International Baccalaureate

BIOLOGY EXTENDED ESSAY

Topic: Examination of the antimicrobial activity of extractions from *Cotinus coggygria* Scop. on *Staphylococcus aureus* prepared by boiling for 30, 40, 50, 60, and 70 minutes in 200 mL (±1 mL) distilled water with 10 gr (± 0.01 gr) of powdered dried leaves.

Research Question: How does the extractions from 10 gr dried leaves of the *Cotinus coggygria* Scop. prepared in 200 mL distilled water by boiling for 30, 40, 50, 60, and 70 minutes affect the activity of *Staphylococcus aureus?*

Word Count: 3965

TABLE OF CONTENTS

INTRODUCTION

The recent increase in the antimicrobial resistance (AMR) of pathogens has accelerated the search for new molecules in production of antibiotics and has drawn the scientists' attention to find new plants effective against microorganisms. Plants produce a variety of chemical compounds with different biological activities, including antimicrobial compounds effective against various pathogenic microorganisms. Development of new, reliable, cost-efficient, and non-toxic herbal anti-microbial agents is also important to minimize environmental and health problems. Therefore some of the recent researches have focused on antibacterial effectiveness of plants and natural products also known to be used in traditional folk medicine with an easier accessibility and less deleterious side effects. *Cotinus coggygria* Scop. has important source of essential oils and extractions with a wide range of health-promoting properties and has been known to have significant usage in traditional medicine since ancient times (Matic , Stanic , Mihailovic , & Bogojevic , 2016, p. 453).

According to recent studies on contemporary medicinal usage of extractions from the leaves and body parts of the *C. coggygria* Scop., it has a wide range of application as antiseptic, anti-inflammatory, antimicrobial, antihemorrhagic, wound healing and anti-diarrhoea. The extractions and oils of the plant have been reported to contain significant phenol and tannin contents showing important antimicrobial effectiveness against various pathogen and non-pathogen bacteria. In a study examining the antibacterial effect of *Cotinus coggygria* Scop. extracts obtained in 6 different solvents against 7 different microorganisms, the plant extractions are found to comprise at least 31 to 42 components, including the limonene as the most dominant one, and to inhibit the reproduction of different bacteria being the most effective against *Staphylococcus aureus* (Tunç, Hoş, & Güneş, 2013, p. 1560). Another study reports the limonene and cis-ocimene as major ingredients by 48.53% and 23.57%, respectively (Kocak & Yıldırım, 2022, p. 130). On the other hand, one study reports antibacterial activity of the ethanol extract of the plant against Gram (+) and Gram (-) bacteria, comparing with the antibacterial effect of different antibiotics such as ampicillin, cefazolin, cefuroxime, meropenem,

colistin, ofloxacin, sulfamethoxazole / trimethoprim, tetracycline, and gentamicin (Goncagül, Güceyü, & Günaydın , 2020, p. 127).

This study explores the botanical descriptions, chemical compounds, pharmacological properties, extraction methods, and antimicrobial activities of *C. coggygria* Scop. against the bacteria *Staphylococcus aureus*. It examines the effectiveness of boiling as the traditional method and compares it with the findings of the studies applying contemporary laboratory-based extraction methods using Soxhlet and rotary evaporation devices. It is designed to test the relationship between the boiling time for extraction, as the independent variable, and the production of inhibition zones against bacteria through disc diffusion, as the dependent variable. This study is expected to show the antimicrobial potential of home-made extractions of *C. coggygria* Scop. prepared by boiling method, and their cost-effective, reliable and environmentally friendly usage for disinfection purposes.

LITERATURE REVIEW

Properties, extraction and usage of *C. coggygria* Scop.

Physical Properties of *Cotinus coggygria* Scop.

C. coggygria Scop. belongs to the *Anacardiaceae* family growing in a wide area stretching from the Mediterranean, Southern Europe, Moldova, the Himalayas and the Caucasus to Central China (Kocak & Yıldırım, 2022, p. 130). It is a round-topped shrub with 3 m height and 4-5 m width and sheds leaves in winter. Owing to the shape of its flowers, it is popularly known as “peruke tree” (Akçalı, Oktav Bulut, Tunçkol, & Buharalı, 2023, p. 369) or “smoke tree” (Tunç, Hoş, & Güneş, 2013, p. 1559) or “tetra tree” in Türkiye as well as in the Balkans. The plant is in demand due to its long flowering and fruiting period in summer, i.e. April and June, and ease of cultivation and longevity. Although there are several audio-visual materials on harnessing and usage of the plant among the population (Tetra Ağacı, Duman Ağacı Nedir?, 2021); (Şifalı Tetra Yaprağı Nedir Nasıl Kullanılır?, 2021)), this study aims at exploring effects of the traditional usage of the plant by applying scientific data and methods in a laboratory environment.

Figure 1: The bushes of *Cotinus coggygria* Scop.

Source : *Serdar Ölez © Sırakaya / Kahta / Adıyaman, July 2021*

https://www.floranatolica.com/eukaria/gui/species.php?ID=Cotinus-coggygria (30.11.2024)

Figure 2: Flowers and leaves of *C. coggygria* Scop.

Source: Serdar Ölez © Dağbek / Pülümür / Tunceli, June 2022

https://www.floranatolica.com/eukaria/gui/species.php?ID=Cotinus-coggygria (30.11.2024)

Chemical components of *C. coggygria* Scop.

Studies on phytochemical properties of *C. coggygria* Scop. show that the plant contains wide range of bioactive components including monoterpenes, high amount of hydrolysable tannins, gallic acid, methyl gallate, pentagalloyl glucose and flavonoids, glycosides of myricetin, quercetin and kaempferol (Matic , Stanic , Mihailovic , & Bogojevic , 2016, pp. 454-455). The extractable compounds content is reported to vary between 5 to 114 mg/g of dried plant extracted in distilled

water, ethanol and acetone by assaying with Folin-Ciocalteu reagent and comparing with α-tocopherol, BHT and BHA while the highest extraction in distilled water is reported to yield with total phenolic compound amounts of 176±0.025-199.07±0.044 mg/g as gallic acid equivalent (Bektaş, 2011, p. ii). Another study reports amount of total phenolics as higher as 518.4mg/g in the acetone extract and 413mg/g in the methanol extract alongside flavonoids and tannins (Marcetić, et al., 2013, pp. 1658-1660). The level of hydrolysable tannins with astringent, anti-inflammatory, and antiseptic properties as secondary metabolites are reported to vary from 6 to 30% depending on the time of collection and the amount of sun absorbed by the plant leaves (Sukhikh, et al., 2021, p. 2).

