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Scientific Research

Investigating the Possibility of Producing Antibacterial Paper for Food Packaging with Rosemary (*Rosmarinus Officinalis l.*) Extract

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ABSTRACT

Change in the food consumption's pattern and the widespread use of the fast foods, as well as, the globalization of the food trade and the transportation of food over the long distances have brought great challenges to the safety and quality of the foods. One of the most important uses of the papers is the food packaging industry to keep the health and quality of the packaged materials from the production step to the customer.The extract of medicinal plants has the ability to create antibacterial properties. In this research, rosemary extract was used to produce the antibacterial papers. Extraction of plant was done by using soxhlet extractor and ultrasonic. In the soxhlet method, the dry powder was extracted at 4 and 6 hours and in the ultrasonic method, 40 and 60 powers were used for extraction. Then the papers were treated with plant extract prepared in two amounts of 15 and 20% based on the dry weight of the paper.The antibacterial properties of the papers were investigated with Escherichia coli and Staphylococcus aureus bacteria. All the papers treated with rosemary extracted by soxhlet method showed antibacterial property. Inhibition zone of bacteria was formed for both of them.The maximum diameter of the Inhibition zone for Escherichia coli and Staphylococcus aureus bacteria with 6 hours' extraction and the consumption amount of 20% was 22 and 20 mm. In the treated paper with rosemary extracted by ultrasonic method, the maximum diameter of the Inhibition zone for Escherichia coli bacteria was 20 mm in 40A and 9 min. Also, the highest diameter of the Inhibition zone for Staphylococcus aureus bacteria was 20 mm in 40A and 9 min. Gas chromatography confirmed the presence of antibacterial substances including, Borneol, Camphor, and Bornyl acetate in rosemary extract.

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1. Introduction

Paper plays an important role in meeting the daily needs of human life. One of the most important cases is the use of paper in the packaging industry, where it is necessary to ensure that the materials that have been packaged do not rot or perish from the production stage until they reach the customer [1]. In the past, materials made of plastic were used for packaging. After extensive research on their properties, scientists came to the conclusion that these materials have many environmental hazards. For this reason, the research to replace these materials has attracted the attention of scientists. After many studies, special papers with a combination of metal and plastic nanomaterials were used for packaging. Plastic materials are synthesized from oil and are not renewable, and this issue has caused many environmental concerns. Scientists are looking for renewable and environmentally friendly materials. Today, the use of biodegradable coatings for paper and cardboard has received a lot of attention, so that it has become a suitable alternative for food and health packaging [2].

The purpose of food packaging is to prevent bacterial spoilage and loss of nutrients and, as a result, to increase their shelf life [1]. Packaging is a protection that preserves the health of its contents after production until the stage of consumption. On the other hand, packaging has the role of introducing the product to the buyer. For materials such as bread, dairy products, meat, dry food, cereals, chocolate, etc., packaging materials such as metals, plastic materials, paper and cardboard are used, and their variety is increasing day by day [3]. In the past, the preservation of the product was considered as packaging, while after some time the advantages of durability, beauty and high-quality printing were added to it. Some protective materials have the necessary resistance properties and some are used due to their lightness. Of course, in food packaging, barrier properties against air (oxygen and carbon dioxide), humidity (water and steam) and heat are necessary to increase the useful life of food, and the world needs packaging. In addition, food packaging will have an upward demand in the future [4]. In addition, the creation of antibacterial properties in the papers used in the packaging of pharmaceutical and food products will prevent the contamination of the product with bacteria and its destruction during storage and transportation [5].

