes, making up around 80-90% of the overall pigment content.

Lycopene, the predominant carotenoid in tomatoes, exhibits the highest antioxidant activity and singlet oxygen scavenging ability of all dietary carotenoids (Di Mascio et al., 1989). Tomato products that have been processed, like pasteurized tomato juice, soup, sauce, and ketchup, provide the most concentrated amounts of lycopene that can be easily

absorbed by the body. The bioavailability of lycopene is higher in tomato soup and tomato paste compared to raw tomatoes.

Lycopene is not a necessary nutrient for humans, however it is frequently present in the diet, primarily in dishes that contain tomato sauce. Lycopene, once ingested, is carried in the bloodstream by different lipoproteins and tends to accumulate in the liver, adrenal glands, and testes. Due to initial research findings indicating a negative relationship between tomato consumption and the risk of cancer, lycopene has been identified as a possible preventive agent for some types of malignancies, specifically prostate cancer.

This study evaluates the total lycopene and carotenoid contents of various commonly consumed tomato products (dried tomato and tomato sauce) from the Turkish cuisine. Additionally, the antioxidant capacity of dried tomatoes and tomato sauce was measured by the CUPRAC method. Also, the amounts of carotenoid in the extracts of dried tomato and tomato sauce were measured using HPLC-DAD system.

2. MATERIAL AND METHOD

2.1. Chemicals

Neocuproine (2,9-dimethyl-1,10-phenanthroline) and lycopene were supplied by Sigma Chemical Co., Steinheim, Germany. Ammonium acetate, iron(II) chloride tetrahydrate, copper(II) chloride, 96% ethanol, and the rest of the chemicals were purchased from E. Merck, Darmstadt, Germany.

2.2. Sample materials

Tomato samples were purchased in a local market (Isparta, Türkiye). We washed them in water to remove any foreign matter, then left some of them to dry in the shade. To prepare the sauce from fresh tomatoes, we pureed them using a blender. Then the pureed tomatoes were transferred to a pot and left to boil for 10 min. Thus, samples were prepared for lycopene analysis (Figure 1).

Figure 1. Dried tomato and tomato sauce

2.3. Solutions

The CUPRAC reagents ($CuCl_2$, Nc and NH_4Ac) were prepared in ethanol.

2.4. Extraction procedure of lycopene from tomato products

Solvent extraction of antioxidants in the samples was performed with a mixture of hexane: ethanol:acetone (H:E:A) at 2:1:1 (*v*/v/v) ratio. Briefly, 50 mL of extraction solvent was added to 5.0 g of sample and shaken for 30 min at 45 °C in ultrasonic bath. Then, 10 mL of water was added to separate polar and non-polar layers. The lypophilic antioxidants were

in the non-polar layer (Martinez-Valverde et al., 2002; Sadler et al., 1990). The supernatants were filtered through 0.45 μm GF/PET microfilters.

2.5. Total lycopene and carotenoid content

To determine the total lycopene content in the tomato products methodology adopted was that described by Carvalho et al., (2005) with minor modifications, exhaustive extraction with H:E:A (2:1:1, v/v/v) performed in triplicate. Quantification of lycopene in the samples was determined by the spectrophotometric method for total carotenoids, considering lycopene which is the major component. Spectrophotometry readings were conducted at the wavelength of 472 nm. The concentration of lycopene (μg lycopene/g sample) was obtained from Eq. (1) as proposed by Rodriguez-Amaya (2001).

$$\textbf{Total lycopene concentration } (\mu g / g) = \frac{A \times V \times 1000000}{A1 \times M \times 100} \quad (1)$$

where: A, V, M are the absorbance of the solution at the wavelength of 472 nm, final volume of the solution, sample mass in the analysis and A1= 3450, the extinction coefficient. The measurements performed at 472 nm used to calculate the content of the rest of carotenes as b-carotene equivalents (BCeq), finally giving total carotenes content 2049 dL g^{-1} cm^{-1} for β -carotene at 475 nm.