Medicinal use of *C. coggygria Scop.*

Due to its antimicrobial effect, the plant leaves is known to date back to the Roman Empire times in healing soldiers of the Roman armies wounded at the battlefield. Most of the recent studies report the plant as being used as antiseptic, anti-inflammatory, antimicrobial, antihemorrhagic and wound healing in traditional medicine in a wider geographical area comprising Türkiye and the Balkans. The extracts from flowers, leaves and body parts of *C. coggygria* Scop. are reported to provide protection against pathogens and wide spectrum of treatment for respiratory diseases such as flu and cold, sinusitis and runny nose, for skin diseases and inflamed wounds, for gastrointestinal discomforts like haemorrhoids as well as gastric, paradontosis, diarrhoea and duodenal ulcers (Ivanova , Gerova , Chervenkov, & Yankova , 2005). It is also reported to have anti-cancer, antigenotoxic, antibacterial and hepatoprotective properties, and to treat stomatitis and pharyngitis thus being included in the pharmacopeia (Sukhikh, et al., 2021, p. 2). Several *in vivo* and *in vitro* studies have recently demonstrated the antioxidative, antibacterial, antifungal, antiviral, anticancer, antigenotoxic, hepatoprotective and anti-inflammatory effects for all parts of the plant as a commercial ornamental plant with high medicinal usages (Matic , Stanic , Mihailovic , & Bogojevic , 2016, pp. 453-454). A very recent study reports the plant to have beneficial effect in prevention of kidney stone and consequent protection against nephrolithiasis, and thus proposes to be considered as a potential prophylactic and therapeutic option in high-risk stone formers (Gümrü, et al., 2023, p. 734).

Pathogens sensitive to *C. coggygria* Scop.

C. coggygria Scop. is reported to have antimicrobial activity against various bacteria including *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* (Tunç, Hoş, & Güneş, 2013, p. 1559) while the extraction in distilled water is reported to show the best antibacterial activity against *S. aureus* (Bektaş, 2011, p. ii).

Extraction methods and dosages of *C. coggygria* Scop.

There are several reported extraction methods of the dried leaves, flowers and body parts of the plant at laboratory conditions. Most of those studies report extraction method of dried powdered leaves kept in different solvents such as ethanol, methanol, and distilled water (Sukhikh, et al., 2021, p. 12) (Kocak & Yıldırım, 2022, p. 131) by using the Soxhlet device for 24 hours and then rotary evaporation (Tunç, Hoş, & Güneş, 2013, p. 1560). Different amounts of material is reported for extract preparation varying from 300 gr in %96 ethanol with extract yield of 41.56% (g/g) (Gümrü, et al., 2023, p. 736) to 25 gr in 200 mL in ethanol (Goncagül, Güceyü, & Günaydın , 2020, p. 129) and 2 gr in 150 ml of ethanol, methanol, distilled water, chloroform, acetone, and petroleum (Tunç, Hoş, & Güneş, 2013, p. 1560).

There are also other techniques for extractions prepared with distilled water at laboratory environment. One of them is hydro-distillation of 100 gr dried leaves in Clevenger apparatus (Kocak & Yıldırım, 2022, p. 131). The other one starts with boiling 20 gr powdered dried leaves in 400 mL distilled water for 30 minutes based on a ratio of 1/20 for solid and liquid substances, and then, applies filtering and lyophilizing (Bektaş, 2011, p. 39). Lyophilization, or freeze drying, "is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase" (U.S. Food and Drug Administration, 2014).

Apart from the abovementioned laboratory methods, brewing and boiling are known to be traditional method or home-made methods for extraction. I found very short information about two of them applied for dermatological purposes. The first one is prepared by boiling 2-3 spoons of dry plant leaves in 1.5 litres of water for 10 minutes while the second one is prepared by boiling 200 gr of dried leaves in 5 litres of water for 10 minutes (Yavuz, 2015).

To test the antibacterial activity of *C. coggygria* Scop. extractions, the cylinder plate method, macro broth dilution method, and disk diffusion method are proposed for the assessment of inhibition zones against various bacteria (Matic , Stanic , Mihailovic , & Bogojevic , 2016, pp. 456-457).

I combined the traditional extraction method of boiling the plant with specific dosage ratio of 1/20 for at least 30 minutes and testing the antibacterial activity by assessing infusion zones through laboratory-based disc diffusion method as described in details under the method development part.

Aim of the Experiment

The main aim of the current study is to test the antimicrobial effectiveness of *C. coggygria* Scop. extracted by boiling in distilled water and applying the disc diffusion method to assess the infusion zone of *Staphylococcus aureus* (ATCC 29213). *S. aureus* are chosen as experimental bacteria in this study because they are reported to be the most sensitive bacteria against *C. coggygria* Scop. extracted in distilled water (Bektaş, 2011, p. ii) (Tunç, Hoş, & Güneş, 2013, p. 1560).

S. aureus are small, round, oval shaped gram (+) cocci. They can easily grow in aerobic or facultative anaerobic environments in liquid media by creating turbidity at different degrees. In blood agar from solid media, pathogenic species grow by haemolysis as facultative anaerobic bacteria at a temperature range between 15℃ to 42℃ (Missiakas & Schneewind, 2013, s. 3). They are quite common in nature and found in dust and soil, and on objects as well as in human and animal skin, nasal mucosa, mouth and nasopharynx flora. They can cause a wide variety of diseases in humans and animals by producing toxins. Staphylococcal toxins are the main agents in food poisoning cases. Although bacteria are killed by cooking, the enterotoxins are heat-resistant and can withstand boiling for several minutes.