Medicinal plants are one of the important and renewable resources for creating antibacterial properties in the papers used in packaging. These plants are among natural resources that are found in abundance. The type, number and variety of species are different based on the conditions and geographical location of each region. The use of herbal products has increased significantly in recent years due to the evidence of harmful and side effects of chemical drugs and the creation of environmental pollution [6]. Among all the substances identified in plant organ compounds, phenolic compounds or nitrogen-free secondary compounds are the most and most important substances that have various biological effects, including antibacterial activity. Studies have shown that many plants of the mint family such as rosemary, thyme and oregano have antimicrobial properties, the extracts of these plants contain compounds such as phenylpropanoid glucoside, polyethylene, diterpenes, flavonoid, polyphenols and flavonglycoside. These compounds are involved in antimicrobial and antioxidant activity [7]. Medicinal plants have a wide range of secondary compounds that often have important biological activity. Due to the wide use of plant extracts in pharmaceuticals, extraction technologies were widely studied. Rosemary*Rosemary Officinalis l.*) belongs to the mint family. Rosemary is rich in phenolic compounds and shows good antimicrobial effects against gram positive and negative bacteria. In addition to antibacterial and antioxidant properties, it also has antimutagenic properties [8]. The extract of this plant shows its antimicrobial effects in the first 4 hours of bacterial growth, and this plant can be used as a natural food preservative [9].

The purpose of this study is to prepare rosemary plant extract by soxole and ultrasonic methods and to investigate the effect of the amount of compounds in the extracts on the antibacterial activity of the prepared packaging papers against the standard bacterial strains of Staphylococcus aureus and Escherichia coli.

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2- Materials and methods

2-1- Materials

Long fiber kraft paper was obtained from Mazandaran wood and paper factory. The dry powder of Shirazi thyme and rosemary plants was obtained from the educational campus and research greenhouse of Sari University of Agricultural Sciences and Natural Resources. Ethanol (purity 99%) was obtained from Merck.

2-2-method

2-2-1-Preparation of cationic starch

To prepare cationic starch, calculate the moisture percentage of starch0.048 grams on a dry weight basis100 added milliliters of distilled water and at temperature90 degrees Celsius and duration30 Minutes were placed under continuous stirring. After the test period,100One milliliter of water at the same temperature was added to the solution[10].

2-2-2-Preparation of long fiber dough

First, the sheet of kraft paper with long fibers was cut into small pieces, about 1 x 1 centimeter, and distilled water was added to it to soak the fibers. Mix water and paper crumbs for a long time24 The watch was set aside and then stirred well with a stirrer to separate the fibers. Pulp with machine Laboratory refinerPFI-MILLMade in Austria to the degreeRefinement of 450 mlbecame.

2-2-3- Preparation of extract by ultrasonic method

10 grams of dry powder of rosemary plant along with 100 milliliters of ethanol solvent were poured into the flask. Then the mixture was treated at laboratory temperature and at three time levels of 3, 6 and 6 minutes by means of ultrasonication with two amplitudes of 140 and 60. Finally, the solvent was evaporated and its solid material was collected [11].

2-2-4-preparation of extract by soxole method

In the extraction method of plant extract using soxole, 10 grams of dry powder of rosemary plant with ethanol solvent was used for 4 and 6 hours. Finally, the solvent was evaporated and its solid material was collected [11].

2-2-5- Making handmade paper

Handmade paper with a weight of 120 grams per square meter was prepared according to T205 sp-95 standard of TAPPI regulations. Therefore, 24 grams of refined paper pulp based on dry weight was transferred to human body and then cationic starch was added to it. For better mixing of cationic starch, pulp and starch suspension was stirred for 10 minutes.Then extract the amount of medicinal plants15 And20 The percentage based on the dry weight of paper pulp was sprayed on the surface of the paper and air dried for 24 hours.

2-2-6-**Antibacterial test**

In this test, the non-growth halo of Gram-positive and negative bacteria Escherichia coli and Staphylococcus aureus was measured and the results were recorded.

2-2-7-Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography (GC-MASS)

In order to investigate the chemical structure of the paper and extract extracted from the Fourier transform infrared spectrum (FTIR) used. Spectra of samples with Fourier transform infrared spectrometer Cary 630, company construction Agilent was taken Also, to check the constituents of rosemary plant extract using gas chromatography device (GC-MASS)ModelN7890Company construction Agilent Done.

