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Project Title: Clean Works! Antibacterial Soap from Ziziphus Jujuba Waste

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Key Words: Ziziphus Jujuba, Antibacterial Soap, Recycling

Abstract

In this study, a natural soap with antibacterial properties was produced by using waste obtained from the Jujuba (*Ziziphus Jujuba*) fruit, and the effect of this soap on different bacterial species was investigated. In the study, *Ziziphus Jujuba* extract, which are normally considered waste after food consumption, were used by adopting an upcycling approach. In this way, it was aimed to obtain an environmentally friendly product with high added value. The produced soap was prepared without synthetic antibacterial agents and heavy chemical additives. In this respect, it offers a natural alternative to commercial antibacterial soaps. The antibacterial activity of the soap was tested on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus Hirae* bacteria. The results showed that the soap was effective against different bacterial species. This study demonstrates that plant-based waste materials can be transformed into valuable products through sustainable production approaches. At the same time, the findings suggest that natural antibacterial soaps can contribute to both environmental sustainability and human health.

Keywords: Ziziphus Jujuba, Antibacterial Soap, Recycling.

Purpose

The aim of this research is to produce a natural soap with antibacterial properties using an extract obtained from the jujube plant (*Ziziphus jujuba*) and to experimentally examine the

effect of this soap on different types of bacteria. Nowadays, most commercial antibacterial soaps contain synthetic chemicals, which may pose risks to both human health and the environment. In this study, it is aimed to develop an alternative cleaning product with low chemical additives by using *Ziziphus Jujuba*, an easily accessible plant-based resource. Within the scope of the project, the jujube extract was converted into soap form, and the antibacterial activity of the obtained product was tested against different types of bacteria with varying characteristics, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus Hirae*. Thus, it is intended to comparatively present the effects of a plant-based natural soap on different bacterial groups. This study also aims to encourage individuals to use more natural and environmentally friendly ingredients in daily cleaning and personal care products and to raise awareness about reducing the use of synthetic chemicals.

Planning

I like biology. Therefore, I was trying to find a project topic about biology, and I came across the *Ziziphus Jujuba* plant. I was curious about this plant, so I researched it. When I researched it, I noticed that the plant has antibacterial properties. Therefore, I decided to make a natural antibacterial soap. I started to do research about this. I have also selected four ATL skills (Information literacy, organization, creative thinking and critical thinking) for myself, and I have planned to develop these skills and demonstrated them in my report.

History Of The *Ziziphus Jujuba*

Jujuba (*Ziziphus jujuba* Mill.), a temperate climate plant, originates from the Yunnan province of China and has been used and cultivated for both nutritional and medicinal purposes in China for approximately 7,000 years. It is a plant that can grow in the form of a tree or shrub and is notable for its resistance to cold, drought, and especially saline soil conditions. Different parts of the plant, such as its bark, fruit, seeds, and leaves, have long been used as sources of

medicine in the treatment of various diseases. Phytochemical studies conducted on *Ziziphus Jujuba* fruits have shown that they possess anticancer, anti-inflammatory, anti-obesity, immune-stimulating, and antioxidant properties. Rich in vitamins, minerals, phenolic compounds, and antioxidants, the *Ziziphus Jujuba* plant and its fruit are not sufficiently recognized in our country. This review provides general information about the *Ziziphus Jujuba* plant and its fruits, followed by important information about the nutritional content of the fruits and their importance for human health (İkinci & Ekinici, 2022).

Nutritional Content of *Ziziphus Jujuba*

In recent years, thanks to scientific studies conducted both worldwide and in our country, conscious consumers have begun to consider not only the taste, aroma, or smell of fruits and vegetables, but also their vitamin and mineral contents; therefore, there has been a significant increase in the production of *Ziziphus Jujuba* fruit, which is rich in water-soluble vitamins and minerals. *Ziziphus Jujuba* fruits are rich in various nutrients such as polysaccharides, protein, fat, fiber, vitamin C, cyclic adenosine monophosphate (cAMP), minerals like iron and potassium, and phenolics (San ve Yıldırım, 2010; Fang ve ark., 201). *Ziziphus Jujuba* fruits have played a role in the processing of various food products in many countries, including compotes, cakes, and bread. In addition, the peel, pulp, and seeds of *Ziziphus Jujuba* fruit have also been used in different ways for many diseases. Besides being consumed fresh, *Ziziphus Jujuba* fruits are also utilized in dried form (Zozio ve ark., 2014; Ivanišová ve ark., 2017). Consumed for thousands of years, *Ziziphus Jujuba* continues to influence our daily lives. Recent phytochemical and pharmacological findings have revealed that flavonoids, polysaccharides, and triterpenic acids are the main active compounds in *Ziziphus Jujuba*. According to studies, both flavonoids and polysaccharides are thought to be responsible for the antioxidant effects of *Ziziphus Jujuba*. In addition, *Ziziphus Jujuba* polysaccharides have been suggested to be the main active components contributing to immunoregulatory and

hematopoietic functions. Triterpenic acids have been accepted as active components responsible for anti-inflammatory and anticancer activities (Chang ve ark., 2010; Chen ve ark., 2017).