They grow in foods stored inappropriately, especially in foods with low water content such as cheese and salami. *S. aureus* can cause meningitis, septicaemia, wound inflammation and significant food poisoning in humans. It is known to be resistant to antibiotics like penicillin, while sensitive to Vancomycin and Nafcillin (Bektaş, 2011, p. 33).

Hypothesis

The extractions of *Cotinus coggygria* Scop. prepared in distilled water by boiling at 100°C for 30, 40, 50, 60, and 70 minutes are effective in producing infusion zones against *Staphylococcus aureus.* The inhibition zone of bacteria will increase when the boiling time increases. This hypothesis will be valid when the results of the experiment show significant differences between inhibition zones based on boiling times.

PLANNING

Variables

Independent Variables: The boiling time of 30, 40, 50, 60, and 70 minutes for extraction of 10 gr *C. coggygria* Scop. leaves in 200 mL distilled water.

Dependent Variables: The diameter of inhibition zones of *S. aureus* at the end of the experiment.

Table 1: Types and features of the variables in the experiment

CONTROLLED VARIABLES	**WHY IS IT CONTROLLED**	**HOW IS IT CONTROLLED**
Incubation time	*S. aureus* bacteria in the experiment can multiply under appropriate lab conditions.	Petri dishes of each bacteria type are left for 24 hours in a sterilized lab incubator in 25℃.
Incubation temperature	Although the ideal temperature for the growth of bacteria is near to human body temperature, temperature limits in the experiment guide book must be applied.	All petri dishes with bacteria are kept in lab incubator to avoid any false result for each trial.
Technical (Lab) equipment	Each petri dish is seeded with bacteria. Each extraction from 10 gr dried leaves of *C. coggygria* Scop. are boiled separately in 200 mL distilled water for 30, 40, 50, 60, and 70 minutes, respectively. Each procedure is applied with technical equipment in a lab environment to ensure the accuracy, reliability and validity of the experiment.	Paper discs saturated with antimicrobial agents in solutions having different concentration are put on each petri dish seeded with bacteria. Then dishes are put in an incubator for 24 hours in 25 ℃, and finally inhibition zones around the discs are measured.
Sterilization	Since the use of pathogenic bacteria has a potential contamination risk, the experiment is conducted in a lab environment.	Every equipment is sterilized before experiment and is treated as medical waste with no further use afterwards.

Material List

Table 2: The types and quantities materials needed for the experiment

Material	**Quantity**	**Uncertainties and Units**	**Size**
Sterilized standard petri dishes	× 10	± 0.1 mL	10 cm radius × 1,5 cm height × 3 mL volume
Distilled water	900 mL (200 mL × 6 cups)	±0.1 mL	_____
Cotinus coggygria Scop.	10 gr x 5 extracts (250 gr in total)	± 0.01 gr	_____
Electronic weighting scale	× 1	± 0.01 gr	_____
Incubator	× 1	± 0.5 ℃	25 ℃
Sterilized bottles	× 5	_____	each 50 mL in volume and with cover
Sterilized disposable inoculation loops	× 30	_____	_____
Glass marker	x 1	_____	_____

METHOD DEVELOPMENT

In an interview on 15 November 2024, my grandfather who served as a health practitioner provided me with information about the extraction of *C. coggygria* Scop. and its usage in traditional medicine. As a shrubby plant growing in the Thrace region of Türkiye and some parts of the Southeastern Europe, he stated that the extractions of the plant was traditionally prepared by boiling and applied mainly in the treatment of wounds by villagers before the emergence of modern medicine. It was thought to heal the wounds because of its antibacterial activity. The plant extractions were also consumed as tea or used as gargle, in case of sore, bleeding or wounded gums in some places.

For the preparation of the extracts, he stated that the dried plant leaves should be boiled in 1.5 litres of water at least for 10 minutes while the woody parts of the plant should be cut into small pieces and boiled in 3 litres of water until it halved. Although not being very specific, an average ratio of 1/3 was used for plant and water.

In this experiment, the extractions of *C. coggygria* Scop. were prepared by boiling only as the traditional method without applying any laboratory-based techniques of extracting, evaporating or lyophilizing. On the other hand, the dosage of solid and liquid substance was based on the ratio of 1/20. Also the assessment of antibacterial activities was based on the inhibition zones through disc diffusion method. Therefore boiling as traditional method and the disc diffusion method as modern assessment technique are both combined and applied in sterile laboratory environment.

Preliminary Experiment

I visited the microbiology reference laboratories of the Public Health General Directorate of the Ministry of Health of the Republic of Türkiye, and conducted the experiment under the supervision of the authorised laboratory staff. In the preparation stage, I prepared extractions by brewing and boiling of dried leaves and body parts of the plant separately for each and every trial. After that, I applied the extractions by using disc diffusion method to the bacteria seeded in the petri dishes. Positive results were obtained from boiling of the plant leaves while brewing of both leaves and body parts produced no results.

For determination of the effective antimicrobial dosage in traditional preparation method, I prepared extractions from 10 gr dried leaves and body parts of plant separately in 200 mL of distilled water in two different ways as described above in the extraction method and dosage part, one brewing at 60°C for one hour, and the other boiling at 100°C for 10, 30 and 60 minutes, respectively as a first trial.

Table 3: The results obtained from preliminary experiment

The extraction time of *C. coggygria* Scop.	**Preparation method (mL±1 and gr ±0.01)**	**Inhibition zone of *Staphylococcus aureus* (mm) (± 0.5 mm)**
Brewed at 60°C for 1 hour	200 mL distilled water + 10 gr plant leaves	No zone
Boiled at 100°C for 10 minutes	200 mL distilled water + 10 gr plant leaves	No zone
Boiled at 100°C for 30 minutes	200 mL distilled water + 10 gr plant leaves	19
Boiled at 100°C for 60 minutes	200 mL distilled water + 10 gr plant leaves	19
Negative control	Distilled water	No zone

The results obtained in the first trial for *S. aureus* were shown in Table 3. When both the leaves and body parts were brewed at 60°C for an hour, no zones were observed. When both the leaves and body parts were boiled at 100°C for 10 minutes, no zones were observed. The only inhibition zone of 19 mm was yielded from the leaves boiled at 100°C for at least 30 minutes. Thereupon, I decided to use the extractions prepared from boiled leaves, which was the independent variable of the experiment.