3-Results and discussion

3-1-Gas chromatography

Figure and Table 1 show the gas chromatogram and chemical compounds in the extract of rosemary plant extracted with an ultrasonic device. Separation of essential oil components in gas chromatography is based on boiling point. The lower and more volatile the molecular weight of the compound is, the sooner it is removed from the device, and the heavier the compound, the slower it is removed. In general, the main compounds in rosemary are 1-8-cineole, borneol, camphor, bornel acetate, alpha and beta pinene [12]. In the chromatographic spectrum of rosemary plant, a total of 21 compounds were identified, most of which are shown in Table 1. The most important observed compounds that have antibacterial properties include borneol, camphor and bornel acetate.

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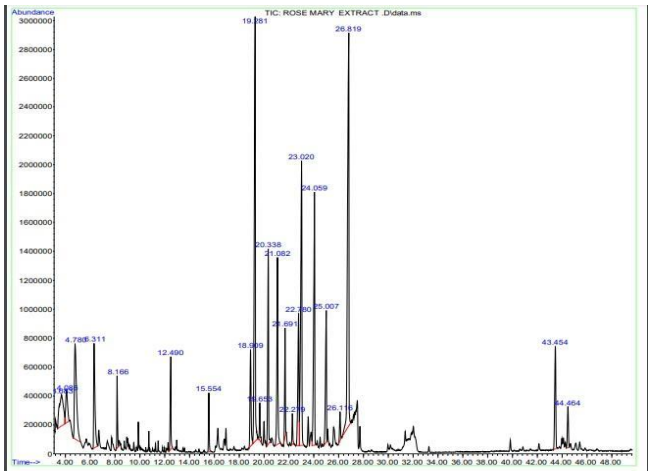

Fig 1 The components chromatogram of Rosemary extraction by ultrasonic.

Table 1 The components of Rosemary extraction by ultrasonic.

Components	hour (min)	Percentage (%)
Camphor	3.684	3.75
Bornyl acetate	4.778	6.14
Borneol	3.684	3.75
Acetyl	18.907	3.06
Silane, dimethyl	20.339	4.85
Comaroine	24.095	7.90
Diisooctyl phthalate	26.820	16.62

2-3-Fourier transform infrared spectroscopy (FTIR)

Figure 2 shows the spectrum obtained from papers treated with extract extracted from rosemary plant by different methods. Peaks in all three control and treated paper samples in the range of cm^{-1}1059 cm^{-1}3311-3414, it was observed that it is related to carbon-oxygen-carbon stretching vibration bond and free hydroxyl group respectively [13 and 14]. Peak in cm $range^{-1}$2900 is related to carbon-hydrogen stretching group. Also, the peak is in the cm $range^{-1}$1385 is related to the glycosidic bond between cellulose units [15]. In two samples treated with rosemary extract, peaks in cm $wavelengths^{-1}$1027.71, 1685.803 and 2851.522 represent the structures of carbon-nitrogen, carbon-oxygen and hydroxyl groups.

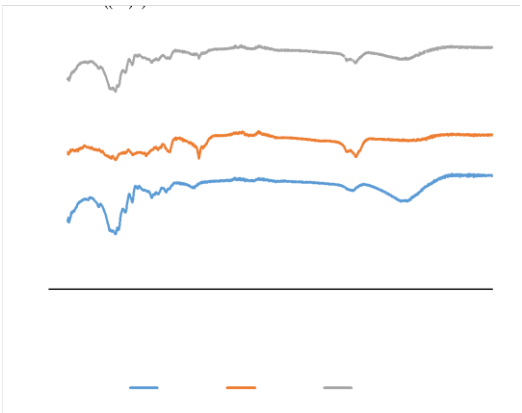

Fig2 FTIR of papers with Rosemary extraction by soxhlet, ultrasonic methods and control paper.