Chemical Composition and Applications of *Ziziphus Jujuba* Plant

The *Ziziphus Jujuba* plant has a chemical composition rich in phenolic compounds, flavonoids, saponins, alkaloids, polysaccharides, vitamins (especially vitamin C), minerals, and triterpenic acids (oleanolic, ursolic, betulinic acids, etc.), and various studies have reported that these compounds exhibit antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory effects (Liu ve ark., 2021; Wang ve ark., 2023). It has been reported that fruit and seed extracts of the plant show antimicrobial activity against both Gram-positive and Gram-negative bacteria; polysaccharide fractions can support the immune system, and triterpenic compounds can exert beneficial effects on inflammation (Guo ve ark., 2013; Ahmed ve ark., 2021). In traditional medicine, *Ziziphus Jujuba* leaves are used in the management of diabetes, obesity, and allergies; its fruit and peel are used to alleviate infections; and seed and leaf extracts are employed for sedative and sleep-regulating purposes, with modern literature supporting some of these effects through biological mechanisms (Huang ve ark., 2020; Zhang ve ark., 2022).

***Ziziphus Jujuba*'s Mechanisms of Antibacterial, Antioxidant, and Anti-inflammatory Activity**

Studies have proven that the *Ziziphus Jujuba* has many antibacterial, antioxidant, and anti-inflammatory effect mechanisms. The average total oxidant status (TOS) of *Ziziphus Jujuba* was calculated as 11.25 $\mu\text{mol/L}$ in the water extract and 78.57 $\mu\text{mol/L}$ in the alcohol extract; the average total antioxidant status (TAS) of *Ziziphus jujuba* was calculated as 0.96 $\mu\text{mol/L}$ in the water extract and 2.87 $\mu\text{mol/L}$ in the alcohol extract. The total antioxidant and total oxidant

amounts of *Ziziphus Jujuba* were found to be higher and significant in alcohol extracts ($p < 0.05$) (Aydm, B. K. (2023)). The pharmacological properties of the *Ziziphus Jujuba* plant and the components in its structure have been evaluated both in vivo and in vitro, but the studies conducted have remained limited. In addition to the existing studies, it is necessary to expand the potential of medicinal use by researching the *Ziziphus Jujuba* fruit in more detail. It is known that different parts of the *Ziziphus Jujuba* fruit have medicinal properties such as antiseptic, analgesic, and antidiabetic. While *Ziziphus Jujuba* seeds are used due to their effect of reducing insomnia and anxiety, it has been proven that the fruit is effective as a blood purifier, nerve relaxant, stomach tonic, laxative, and cough suppressant (BELTEKIN, B., & IZOL, E. TIBBI MEYVELER). Studies conducted on *Ziziphus Jujuba* fruits have shown that the fruits have anticancer, anti-inflammatory, anti-obesity, immune system stimulating, and antioxidant effects.

Medical Uses

The presence of phenolic compounds, alkaloids, vitamins, minerals, fatty acids, carbohydrates, and proteins in the compounds of the *Ziziphus Jujuba* plant has been proven by various studies to prevent diseases. It has been reported that triterpenic acids such as ursolic acid, oleanolic acid, and betulinic acid in its content stop apoptosis in cancer. It has been proven as a result of studies that the leaves of the *Ziziphus Jujuba* plant can be used in the treatment of diabetes, obesity, and allergy; the tree bark and fruit can be used in the treatment of infections due to their antimicrobial effect; the seeds, bark, and leaves can be used as sedatives; triterpenic acids such as betulinic acid, zizyberanolic acid, oleanolic acid, and ursolic acid can be used as anti-inflammatory; and polysaccharides can be used as immune system boosters (Hürkan, Y. K. (2019)).