Methodology and conduct of the experiment

Preparation of the extract

1. Dry leaves of *C. coggygria Scop*. at room conditions, and then make them crushed and powdered (Figure 3).
2. Boil 10 gr crushed leaves in 200 mL distilled water for 30, 40, 50, 60 and 70 minutes respectively to prepare the extract.
3. Filter the boiled solution with paper filters (Figure 4) separately.
4. Fill the extracts in sterile bottles. Labelled as 1,2,3,4,5 for 30, 40, 50, 60 and 70 minutes respectively. (Figure 5).
5. Keep the extract bottles at 4°C.

Figure 3: Dried *C.coggygria* Scop. leaves (green).

Figure 4: Filtering the boiled extract

Preparation of discs and petri dishes

1. Obtain bacteria strains (*S. aureus*) from an authorized laboratory.
2. Obtain 5 petri dishes of 150 mm with %5 sheep blood agar.

Figure 5: Boiled extract filled in sterile bottles and labelled

3. Adjust the bacteria density to 0.5 MacFarland before seeding into agar petri dishes (Figure 7).
4. Seed the adjusted bacteria into agar petri dishes (Figure 7).
5. Mark petri dishes into 6 zones and label each zone as 1, 2, 3, 4 and 5 to put paper discs saturated with extracts of 30, 40, 50, 60 and 70 minutes, respectively. Put the distilled water at zone 6 for negative control.
6. Saturate paper discs with 30 µl of each extract of 30, 40, 50, 60 and 70 minutes and leave them to dry, (Figure 8).
7. Use one disc saturated with distilled water only as a negative control.
8. Transfer each saturated disc with inoculation loop to each agar petri dish seeded with *S. aureus* bacteria.
9. Repeat the steps from 3 to 8 for 4 more times.
10. Keep the petri dishes in a laboratory incubator for 24 hours in 25 ℃.
11. Measure the diameter of the inhibition zone surrounding each sample at the end of incubation.

Figure 6: Bacteria density adjusted to 0.5 MacFarland

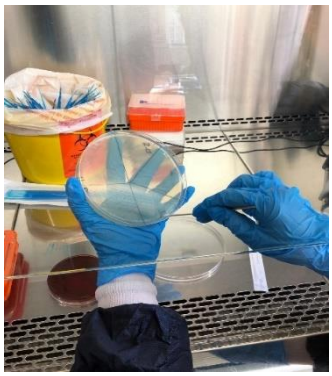


Figure 7: Seeding bacteria in petri dish

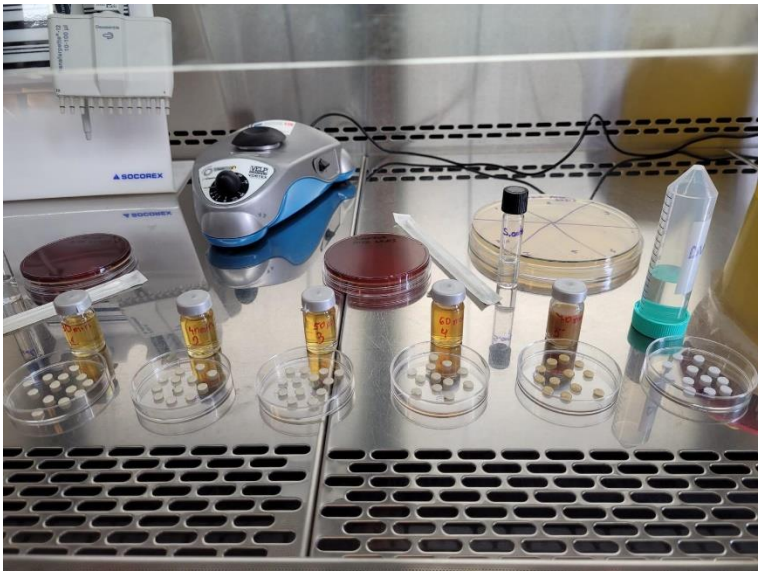


Figure 8: Saturating paper discs with extracts and apply disc diffusion method in a laboratory

Assessing the bacteria growth and inhibition zones

Incubation process is successful if the bacteria growth are observed in the agar petri dishes. On the other hand, inhibition zones around the extract saturated paper discs shows the effectiveness of antibacterial activity of the relevant extraction. As the results of incubations are shown in Figure 10, the extractions of boiling for different times have produced inhibition zones with different diameters against *S. aureus*.

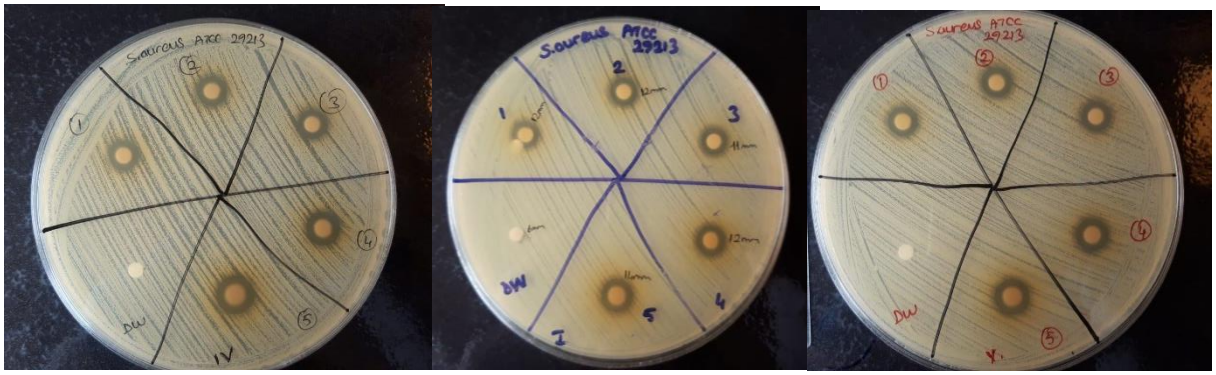


Figure 9: Results of the trials

Risk Assessment, Environmental and Ethical Concerns

Masks, gloves and white coat must be worn throughout the experiment and the inoculation must be performed in biosafety cabinet (see Appendix). Items should be disposed after usage to biohazard disposable bins to prevent uncontrolled distribution. Therefore, there is no concern for environmental contamination. No animals or humans are included in this experiment. It has been conducted under ethical conditions with taking utmost care of human safety.

