

Orange Peel	<i>Citrus sinensis</i>	<i>Bacterial and fungal pathogens</i>
Tea Tree (Indian bay)	<i>Melaleuca alternifolia</i>	<i>Staphylococcus aureus and Escherichia coli</i>
Rose	<i>Rosa</i>	<i>Intestinal and pathogenic bacteria</i>
Cinnamon	<i>Cinnamomum verum</i>	<i>Pathogenic</i>

et al., 2013. ; Kamijo, Kanazawa, Funaki, Nishizawa, &, Yamagishi, 2008; Kwak, Kim, & Kim, 2017; El-Desoukey, Saleh, & Alhowamil, 2018; Hammer, Carson, & Riley, 2011; Okmen, Mammadhkanli, & Vurkun, 2018)

The purpose of this project is to identify the types of bacteria that may threaten the health of students who spend most of their time in school and produce herbal disinfectant for this purpose. The expected result of the experiments is that bacteria or microorganisms that threaten student health will be destroyed with the prepared herbal disinfectant or their breeding will be slowed down. As a result of the project, a disinfectant will be produced that can be used in schools easily, that has no chemical ingredient, completely herbal and can destroy harmful bacteria and other types of microorganisms in order to protect students' health. This disinfectant will be a substance that can be used in detergent form, soap form or in the form of instantly used cleaning materials such as wet wipes.

2. METHOD

The method determined as a result of the research consists of 4 stages.

2.1.) Isolation of bacteria from the most frequently used environments in the school

Bacteria samples are taken by using sterile swabs from school environment including; classroom (smart board, door, window handle, student locker, desk, light switch),

Robotic room, corridor stair railing, mouse-keyboard in Computer classroom, corridor grip, vending button, and toilet flush and inoculated in petri dishes filled with agar media on the same day. Samples are incubated in the incubator under 36 degrees for 48 hours.

2.2.) Determination of bacterial species according to the Gram staining method

After inoculation, bacterial colonies were stained and was determined whether there they are Gram (+) or Gram (-) according to whether the cell walls were stained, observed under microscope.

Gram staining steps were carried out in the following order:

1. Preparations were fixed by heat fixing method.
2. Crystal violet dye solution was added to the prepartate and coated for 1 minute. The preparation was then washed with plenty of distilled water.
3. Lugol solution was added to the prepartate and coated for 1 minute, then washed with distilled water.
4. 95% ethanol or acid-alcohol mixture was added to the preparation and waited for 10-15 seconds. The prepartate was washed with distilled water.

5. Aqueous fuxin was added to the preparation and coated for 30 seconds. The preparation was washed with plenty of distilled water. The prepate was dried in air or with a blotter paper and examined under a microscope.
6. The microorganisms seen in purple are defined as gram (+), and those seen in pink-red are defined as gram (-).

It has been determined whether the cell walls of the bacteria are Gram (+) or Gram (-) after Gram staining; and assistance was obtained from the Microbiology Lab of a university in order to identify these bacterial species precisely.

2.3.) Preparation Stage of the Extracts

10 grams of rose, orange peel, cinnamon, clove, chamomile and tea tree leaf plants were weighed from the plant samples. These plants were beaten with the help of mortar and the surface area of each plant was increased. By increasing the surface area, it was aimed to make the active substances more active in 100 milliliters of methanol and water mixture solutions. 30 milliliters of the 100 milliliter methanol + water mixture was treated as methanol and 70 milliliters as water. The created solutions were placed in the evaporator for 24 hours to fly.

3.4.) Preparation Stage of the Herbal Mixture

The disinfectant mixture was prepared with the prepared extracts. Disinfectant mixture was created by mixing the extracts of rose, orange peel, cinnamon, clove, chamomile, tea tree leaf with equal milliliters. The mixture was revealed by taking five milliliters of each extract.

3.5.) Observing the effect of herbal extracts and mixture by applying disc diffusion method to bacterial species

Six different herbal extracts (cinnamon, cloves, chamomile, rose, tea tree, orange peel) and mixture extracts are applied by disk diffusion method. After 48 hours, bacterial colonies' (*Streptococcus pyogenes* and *Streptococcus epidermidis* applied to the) zone diameters were measured and the results were interpreted accordingly.

5. RESULTS

Bacteria species in 5 different environments at school were determined. Gram (+) *Streptococcus pyogenes* were determined under the Classroom desk and Gram (+) *Staphylococcus epidermidis* bacteria were found on class board, classroom window handle, corridor handles and keyboard-mouse. Effect of herbal extracts on bacteria was determined by Disc diffusion method, interpreted results according to the zone diameters formed in Table 3 (see Fig.7).