2.6. CUPRAC assay of total antioxidant capacity

The total antioxidant capacity of the infusions was assessed using the CUPRAC test, as described by Apak et al. (2006). To summarize, the following substances were sequentially introduced into a glass tube: 1 mL of copper(II) solution (Cu(II)), 1 mL of neocuproin solution (Nc), 1 mL of ammonium acetate buffer (NH_4Ac), 0.5 mL of a sample solution and 0.6 mL of distilled water. Samples incubated at 25 °C in the dark for 30 min. Absorbance values were recorded at 450 nm against reagent blank solution. TAC was given as trolox equivalents (μmol TR/g-sample) depending on the trolox standard curve, calculated with Eq. (2):

$$TAC\ (mmol\ TR/g\ -sample) = \frac{A}{\varepsilon_{TR}} x\ \frac{V_m}{V_s}\ x\ D_f\ x\ \frac{V_E}{m} \quad (2)$$

where ε_{TR}: molar absorption coefficient of Trolox compound (1.67×10^4 L $mol^{-1}.cm^{-1}$),Vs is the sample volume, Vm is the total volume of method (4.1 mL), Df is dilution factor (when needed), V_E is the infusion volume (60 mL) and, m is the mass of sample [5 g].

3. RESULTS AND DISCUSSION

3.1. Total lycopene and carotenoid content

The total lycopene and carotenoid concentration was expressed as mg/g sample. In tomato products the lycopene content had the folowing values: in dried tomato approximately 2.04 mg/g and in tomato sauce approximately 0.24 mg/g. Total carotenoid contents of dried tomato and tomato sauce were calculated to be 0.39 /g and 3.14 mg/g, respectively (Figure 2). Comparing the results found that dried tomato's total lycopene content was nearly ten times higher than tomato sauce's. Similarly, the carotenoid content of dried tomato was 8 times higher than that of tomato sauce. The data revealed that dried tomato was richer in total lycopene and carotenoid content compared to tomato sauce.

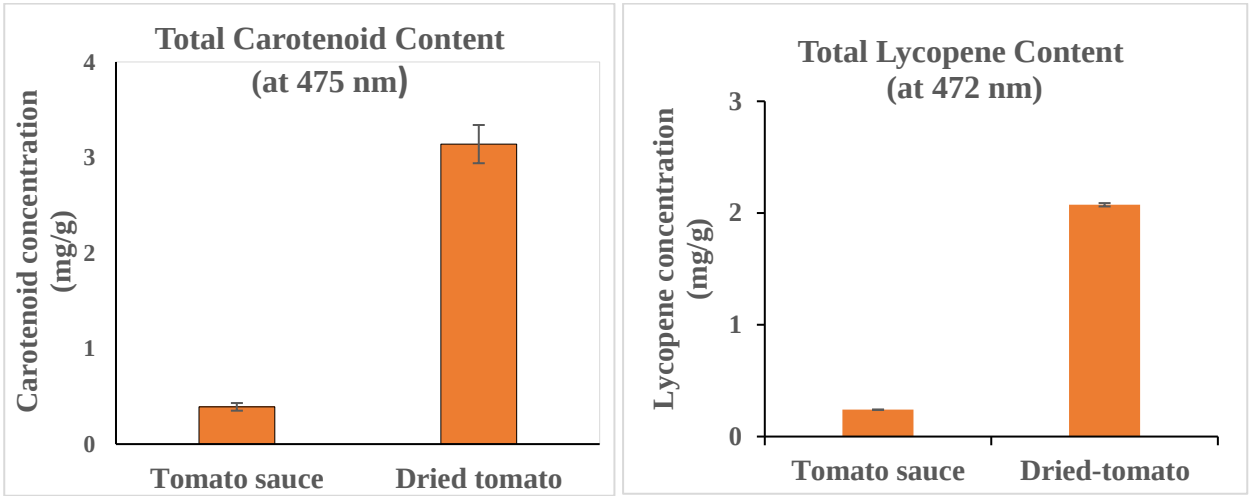

Figure 2. Total lycopene and carotenoid contents of dried tomato and tomato sauce

3.2. Antioxidant activity

The Cu(II)-neocuproin (Nc) reagent is used as a chromogenic oxidant in the CUPRAC test to accurately measure the total antioxidant capacity of plasma antioxidants, flavonoids, and dietary polyphenols (Apak et al., 2004). Dried tomato and tomato sauce prepared from fresh tomatoes gave CUPRAC values of total antioxidant capacity of 19.1 and 3 ɥmol TR/g-sample, respectively.