DATA COLLECTION AND PROCESSING

Raw Data

5 trials were conducted for each of 5 extractions prepared at different boiling times. The inhibition zone data for each extract are shown at Table 4.

Table 4: The inhibition zone (mm) of S.aureus in 30, 40,50,60 and 70 minutes

The boiling time for extraction of 10 gr dry powdered leaves of *C. coggygria* Scop. in 200 mL distilled water (minutes) (±1 second)	**Trials**	**Inhibition zone of *S. aureus* (mm) (±0.5 mm)**
30	1	12
	2	13
	3	12
	4	13
	5	12
40	1	13
	2	13
	3	13
	4	13
	5	13
50	1	13
	2	12
	3	12
	4	12
	5	12
60	1	14
	2	13
	3	14
	4	12
	5	14
70	1	15
	2	15
	3	15
	4	13
	5	15

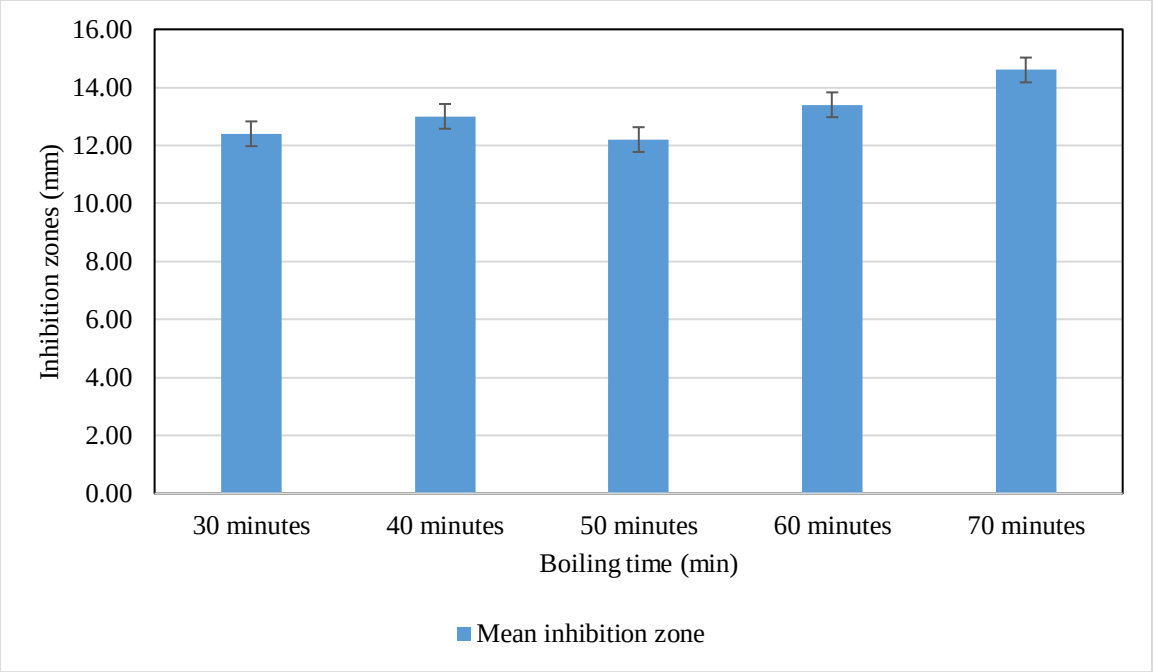
Processed Data

The calculation of all means and standard deviations of inhibition zones are shown at Table 5.

Table 5: The mean and standard deviation of inhibition zones (mm)

The boiling time for extraction of 10 gr dry powdered leaves of *C. coggygria* Scop. in 200 mL distilled water (min) (±1 sec)	**Mean inhibition zone of *S. aureus* (mm) (± 0.5 mm)**	**Standard Deviation**
30	12.4	0.55
40	13	0
50	12.2	0.45
60	13.4	0.89
70	14.6	0.89

Graph 1: The means and standard deviations of inhibition zones (mm)



Standard deviations are shown in Graph 1.

ANOVA One-Way Test

The data were processed by one-way, or one-factor, ANOVA test designed to compare the means of three or more independent samples (treatments) simultaneously (ANOVA- One Way Calculator, 2025). The f-ratio value is 10.85714. The p-value is 0.000076. The result is significant at $p < 0.05$.

It will mean that the null hypothesis (H_0) is rejected and my hypothesis (H_1) is supported provided as follows:

H_0: There is no significant difference between the variances.

H_1: There is statistically significant difference between the variances.

Table 6: Statistical significance of means processed by one-way ANOVA

Summary of Data						
	Treatments					
	1	2	3	4	5	Total
N	5	5	5	5	5	25
∑X	62	65	61	67	73	328
Mean	12.4	13	12.2	13.4	14.6	13.12
∑X2	770	845	745	901	1069	4330
Std.Dev.	0.5477	0	0.4472	0.8944	0.8944	1.0536

Result Details					
Source	SS	df	MS	F ratio	p-value is
Between-treatments	18.24	4	4.56	10.85714	0.000076
Within-treatments	8.4	20	0.42		
Total	26.64	24			

Post-Hoc Testing

Although ANOVA test shows that there is statistically significant difference between means of data groups, it does not provide specific information on which data groups are different. In such case, the Post-Hoc testing is applied to identify exactly which groups differ from each other. Levene's test and Tukey HSD / Tukey Kramer are applied in this study (Levene's test calculator, 2024). The labels of x1, x2, x3, x4, and x5 stand for boiling times of 30 min., 40 min., 50 min., 60 min., and 70 min., respectively.