3-3- Antibacterial test of paper containing rosemary extract extracted by Soxole method

Table 2 and Figure 3 respectively show the value of the diameter of the non-growth halo of Escherichia coli and Staphylococcus aureus bacteria and the antibacterial test of treated papers for papers treated with rosemary extract. The diameter of the halo of non-growth for 4 hours and the percentages of the extract applied to the paper with amounts of 15 and 20% on Escherichia coli and Staphylococcus aureus bacteria are 18, 20, 13 and 14 mm, respectively, and the diameter of the halo of non-growth for the extraction time of 6 hours and With values of 15 and 20%, it is 20, 22, 13 and 20 mm on Escherichia coli and Staphylococcus aureus, respectively. As can be seen in the graphs, the diameter of the halo of non-growth of bacteria has increased with the increase in the extracting time and also the increase in the amount of extract used in the papers. Among all the identified substances present in the effective compounds of plant organs, phenolic compounds or secondary compounds without nitrogen are the most and most important substances that have various biological effects, including effective antibacterial activity [16]. The most important phenolic compounds in rosemary include carnosol, rosmarinic acid and caffeic acid. Also, the most important flavonoid compounds include diosmin, luteolin, camphor, borneol, cineole, and various terpenes that produce antibacterial properties in this plant [17]. These terpene and parthenoid compounds, in addition to being an antipathogen in eliminating bacterial, fungal and

pest infections, are considered as an effective substance in the treatment of migraine and cancer [7]. Walid et al., 2021, in a research on the antibacterial property of films containing rosemary methanol extract, reached a similar result against Escherichia coli bacteria. The treated films inhibited the growth of bacteria. The diameter of the non-growth halo for the film sample containing 2% rosemary extract was 14.33 mm [18].

Table 2 The inhibition zone diameter of papers with Rosemary extraction by soxhlet method for Escherichia coli and Staphylococcus aureus bacteria.

Time (h)	consumption	Inhibition zone diameter (mm)	
		Escherichia coli	Staphylococcus aureus
4	15 %	18	13
	20 %	20	14
6	15 %	20	13
	20 %	22	20