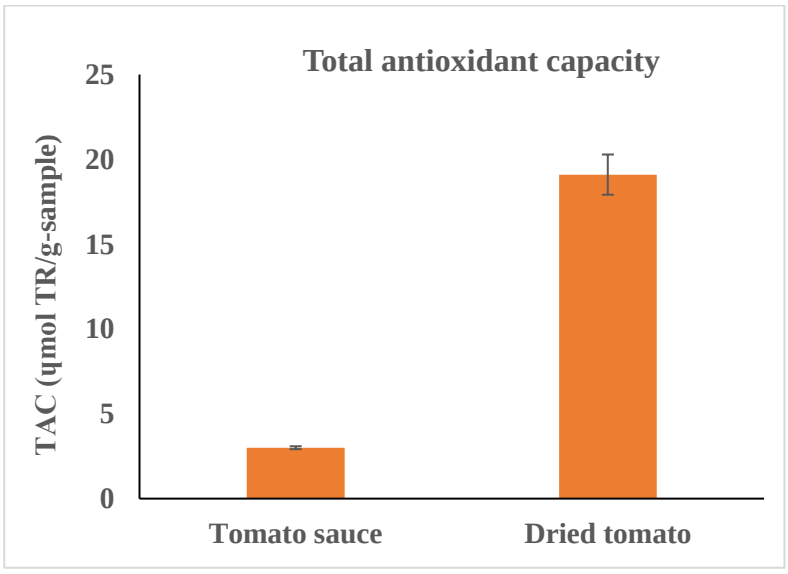

Figure 3. CUPRAC value of tomato products (Dried tomato and Tomato sauce)

4. CONCLUSIONS

The results indicated that dried tomatoes exhibited superior levels of both total antioxidant capacity and total carotenoids in comparison to tomato sauce. Additional research is required to ascertain the tomato product with the greatest bioavailability when ingested in the diet. These findings have consequences for assessing the daily consumption of lycopene in Turkey in order to produce a recommendation for the ideal daily lycopene intake.
These components possess the capacity to protect consumers from harm produced by free radicals and disorders linked to oxidative stress. As a result, the use of tomato in traditional medicine will be improved, and significant information on identifying antioxidant-rich foods and developing safe food items and additives with appropriate antioxidant properties will be gathered.

Author Contributions
Conceptualization: Ü.E.; Investigation: Ü.E., T.E.; Material and Methodology: Ü.E., T.E.; Supervision: Ü.E., T.E..; Visualization: Ü.E.; Writing-Original Draft: Ü.E., T.E..; Writing-review & Editing: Ü.E., T.E.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Alda, L. M., Gogoasa, I., Bordean, D. M., Gergen, I., Alda, S., Moldovan, C., Nita, L. (2009). Lycopene content of tomatoes and tomato products. Journal of Agroalimentary Processes and technologies, 15(4), 540-542.

Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S., Erçağ, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. International journal of food sciences and nutrition. 57(5-6), 292-304.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of agricultural and food chemistry, 52(26), 7970-7981

Barros, L., Due˜nas, M., Pinela, J., Carvalho, A. M., Buelga, C. S., Ferreira, I. C. (2012). Characterization and quantification of phenolic compounds in four tomato (Lycopersicon esculentum L.) farmers' varieties in northeastern Portugal homegardens. Plant Foods for Human Nutrition. 67, 229–234.

Carvalho, W., Fonseca, M. E. D. N., Silva, H. R. D., Boiteux, L. S., Giordano, L. D. B. (2005). Estimativa indireta de teores de licopeno em frutos de genótipos de tomateiro via análise colorimétrica. Horticultura Brasileira, *23*, 819-825.

Di Mascio, P., Kaiser, S., Sies, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Archives of biochemistry and biophysics, *274*(2), 532-538.

Gupta, S., Jawanda, M. K., Arora, V., Mehta, N., Yadav, V. (2015). Role of lycopene in preventing oral diseases as a nonsurgical aid of treatment. International journal of preventive medicine, *6*(1), 70.

Liu, W., Wu, Z., Wang, Y., Li, R., Ding, L., & Huang, D. (2015). Rhamnolipid assisted recovery of lycopene from the tomato-based processing wastewater using foam fractionation. Journal of Food Engineering, *164*, 63-69.

Martinez-Valverde, I., Periago, M. J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture, 82, 323–330.