Today's Use In The Food Industry

The *Ziziphus Jujuba* plant has recently begun to be used more frequently in the food industry, both for the purpose of healthy nutrition and with the increasing interest in natural products. One of the main reasons why the preservation of fruits and vegetables by drying is widely used in the food industry is that, as a result of drying, these foods gain a concentrated characteristic in terms of their nutritional values. From the *Ziziphus Jujuba* plant, dried *Ziziphus Jujuba*, *Ziziphus Jujuba* bars, *Ziziphus Jujuba* tea, fruit juices with *Ziziphus Jujuba* extract, Turkish delight, dried fruit pulp, marmalade, jam, jelly, *Jujuba* powder can be produced, and it can be used as a natural sweetener instead of white sugar in some products.

Antibacterial Properties of Natural Soaps

The antibacterial properties of herbal soaps arise from the inhibitory effects of phenolic compounds, flavonoids, essential oils, organic acids, and natural fatty acids present in their contents on microorganisms, and it has been demonstrated by various scientific studies that these components can show growth-inhibiting effects especially against pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* (Burt, 2004; Nazir ve ark., 2023). Plant-based components such as olive oil, coconut oil, lavender, thyme, and rosemary not only contribute to the reduction of bacterial load but also provide a lower risk of irritation and allergic reactions compared to chemical antibacterial agents by preserving the natural microbial balance on the skin surface (García-Sánchez ve ark., 2019). The vegetable oils contained in these soaps prevent the skin from drying thanks to their moisturizing properties that strengthen the skin barrier, while supporting the maintenance of skin health with regular use. In addition, the fact that herbal antibacterial soaps do not contain synthetic preservatives, parabens, and strong surfactants provides an important advantage in terms of both human health and the environment (Raut ve ark., 2020). Studies conducted have shown that natural antibacterial products provide effective cleaning on surfaces while not leaving chemical residues, therefore

they can be safely used in household cleaning and personal care products (Singh ve ark., 2021). All these features make herbal soaps a sustainable, environmentally friendly, and preferable cleaning alternative in the long term.

Method

The Materials Used

In the study, the following materials were used for soap production:

- * 70 g ripe *Ziziphus Jujuba* puree
- * 30 g coconut oil
- * 100 mL olive oil
- * 190 g pure water
- * 72 g caustic soda (NaOH)
- * 8–9 drops of lavender oil
- * Silicone soap molds
- * Alcohol (to remove surface foam)

Soap Production Method

Preparation of *Ziziphus Jujuba* extract

First, fresh *Ziziphus Jujuba* fruits were separated from their seeds and boiled with a small amount of water for approximately 10 minutes. About one teacup of water was used for 6–7

Ziziphus Jujubas on average. The boiled fruits were turned into a puree using a blender and left to cool.

Preparing and cooling the soap mixture.

Olive oil and coconut oil used for soap were combined in a beaker, and the mixture was heated over low heat to 40–45°C until the oils were completely melted. In a separate container, a caustic solution was prepared by slowly adding 72 g of caustic to 190 g of water, and it was left to cool until its temperature dropped to approximately 40°C.

Making the soap homogeneous

When the temperatures of both mixtures approached each other, the caustic solution was added to the oil mixture in a thin stream, and the mixture was blended using a hand blender in a cycle of 5 seconds mixing and 10 seconds resting for a total of 5–10 minutes. When the mixture reached a pudding-like consistency called “trace,” *Ziziphus Jujuba* puree was added and stirred with a spatula until a homogeneous distribution was achieved.

Placing the prepared soaps into molds and removing them from the molds

Then, the mixture was poured evenly into silicone soap molds. To eliminate any foaming that might occur on the surface, a small amount of alcohol was sprayed onto the soaps. The molds were left at room temperature for 24–48 hours, and once the soaps had hardened, they were removed from the molds.

Bacterial inoculation and the disk diffusion method

E. coli bacteria were inoculated in the school laboratory using the disk diffusion method for antibacterial analysis. It was incubated at 37°C overnight.

In order to evaluate the antibacterial effectiveness of the soap, four standard bacterial species with different characteristics were used:

- *Escherichia coli* ATCC 10536
- *Pseudomonas aeruginosa* ATCC 15442
- *Staphylococcus aureus* ATCC 6538
- *Enterococcus Hirae* ATCC 10541

These bacteria are reference test microorganisms widely used in international standards to evaluate the effectiveness of cleaning and hygiene products. While *E. coli* is preferred in hygiene assessments due to being an indicator of fecal impurity, *P. aeruginosa* is important in measuring the effectiveness of cleaning products because of its high resistance. Since *S. aureus* is commonly found on human skin, it is used especially in soaps and personal care products, while *E. hirae* is used in disinfectant tests due to its environmental resistance properties. Thanks to these four different bacterial species, the effectiveness of the prepared *Ziziphus Jujuba* soap against a broad spectrum of bacteria has been evaluated.