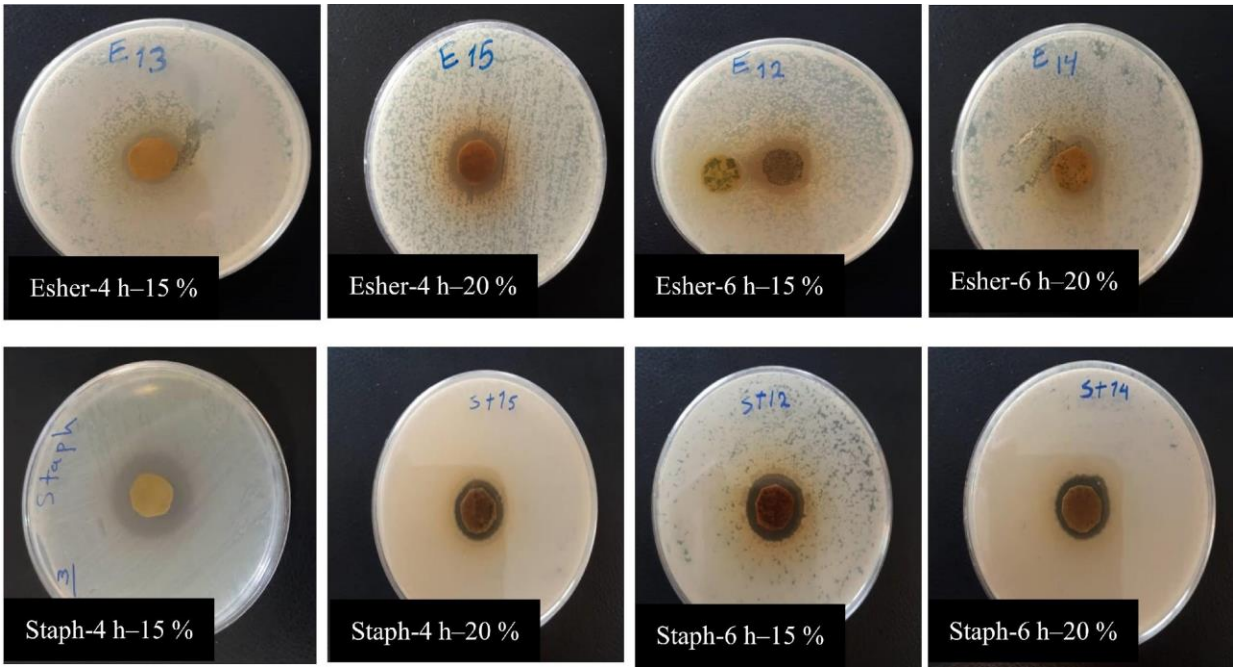

Fig 3 Theantibacterial reaction test of paperswith Rosemary extraction by soxhlet method for Escherichia coli and Staphylococcus aureus bacteria.

3-4- Antibacterial test of paper containing rosemary extract extracted by ultrasonic method

Table 3 and figure a, 4 and b, respectively, the value of the diameter of the lack of growth halo Bacteria Escherichia coli and Staphylococcus aureus show the antibacterial test of papers treated with rosemary extract extracted at 3, 6 and 9 minutes with ultrasonic device at 40 range.

Table 3 The inhibition zone diameter of papers with Rosemary extraction by ultrasonic method with 40 amplitudes for Escherichia coli and Staphylococcus aureus bacteria.

Amplitudes	hour (min)	consumption	Escherichia coli	Staphylococcus aureus
40	3	15 %	14	11
		20 %	16	14
	6	15 %	15	16
		20 %	15	14
	9	15 %	17	17
		20 %	20	20

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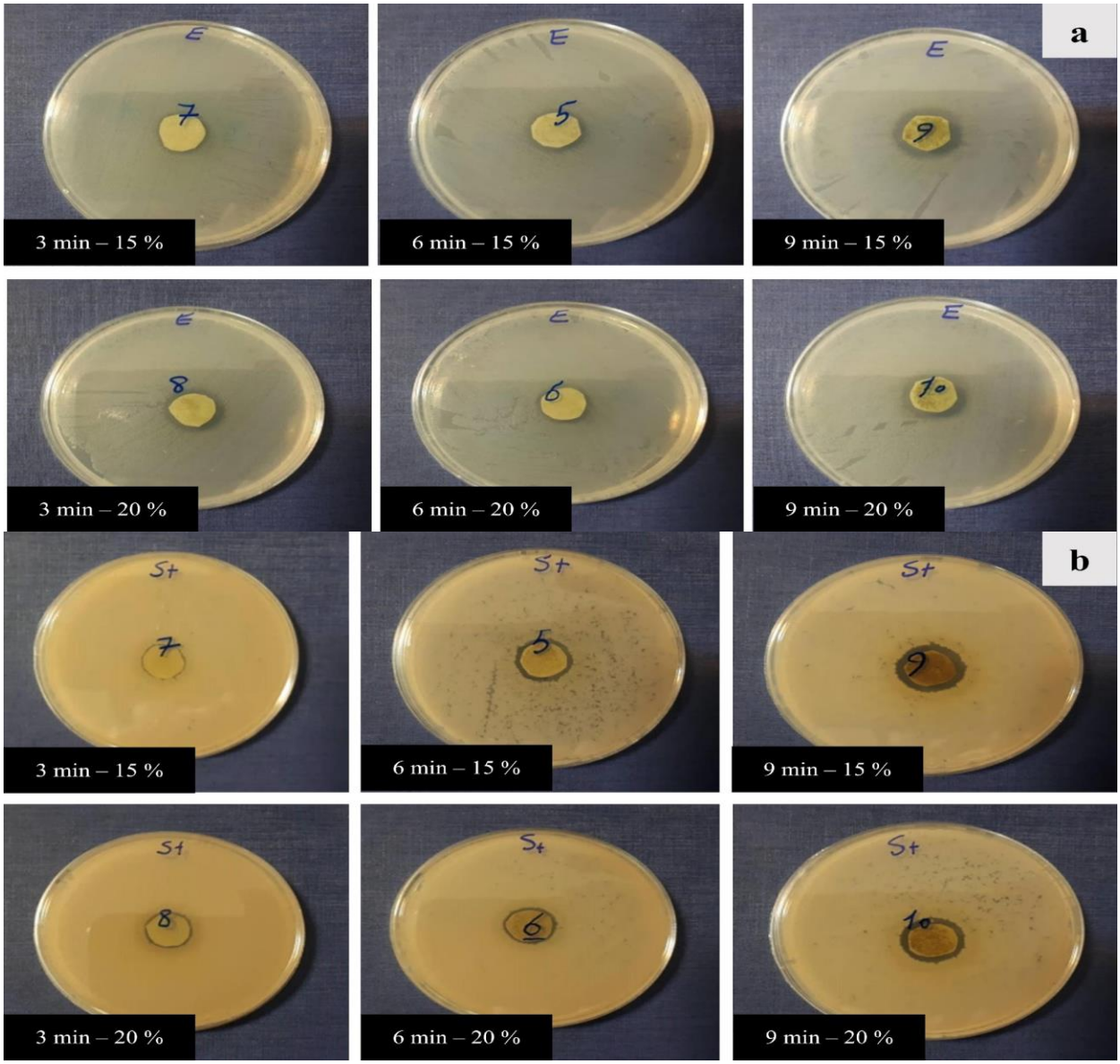