Pinela, J., Barros, L., Carvalho, A. M., Ferreira, I. C. (2012). Nutritional composition and antioxidant activity of four tomato (Lycopersicon esculentum L.) farmer'varieties in northeastern Portugal homegardens. Food and Cemical Toxicology. 50, 829–834.

Pinela, J., Prieto, M. A., Barreiro, M. F., Carvalho, A. M., Oliveira, M. B. P., V´azquez, J. A., & Ferreira, I. C. (2016). Optimization of microwave-assisted extraction of hydrophilic and lipophilic antioxidants from a surplus tomato crop by response surface methodology. Food and Bioproducts Processing. 98, 283–298.

Rodriguez-Amaya, D. B. (2001). A guide to carotenoid analysis in foods (Vol. 71). Washington, DC, USA: ILSI press.

Sadler, G., Davis, J., Dezman, D. (1990). Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. Journal Food Science, 55, 1460–1461

Identification and Characterization of *Gymnosporangium* sp. Causing Rust on *Juniperus Excelsa* in Denizli, Türkiye

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Abstract: This study focuses on the rust disease observed on *Juniperus excelsa*, a tree native to the Mediterranean region. Field surveys in the Denizli area identified several trees with dry branches or twigs, and some trees appeared dead or in decline; however, it is not certain whether these symptoms are directly caused by the rust disease. Macroscobic and microscopic examination of samples collected from these symptomatic trees confirmed the presence of telial structures and spores associated with *Gymnosporangium fuscum*. Characteristic orange-brown galls on branches and twigs indicated a widespread infection across *J. excelsa* trees in the area, disease incidence of 80–90%. These findings underscore the need for further studies to evaluate the potential impact of this rust disease on *J. excelsa* populations. Research into the pathogen's life cycle, possible alternative hosts, and management strategies, as well as the importance of conducting pathogenicity tests, is crucial for protecting the health of Juniperus excelsa populations. Pathogenicity tests are essential to confirm the direct impact of the rust fungus on tree decline or mortality, providing vital insights for developing effective management strategies.

Keywords: *Gymnosporangium*, Junipers, rust disease, Denizli, Türkiye.

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1. INTRODUCTION

Plant parasitic rust fungi, belonging to the order Pucciniales, represent one of the largest groups of fungal pathogens, infecting a wide range of hosts from ferns to advanced monocots and dicots (Aime, 2006; Webster & Weber, 2007). Approximately 7,800 rust species have been identified worldwide, many of which cause significant economic losses in agricultural and forest crops (Arthur, 1934; Hiratsuka et al., 1992; Cummins & Hiratsuka, 2003). Among these, cedar rusts are particularly important phytopathogens (Helfer, 2005).

Fungi of the genus Gymnosporangium, previously classified under the family Pucciniaceae, are obligate biotrophic pathogens (Cummins & Hiratsuka, 1983, 2003). To date, over 64 species have been described, most exhibiting a demicyclic life cycle, while some are macrocyclic or microcyclic. Their life cycle alternates between a telial stage on gymnosperms in the family Cupressaceae and an aecial stage on members of the apple tribe (Maleae) within the family Rosaceae (Kern, 1973; Shen et al., 2018; Farr & Rossman, 2019).

Gymnosporangium fuscum primarily infects Juniperus species and completes its heteroecious life cycle on secondary hosts within the Rosaceae family, such as apple and pear trees. During spring, orange-brown, gelatinous galls develop on juniper branches, which swell under humid conditions to produce yellow-orange telial structures. These structures release spores that are dispersed by wind to infect Rosaceae hosts, resulting in the formation of orange-red spots on their leaves (Kern, 1973; Shen et al., 2018). Infected plants often experience reduced photosynthetic capacity and, in the case of fruit crops, diminished yields, leading to significant economic losses in commercial apple and pear production (Helfer, 2005).

In Türkiye, Juniperus excelsa is an ecologically significant host for macrofungi, supporting 127 fungal species belonging to Ascomycota and Basidiomycota (Doğan et al., 2011). This tree species thrives at high altitudes (>1,000 m) and in areas with high surface stoniness, avoiding Eu-Mediterranean and Supramediterranean plant communities (Özkan et al., 2010). The ecological conditions in Denizli are conducive to the proliferation of Gymnosporangium species. This study aims to identify and characterize Gymnosporangium species causing rust disease on J. excelsa in Denizli, Türkiye.