Table 7: Levene's test on inhibition zone data

Source	**DF**	**Sum of Square**	**Mean Square**	**F Statistic**	**P-value**
Groups (between groups)	4	1.6384	0.4096	3.9084	0.016714
Error (within groups)	20	2.096	0.1048		
Total	24	3.7344	0.1556		

Table 8: Tukey HSD / Tukey Kramer test on inhibition zone data

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	0.48	0.14478	3.31547	-0.13267	1.09267	0.61267	0.1722
x1-x3	0.16	0.14478	1.10516	-0.45267	0.77267	0.61267	0.93303
x1-x4	0.24	0.14478	1.65774	-0.37267	0.85267	0.61267	0.76659
x1-x5	0.16	0.14478	1.10516	-0.45267	0.77267	0.61267	0.93303
x2-x3	0.32	0.14478	2.21032	-0.29267	0.93267	0.61267	0.53631
x2-x4	0.72	0.14478	4.97321	0.10733	1.33267	0.61267	0.016452
x2-x5	0.64	0.14478	4.42063	0.02733	1.25267	0.61267	0.037941
x3-x4	0.4	0.14478	2.7629	-0.21267	1.01267	0.61267	0.3231
x3-x5	0.32	0.14478	2.21032	-0.29267	0.93267	0.61267	0.53631
x4-x5	0.08	0.14478	0.55258	-0.53267	0.69267	0.61267	0.99466

Group	x2	x3	x4	x5
x1	0.48	0.16	0.24	0.16
x2	0	0.32	0.72	0.64
x3	0.32	0	0.4	0.32
x4	0.72	0.4	0	0.08

ANALYSIS AND DISCUSSION

Analysis of the results and comparison with other studies

The findings of this study showed that extractions of *C. coggygria* Scop. prepared by boiling for different durations produced inhibition zones against *S. aureus* with different variances. The results of both ANOVA and post hoc tests are clearly stated and significant p-values are reported. One-Way ANOVA test normally assumes that variances are equal across samples. However, the results of this study showed that mean inhibition zones were different depending on boiling times. The p-value both for ANOVA test (0.000076) and Levene's test (0.0167139), [$p(x \leq F) = 0.983286$] were less than 0.05. It means H_0 is rejected and H_1 is supported. There is statistically significant difference between the average mean of inhibition zones. The smaller the p-value, the stronger support H_1. The variances of the following pairs are significantly different: x2-x4, x2-x5.

The mean inhibition zones increased from 12.4 mm to 14.6 mm as the boiling time increased from 30 to 70 minutes (Table 5) . This could mean that longer boiling time increases the concentration of chemical substance which, in turn, produces wider inhibition zones. This is also in conformity with the inhibition zone of 13 mm in one study (Bektaş, 2011, p. 57), and ranging between 8-16 mm in another study (Tunç, Hoş, & Güneş, 2013, p. 1560).

Strengths, Limitations and Source of Error

Testing antibacterial activity of *C. coggygria* Scop. is a laboratory-based experiment conducted with adequate laboratory equipment including petri dishes, diffusion discs, incubator, etc.. It is easier to replicate and is reliable as being based on validated and reliable scientific data and resources. It also allows precise control of independent and dependent variables.

Besides its strengths, laboratory-based experiment also has some limitations. As in this study on *C. coggygria*, it may not be a complete reflection of real-world situations and thus may limit the generalizability of the experiment to real life. Additionally, ethical concerns may arise when manipulating variables. It should also be underlined that this study was aimed to show only the production of inhibition zone, while minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were not examined. One study reports 7.5 mg/mL and 15 mg/mL for MIC and MBC rates, respectively (Bektaş, 2011, p. 60). Future studies can be formulated to determine those rates for traditionally prepared extractions.

CONCLUSION

In conclusion, this study shows that extractions of *C. coggygria* Scop. prepared by boiling in distilled water has a certain degree of antimicrobial effectiveness and produces inhibition zones against *S. aureus*. Thus, the hypotheses of this study have been proven to be valid for preparations by boiling method. For future studies, both the number of boiling times (number of groups) and number of trials (number of components in each group) can be increased in order to obtain more data to test differences within and between groups. It is promising for future research in AMR and pharmacopeia.

REFERENCES

Akçalı, K., Oktav Bulut, M., Tunçkol, B., & Buharalı, G. (2023, January 31). Cotinus coggygria Scop. Bitkisinden Elde Edilmiş Bitki Ekstratı ile Boyanmış Yünlü Kumaş Numunelerinin Boyama Özelliklerinin İncelenmesi. *Düzce University Science and Technology Journal, 11*(1), pp. 369-380. doi:DOI: 10.29130/dubited.1076850

Anatolmedia. (2022). *Staph Infections*. Retrieved 02 03, 2025, from Anka Hospital: https://ankahastanesi.com/tr/staph-enfeksiyonlari#:~:text=%C3%87o%C4%9Fu%20zaman%2C%20bu%20bakteriler%20hi%C3%A7bir,girerse%20staph%20enfeksiyonlar%C4%B1%20%C3%B6l%C3%BCmc%C3%BCl%20olabilir.

ANOVA- One Way Calculator. (2025). Retrieved 02 03, 2025, from Social Science Statics: https://www.socscistatistics.com/tests/anova/default2.aspx

Association, C. F. (2024). *Microbes and staying healthy*. Retrieved 02 03, 2025, from Kifder: https://www.kifder.org.tr/mikroplar-ve-saglikli-kalma/#:~:text=Pseudomonas%20aeruginosa&text=Bir%20ya%C5%9F%C4%B1nda%20hastalar%C4%B1n%20%30'unun,KF%20hastalar%C4%B1)%20ba%C5%9Fl%C4%B1ca%20bula%C5%9Fma%20kayna%C4%9F%C4%B1d%C4%B1r.

Aydın, F. (2024, August 05). *E.coli (EHEC)*. Retrieved 02 03, 2025, from İstanbul Medipol University, Medipol Health Group: https://medipol.com.tr/bilgi-kosesi/adan-zye-saglik/ehec#:~:text=Escherichia%20coli%20(E.,tehdit%20edebilen%20hastal%C4%B1klara%20neden%20olabilmektedir.