Fig 4 Theantibacterial reaction to Escherichia coli (a), and Staphylococcus aureus bacteria (b)by ultrasonic method with 40 amplitudes.

The maximum size of the halo of non-growth in the range of 40 for Escherichia coli and Staphylococcus aureus in 9 minutes and 20% concentration is 20 mm. Studies have shown that many plants of the mint family, such as rosemary, have antimicrobial effects, the extracts of these plants contain compounds such as phenylpropanoid glucoside, polyethylene, diterpenes, flavonoids, polyphenols, and flavonol glycosides. These compounds are involved in antimicrobial and antioxidant activity and have been used in traditional medicine since the past [7]. Rosemary is one of the plant species that has a large amount of antioxidants and can be used to fight against all types of cancer and microbes. The properties of antioxidants in the extract of this plant are different according to the growth conditions, geographical area of growth, climate,

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genetics, harvest date and extraction process [17]. Table 4 and Figure a5 and b respectively show the diameter of the halo of non-growth of Escherichia coli and Staphylococcus aureus bacteria and the antibacterial test of papers treated with rosemary extract extracted at 3, 6 and 9 minutes with an ultrasonic device in the range of 60. The largest diameter The halo of non-growth in the range of 60 for Escherichia coli and Staphylococcus aureus in 3 minutes and 20% concentration is better 19 and 18 mm. Extracts and essential oils of medicinal plants such as rosemary can be used for the healthy preservation of ready-to-eat foods due to their microbial properties against pathogenic bacteria such as Staphylococcus aureus in food [8]. In general, there are some properties in plant essential oils that lead to antibacterial properties. The first properties of plant extracts and essential oils are hydrophobic properties, which cause this substance to penetrate into the lipids of bacterial cells and disrupt the cell structure and cause ions and other cell contents to leak out. Also, phenolic substances present in plant extracts, such as carvacrol, eugenol and thymol, cause damage to the cytoplasmic end of the cell and disturb the proton force and electric current of the cell and coagulate the cell contents. Another feature of these extracts is the binding of their carbonic group to cell proteins and preventing the role of amino acid and decarboxylase in bacteria [8]. The antibacterial properties of rosemary extract are more effective against gram-negative bacteria than gram-positive ones. This feature increases with increasing the concentration of rosemary extract [17]. As can be seen in Table 4, the antibacterial performance of rosemary extract at a dosage of 20% based on the dry weight of the paper is better for Gram-negative bacteria Escherichia coli. Samples treated with rosemary extract extracted in 9 minutes did not show antibacterial activity against two bacteria. This reaction may be due to the destruction of antibacterial substances in rosemary extract during extraction.