2.MATERIAL and METHOD

2.1. Study Area and Sample Collection

This study was conducted in the Tavas district of Denizli province, located in the southwestern part of Türkiye. In April 2024, field surveys were carried out in a mixed forest area where pine and Juniperus species coexist. During these surveys, samples were collected from *J. excelsa* trees showing signs of drying and mortality (Fig 1). Rust disease symptoms are characterized by the presence of orange/brown galls or spore masses, which were visually inspected. The presence of rust lesions on the trunks and branches of the trees was meticulously examined. The assumption that the lesions were caused by Gymnosporangium was based on the presence of fusiform swellings with rough or flaky bark, or cankers. Telial scars visible from the ground were used to support this determination. Infected samples, including twigs, leaves, and galls, were placed in sterile plastic bags and labeled with GPS coordinates. The samples were transported to the laboratory for further analysis. To understand the prevalence of rust infection in the investigated area, the number of trees showing lesions was recorded.

2.2. Morphological Identification

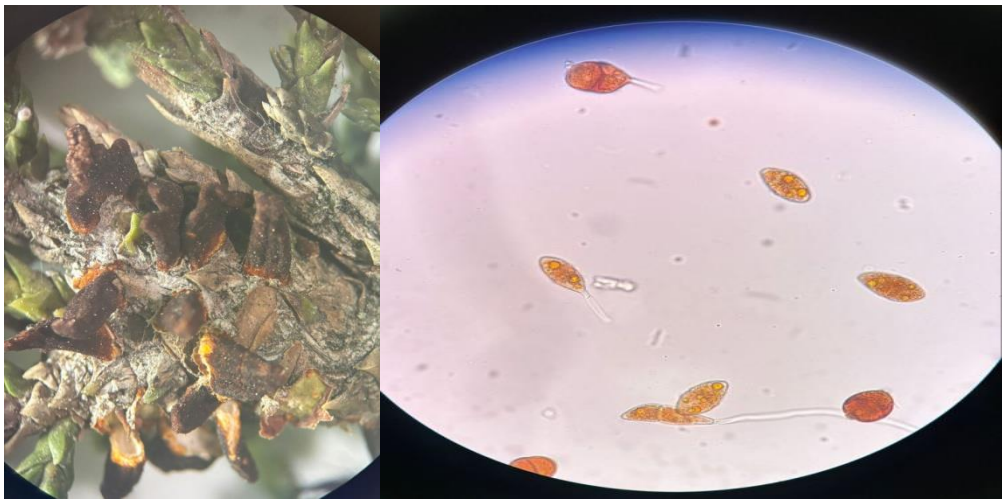
The morphological identification of Gymnosporangium species was performed using stereo and light microscopy to examine the characteristic structures and spore stages of the rust fungi. The collected samples were first observed under a Leica M205 FA stereomicroscope to assess key features, including the shape, color, and size of telial galls and aecial structures. For detailed morphological analysis, spores from the sori were mounted in a drop of lactophenol cotton blue on glass slides and examined under a Leica DM3000 light microscope to measure spore dimensions and study morphological characteristics.

The observed morphological traits were compared with type specimens, original descriptions, and relevant published literature (e.g., Kern, 1908; Sydow & Sydow, 1915; Arthur, 1934; Kuprevich & Tranzschel, 1957; Wilson & Henderson, 1966; Kern, 1973; Hiratsuka et al., 1992; Lee & Kakishima, 1999a,b; Yun et al., 2009; Zhao et al., 2017).

3. RESULTS

Field surveys conducted in the Denizli region revealed multiple Juniperus excelsa trees exhibiting symptoms of rust infection. The visible symptoms included distinct orange to reddish-brown galls on the twigs and branches of the affected trees, ranging in size from 1 to 4 cm. These galls were predominantly observed during the spring, following periods of high humidity, which favor the development of rust fungi.