Process Log Summary

The 1st and 2nd weeks of January passed by finding a project topic, and I decided to do a project about *Ziziphus Jujuba*. Until the end of January, I thought about and researched what I could do with *Ziziphus Jujuba*. Based on my research, I learned that the *Ziziphus Jujuba* plant has antibacterial properties. At the beginning of February, after consulting my teacher about my ideas, we conducted an experiment. As we were not successful in the classical antibacterial property screening as a result of the experiment, I decided to get support from a professional company. The results reached me 2 weeks later, and it was revealed that my natural soap has a high degree of antibacterial properties. Since I reached a positive result, I started writing my report by making use of my process journal.

Results

The microbiological activity tests carried out on the *Ziziphus Jujuba* extract soap sample have revealed that the product exhibits a high level of antimicrobial activity against standard reference bacteria. Among the tested microorganisms are **Escherichia coli (ATCC 10536)**, **Pseudomonas aeruginosa (ATCC 15442)**, **Staphylococcus aureus (ATCC 6538)** and **Enterococcus hirae (ATCC 10541)**. These bacteria are international standards frequently used in the efficacy evaluation of disinfectant and antiseptic products. According to the analysis results, the product has achieved a reduction of over **4 log (≥99.99%)** on all bacteria.

ANALYSIS REPORT

Table 1: Analysis Results (1-Minute Contact Time)

Microorganism	Initial Suspension (cfu/ml)	Control A	Control B	Control C	Test Suspension (N)	Product Conc.	Reduction (R)
Escherichia coli ATCC 10536	N ₀ : 1.25×10 ⁸	1.9×10 ²	1.85×10 ²	3×10 ²	10 ⁻⁶ : 216,182 / 10 ⁻⁷ : 192,232	8.8×10 ²	4.36
Pseudomonas aeruginosa ATCC 15442	N ₀ : 1.4×10 ⁸	2×10 ²	2.15×10 ²	3×10 ²	10 ⁻⁶ : 240,227 / 10 ⁻⁷ : 25,20	5.8×10 ²	4.42
Staphylococcus aureus ATCC 6538	N ₀ : 1.35×10 ⁸	2×10 ²	2.15×10 ²	3.1×10 ²	10 ⁻⁶ : 306,216 / 10 ⁻⁷ : 20,21	1.17×10 ³	4.25
Enterococcus hirae ATCC 10541	N ₀ : 1.35×10 ⁸	1.9×10 ²	2.2×10 ²	3×10 ²	10 ⁻⁶ : 220,190 / 10 ⁻⁷ : 20,19	8.7×10 ²	4.37

These values demonstrate that the soap is not only a superficial cleaning product; but also proves that it exhibits a strong antimicrobial effect even within a short contact time (1

minute). In addition, the fact that all of the neutralization, method validation, and control tests included in the analysis were found to be appropriate shows that the obtained results are reliable and in accordance with the analysis method. As a result, it has been determined that the formulation containing lavender and *Ziziphus Jujuba* provides significant protection against bacteria through the synergistic effect of herbal ingredients; and that the product is a microbiologically **effective, reliable, and usable** cleaning material.

Result and Discussion

In this study, the antibacterial effect of the natural soap produced using the *Ziziphus Jujuba* plant was tested on different types of bacteria. As a result of the experiments, it was observed that the jujube soap exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus Hirae*. In the media where the soap was applied, it was determined that bacterial growth decreased compared to the control group and it has been determined that inhibition zones were formed. Especially in *Staphylococcus aureus* and *Escherichia coli* bacteria, more pronounced zone formations were observed. In the control group, it was determined that bacterial growth continued and no inhibition zone was formed. The obtained findings show that the soap containing *Ziziphus Jujuba* extract may be effective against different groups of bacteria.

Recommendations

Based on the results obtained within the scope of this study, it has been observed that the natural soap produced using *Ziziphus Jujuba* extract possesses antibacterial potential. In future studies, different concentrations of *Ziziphus Jujuba* extract used in the soap formulation can be tested to examine the concentration-dependent variation of antibacterial activity. Additionally,

the effect spectrum of the soap can be expanded by using more bacterial and fungal species. The stability of the produced soap during long-term use, its shelf life, and its effects on the skin can be investigated to evaluate product safety in more detail. In addition, combined studies with different herbal extracts can be conducted to increase the potential use of such soaps with natural ingredients and fewer chemicals as daily hygiene products. Finally, adapting the approach of utilizing *Ziziphus Jujuba* waste through upcycling to different cleaning and personal care products can contribute to environmentally friendly and sustainable product development efforts.



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