Table 4 The inhibition zone diameter of papers with Rosemary extraction by ultrasonic method with 60 amplitudes for Escherichia coli and Staphylococcus aureus bacteria.

Amplitudes	hour (min)	consumption	Escherichia coli	Staphylococcus aureus
60	3	15 %	15	17
		20 %	19	18
	6	15 %	13	12
		20 %	17	14
	9	15 %	0	0
		20 %	0	0

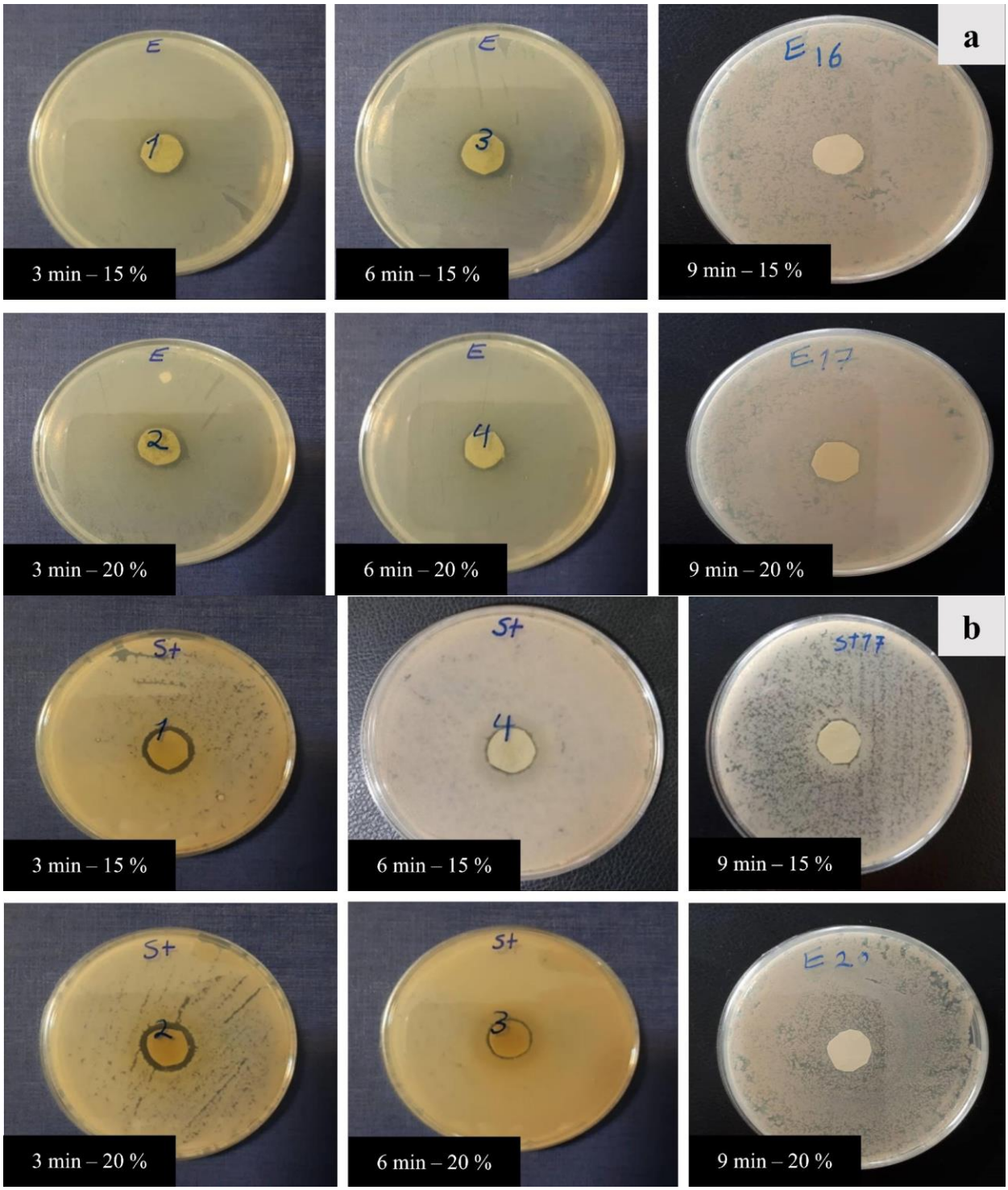

Fig 5 Theantibacterial reaction to Escherichia coli (a), and Staphylococcus aureus bacteria (b)by ultrasonic method with 60 amplitudes.