Microscopic examination and morphological analysis of the collected samples confirmed that the rust pathogen responsible for the infection was Gymnosporangium fuscum (Fig. 1). Telial galls and aecial structures were clearly observed, and the teliospores exhibited elongated, multi-celled morphology, consistent with the characteristics of Gymnosporangium fuscum. Additionally, other morphological features observed were in agreement with the known descriptions of this species, which is recognized for infecting Juniperus species. The widespread occurrence of rust symptoms across multiple survey sites indicates that Gymnosporangium fuscum is a significant pathogen of Juniperus excelsa in the Denizli region. The findings suggest that rust infections negatively impact tree health by reducing photosynthetic capacity and weakening the host, potentially leading to significant ecological and economic consequences.



a) b)

Figure 1. a) *Gymnosporangium fuscum* observed under a stereomicroscope showing characteristic orange-brown galls on *Juniperus excelsa* twigs b) Teliospores of *Gymnosporangium fuscum* under a light microscope

4. DISCUSSION AND CONCLUSIONS

The widespread occurrence of rust symptoms observed across multiple surveyed sites in the Denizli region suggests that Gymnosporangium sp. is a prevalent pathogen affecting Juniperus excelsa. The characteristic formation of orange to reddish-brown galls on the branches of affected trees confirms the presence of a Gymnosporangium species. These galls, typical of this pathogen, cause a reduction in photosynthetic capacity, potentially weakening the trees and compromising their overall health. Such rust infections could have a significant ecological and economic impact, especially considering the role of J. excelsa in local ecosystems.

While this study primarily relied on morphological observations, the characteristics of the telial and aecial stages strongly suggest that Gymnosporangium fuscum is the causative agent of the rust observed in the region. The morphological features observed, including the shape and size of the telial galls and aecia, are consistent with descriptions of Gymnosporangium species known to infect Juniperus species. However, the lack of molecular analysis and pathogenicity testing in this study highlights the need for further research to definitively confirm the identity of the pathogen and assess its pathogenicity. Molecular techniques, such as DNA sequencing, would provide more accurate identification and could help in understanding the genetic diversity of the pathogen across different populations.

The findings from this study highlight the potential ecological consequences of Gymnosporangium sp. infections in J. excelsa, a species that is ecologically important in Mediterranean ecosystems. Juniperus excelsa provides habitat for numerous species and plays a critical role in maintaining biodiversity. Given its importance in the ecosystem, the impact of Gymnosporangium infections on this tree species could have cascading effects on the entire ecosystem. Thus, it is crucial to explore the dynamics of Gymnosporangium infections, not only to understand their direct impact on tree health but also to inform management and conservation strategies.

Future research should focus on molecular characterization to accurately identify the Gymnosporangium species affecting J. excelsa, as well as pathogenicity testing to determine the virulence of the pathogen. Investigating the host range of Gymnosporangium sp. will also be important to assess its potential spread to other species, especially those within the Cupressaceae and Rosaceae families. Additionally, developing effective management strategies, including the use of resistant cultivars and integrated pest management practices, will be essential for controlling the spread of rust infections. Finally, understanding the broader ecological impacts of these infections on forest ecosystems will provide valuable insights into their effects on biodiversity, soil health, and forest resilience.

REFERENCES

Dogan, Y., Ugulu, I., & Durkan, N. (2013). Wild edible plants sold in the local markets of Izmir, Turkey. *Pakistan Journal of Botany*, 45(SPL.ISS), 177–184.

Leppik, E. E. (1953). Some Viewpoints on the Phylogeny of Rust Fungi. I. Coniferous Rusts. *Mycologia*, 45(1), 46–74. https://doi.org/10.1080/00275514.1953.12024250

Ozkan, G., Baydar, H., & Erbas, S. (2010). The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (Origanum onites L.). *Journal of the Science of Food and Agriculture*, 90(2), 205–209. https://doi.org/10.1002/jsfa.3788

Tao, C., Wang, Z., Liu, S., Lv, N., Deng, X., Xiong, W., … Kowalchuk, G. A. (2023). Additive fungal interactions drive biocontrol of Fusarium wilt disease. *New Phytologist*, 238(3), 1198–1214. https://doi.org/10.1111/nph.18793

Yuan, H. S., Lu, X., Dai, Y. C., Hyde, K. D., Kan, Y. H., Kušan, I., … Zhou, L. W. (2020). Fungal diversity notes 1277–1386: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity*, 104(1), 1–266. https://doi.org/10.1007/s13225-020-00461-7

Zheng, Y. K., Qiao, X. G., Miao, C. P., Liu, K., Chen, Y. W., Xu, L. H., & Zhao, L. X. (2016, June 1). Diversity, distribution and biotechnological potential of endophytic fungi. *Annals of Microbiology*. *Springer Verlag*. https://doi.org/10.1007/s13213-015-1153-7