Bektaş, E. (2011, October 14). Cotinus coggygria Scop. Bitkisin Antioksidan ve Antimikrobiyal Aktivitesinin Belirlenmesi (Determination of Antioxidant and Antimicrobial Activity of

Cotinus coggygria (Scop.) Plant). *Master Thesis*. Edirne, Türkiye: Faculty of Science, Trakya University .

Cornell University. (2023, 11 30). *Staphylococcus aureus Biological Agent Reference Sheet (BARS)*. Retrieved 11 15, 2024, from Cornell University Environment, Health and Safety: https://ehs.cornell.edu/research-safety/biosafety-biosecurity/biological-safety-manuals-and-other-documents/bars-other/staphylococcus-aureus

Dulger, B., Hacıoğlu, N., & Bilen, S. (2009, May). Antimicrobial activity of cotinus coggyria from Turkey. *Asian Journal of Chemistry, 21*(5), 4139-4140. Retrieved from Antimicrobial activity of Cotinus coggygria from Turkey: https://www.researchgate.net/publication/288381692_Antimicrobial_activity_of_cotinus_coggyria_from_Turkey?enrichId=rgreq-aa9f070435ece6346020f519ba3964c8-XXX&enrichSource=Y292ZXJQYWdlOzI4ODM4MTY5MjtBUzo1OTM0NzAwNjY1NDg3MzZAMTUxODUwNTY5ODI2NA%3D%3D&el=1_x_

El-Dawy, E., Gherbawy, Y., Hassan, S., & Hussein, M. (2021, April 7). Molecular Identifcation of Penicillium sp. Isolated from Citrus Fruits. *Current Microbiology, 78*, 1981-1990. doi:10.1007/s00284-021-02463-3

Goncagül, G., Güceyü, Ç., & Günaydın , E. (2020, August 28). Cotinus coggygria Scop. (Tetra) Bitkisinin Etanol Ekstraktının Antibakteriyel Aktivitesinin İn Vitro Araştırılması (Investigation of In-Vitro Antibacterial Activity of Cotinus coggygria Scop. Extracts). *Van Veterinary Journal, 31*(3), 127-132. doi:https://doi.org/10.36483/vanvetj.742535

Gümrü, S., Özgür , G., Ertaş , B., Şen, A., Eker , P., Şener , T., & Şener , G. (2023, November 28). Ethanolic extract of cotinus coggygria leaves attenuates crystalluria and kidney damage in ethylene glycol-induced urolithiasis in rats. *North Clin Istanb, 10*(6), 734–744. doi:doi: 10.14744/nci.2023.29794

Ivanova , D., Gerova , D., Chervenkov, T., & Yankova , T. (2005, January 4). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology, 96*(1-2), 145-150. doi:https://doi.org/10.1016/j.jep.2004.08.033

Kamburoğlu, İ. (2024, 11 15). Traditional use of Cotinus coggygria tree. (F. ŞEN, Interviewer) Ankara/Çankaya, Türkiye.

Karadağ, R. (2007). *Doğal Boyamacılık.* Ankara: T.C. Kültür ve Turizm Bakanlığı Döner Sermaye İşletmesi Merkez Müdürlüğü (Ministry of Culture and Tourism of the Republic of Türkiye). Retrieved from T.C. Kültür ve Turizm Bakanlığı.

Kocak, M. Z., & Yıldırım, B. (2022, March 24). Essential oil content and chemical profile of Cotinus coggygria Scop. from Eastern Anatolia Türkiye. *International Journal of Agriculture, Environment and Food Sciences, 6*(1), 130-134. doi:https://doi.org/10.31015/jaefs.2022.1.17

Levene's test calculator. (2024). Retrieved from Statistics Kingdom: https://statskingdom.com/230var_levenes.html

Marcetić, M., Bozić, D., Milenković, M., Malesević, N., Radulović, S., & Kovacević, N. (2013). Antimicrobial, antioxidant and anti-inflammatory activity of young shoots of the smoke tree, Cotinus coggygria Scop. *Phytotherapy Research, 27*, 1658–1663. doi:10.1002/ptr.4919

Matic , S., Stanic , S., Mihailovic , M., & Bogojevic , D. (2016, July). Cotinus coggygria Scop.: An overview of its chemical constituents, pharmacological and toxicological potential. *Saudi Journal of Biological Sciences, 23*(4), 452-461. doi:https://doi.org/10.1016/j.sjbs.2015.05.012

Missiakas, D. M., & Schneewind, O. (2013, 02). Growth and laboratory maintenance of Staphylococcus aureus. *Current protocols in microbiology*, pp. 1-12. doi:https://doi.org/10.1002/9780471729259.mc09c01s28

Ölez , S. (2021). Cotinus coggygria Scop. *Cotinus coggygria*. Floranatolica, Adıyaman, Sırakaya/ Kahta. Retrieved November 30, 2024, from https://www.floranatolica.com/eukaria/gui/species.php?ID=Cotinus-coggygria

Ölez, S. (2022). Leaves and flowers of C.coggygria. *Cotinus coggygria*. Floranatolica, Tunceli, Dağbek/ Pülümür. Retrieved November 30, 2024, from https://www.floranatolica.com/eukaria/gui/species.php?ID=Cotinus-coggygria

Özdoğru, D. E. (n.d.). *Türkiye Ulusal Alerji ve Klinik İmmünoloji Derneği*. Retrieved 01 25, 2025, from https://www.aid.org.tr/kuf-alerjisi-nedir/#:~:text=K%C3%BCf%20alerjisi%2C%20burunda%20ak%C4%B1nt%C4%B1%2C%20ka%C5%9F%C4%B1nt%C4%B1,gereksinim%20duyan%20mantar%20t%C3%BCr%C3%BCnde%20canl%C4%B1lard%C4%B1r.