4 - Conclusion

In this research, the effect of using rosemary plant extract extracted by soxole and ultrasonic methods in the production of antibacterial wrapping paper on Escherichia coli and Staphylococcus aureus was studied. Papers treated with rosemary extract extracted by both methods and on both bacteria showed antibacterial properties. But the antibacterial performance of the papers treated with the

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extracted extract using the ultrasonic device method was better. Therefore, the use of rosemary extract extracted by ultrasonic method is suggested to disinfect packaging papers due to the short time required for extracting and creating a halo of non-growth suitable for both bacteria.

5-Resources

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بررسی امکان تولید کاغذ ضدباکتری جهت بسته‌بندی مواد غذایی با عصاره گیاه رزماری (*RosmarinusOfficinalisL.*)

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چکیده

تغییر الگوی مصرف غذا و استفاده گسترده از غذاهای بیرونی و فست فودها، از طرفی جهانی شدن تجارت غذا و حمل غذا در مسافت‌های طولانی، چالش‌های بزرگی را متوجه ایمنی و کیفیت غذا کرده است. یکی از مهم‌ترین موارد استفاده از کاغذ در صنایع بسته بندی مواد غذایی می‌باشد طوریکه بتواند مواد بسته‌بندی شده را از مرحله تولید تا رسیدن به دست مشتری سالم نگه دارد. عصاره گیاهان دارویی، قابلیت ایجاد خاصیت ضدباکتریایی دارند. در این پژوهش از عصاره گیاه رزماری جهت ایجاد خاصیت ضد باکتریایی استفاده شد. استخراج عصاره گیاهی با دو روش سوکسوله و التراسونیک انجام گردید. در روش سوکسوله، از پودر خشک رزماری در دو زمان ٤ و ٦ ساعت و در روش التراسونیک از دو دامنه آمپلیتوت ٤٠ و ٦٠ برای عصاره‌گیری استفاده شد. سپس کاغذها با عصاره تهیه شده در دو مقدار ۱۵ و ۲۰ درصد بر اساس وزن خشک کاغذ تیمار شدند. در نهایت بررسی ویژگی ضدباکتریایی در کاغذ مورد آزمون با دو باکتری اشرشیاکلای و استافیلوکوکوس اورئوس انجام شد. در کاغذهای تیمار شده با عصاره رزماری استخراج شده به روش سوکسوله، هاله عدم رشد باکتری برای هر دو باکتری تشکیل شد. بیشترین قطر هاله عدم رشد برای باکتری‌های اشریشیاکلای و استافیلوکوکوس ارئوس، در زمان استخراج ٦ ساعت و مقدار مصرف ۲۰ درصد بر اساس وزن خشک کاغذ بترتیب به اندازه ۲۲ و ۲۰ میلی‌متر مشاهده شد. در نمونه‌های کاغذ تیمار شده با عصاره استخراج شده به روش التراسونیک، بیشترین قطر هاله عدم رشد برای باکتری اشریشیاکلای در آمپلیتوت ٤٠ و زمان ۹ دقیقه و به اندازه ۲۰ میلی‌متر بود. همچنین بیشترین قطر هاله عدم رشد برای باکتری استافیلوکوکوس اورئوس در آمپلیتوت ٤٠ و زمان ۹ دقیقه و به اندازه ۲۰ میلی‌متر بوده است. کروماتوگرافی گازی وجود مواد ضدباکتری بورنئول، کامفر و بورنیل استات در عصاره رزماری را تأیید کردند.