Sönmez , N. (Director). (2018). *Boyacı Sumağı Duman Ağacı Sarıcan* [Motion Picture]. Retrieved 01 25, 2025, from https://youtu.be/1bQjXScymQ0?feature=shared

Sukhikh, S., Asyakina, L., Korobenkov, M., Skrypnik, L., Pungin , A., Ivanova , S., . . . Babich, O. (2021). Chemical Composition and Content of Biologically Active Substances Found in Cotinus coggygria, Dactylorhiza maculata, Platanthera chlorantha Growing in Various Territories. *Plants, 10*(12), 2806. doi:https://doi.org/10.3390/plants10122806

Şifalı Tetra Yaprağı Nedir Nasıl Kullanılır? (2021). [Motion Picture]. Retrieved 01 25, 2025, from https://www.youtube.com/watch?v=Sl5raHFQ1i8

Tetra Ağacı, Duman Ağacı Nedir? (2021). [Motion Picture]. Retrieved 02 03, 2025, from https://www.youtube.com/watch?v=OPMrSZcsC7Q

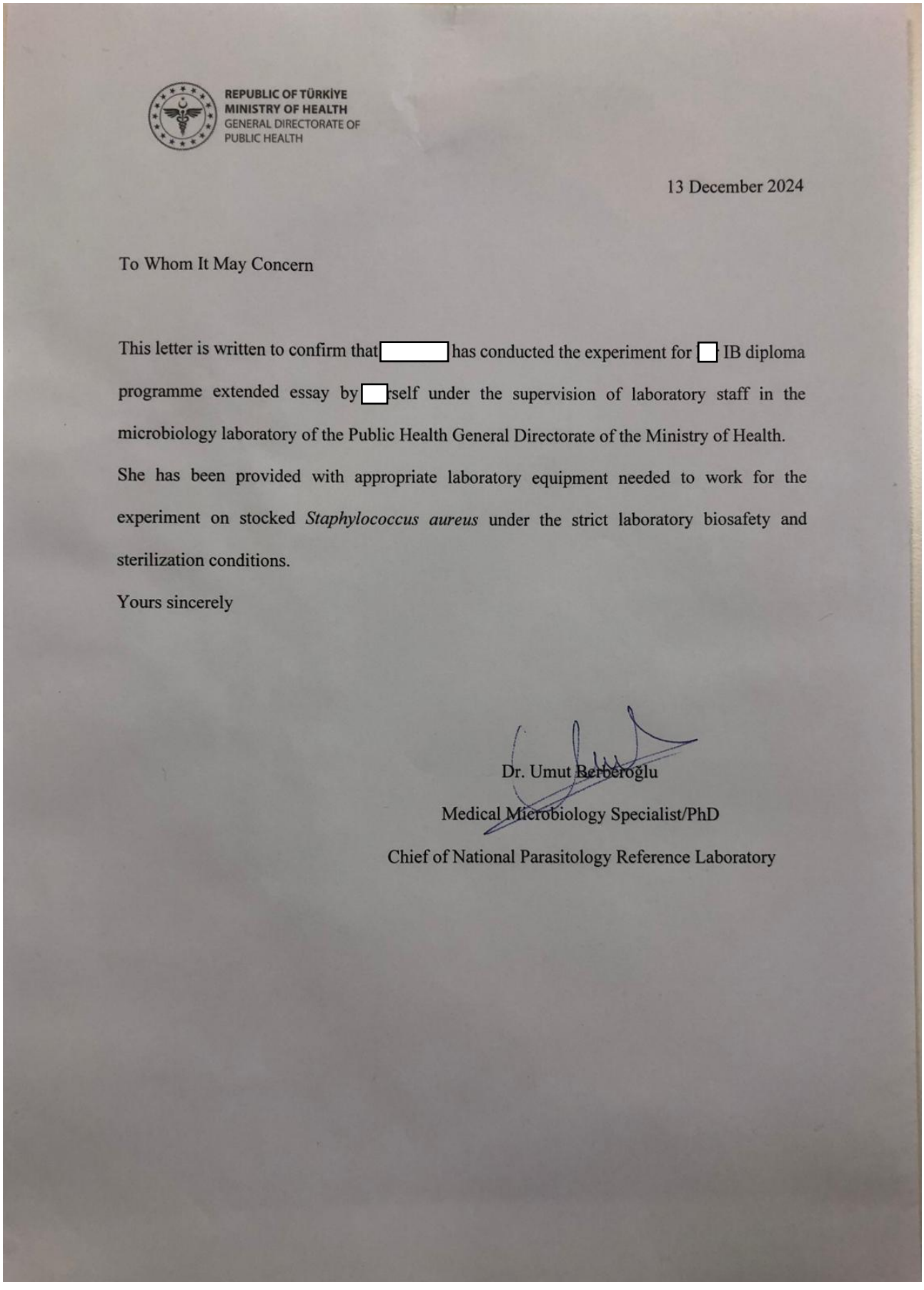
Tunç, K., Hoş, A., & Güneş, B. (2013, April 23). Investigation of Antibacterial Properties of Cotinus coggygria from Turkey. *Polish Journal of Environmental Studies, 22*(5), pp. 1559-1561. Retrieved from Investigation of Antibacterial Properties:

https://www.pjoes.com/Investigation-of-Antibacterial-Properties-r-nof-Cotinus-coggygria-from-Turkey,89123,0,2.html

U.S. Food and Drug Administration. (2014, 11 11). *Guide to Inspection of Lyophilization of Parenterals*. Retrieved 11 15, 2024, from U.S. Food and Drug Administration Website: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-guides/lyophilization-parenteral-793#:~:text=Lyophilization%20or%20freeze%20drying%20is,passing%20through%20a%20liquid%20phase.

Yavuz, T. (2015, 11 25). *Cotinus coggygria leaves*. Retrieved from Atafen Blog: https://atafenblog.com/tetra-agaci-yapragi/

APPENDIX



REPUBLIC OF TÜRKİYE
MINISTRY OF HEALTH
GENERAL DIRECTORATE OF
PUBLIC HEALTH

13 December 2024

To Whom It May Concern

This letter is written to confirm that [redacted] has conducted the experiment for [redacted] IB diploma programme extended essay by [redacted]rself under the supervision of laboratory staff in the microbiology laboratory of the Public Health General Directorate of the Ministry of Health.

She has been provided with appropriate laboratory equipment needed to work for the experiment on stocked *Staphylococcus aureus* under the strict laboratory biosafety and sterilization conditions.

Yours sincerely

Dr. Umut Berberoğlu

Medical Microbiology Specialist/PhD

Chief of National Parasitology Reference Laboratory