Table 3. Inhibition diameters obtained by disk diffusion method

Herbal Extracts	<i>Streptococcus pyogenes</i>	<i>Staphylococcus epidermidis</i>
Chamomile extract	4mm	8mm
Clove extract	4mm	10mm
Tea tree extract	1mm	3mm
Cinnamon extract	2mm	2mm
Orange peel extract	0mm	0mm
Rose extract	0mm	0mm

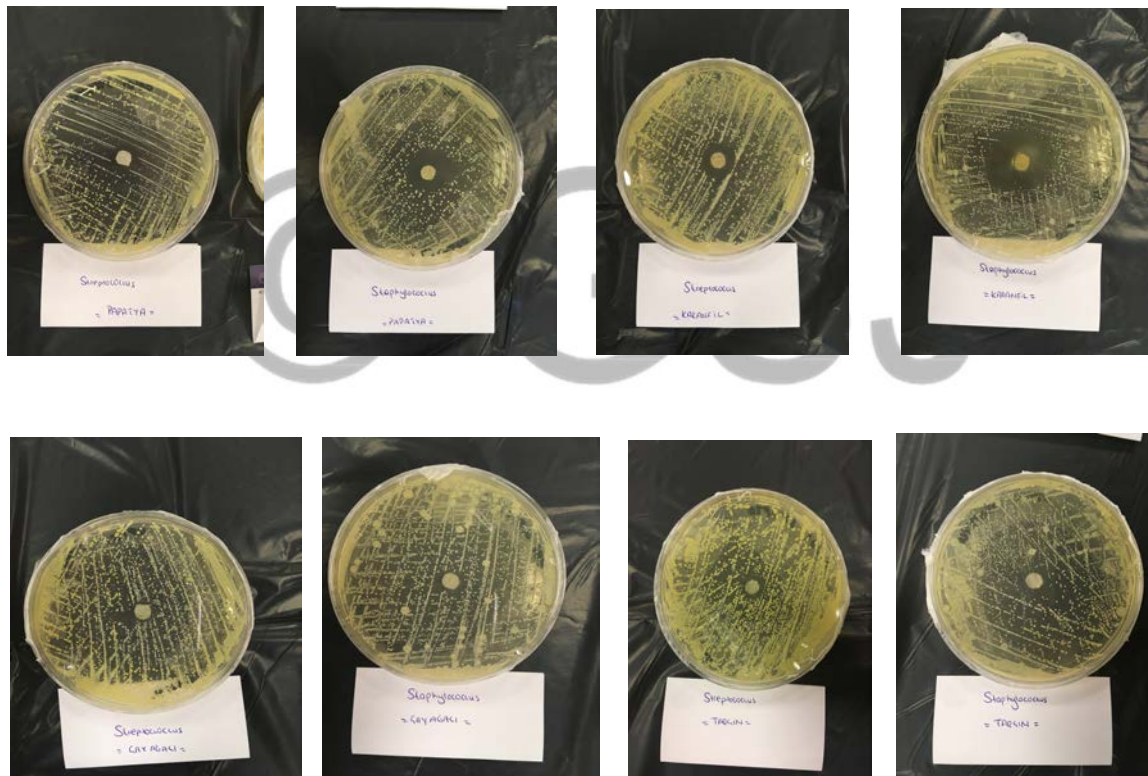
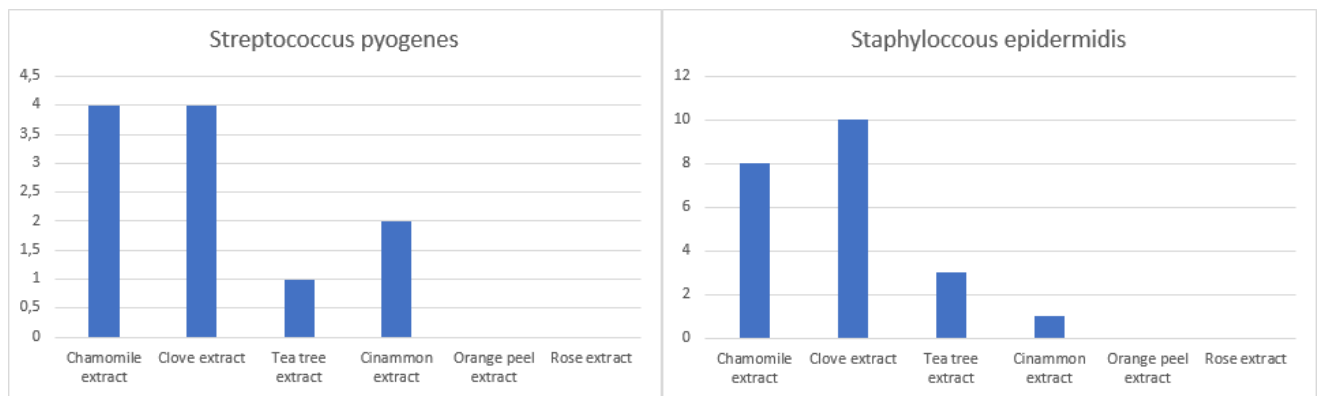


Figure 8. Inhibition diameters obtained by the disc diffusion method

Graphic 1 and Graphic 2. Inhibition zone diameters formed by bacterial species against herbal extracts



7. RESULT AND DISCUSSION

In our project, many bacterial colonies were observed in the places where students frequently spend their time in the school environment. This indicates that there may actually be many types of harmful or harmless bacteria species in the places where students most frequently present and poses a threat to health. Unlike previous research (Labelle, et al., 2019), our project *Staphylococcus epidermidis* and *Streptococcus pyogenes* bacteria have

been observed in school environment. However, to support the findings of another study, *Staphylococcus epidermidis* bacteria were found on the class window handle, class smart board, corridor handles and keyboards (Köljalg, Mändar, Söber, Rööp, & Mändar, 2017). It is known that this bacterial species is frequently found in school environment and outside school and it is a non-pathogenic bacteria; however, it is also known that they can become pathogenic under certain environmental conditions. According to

the inhibition zone diameters determined after disc

diffusion method of applied herbal extracts; *Staphylococcus epidermidis* had the greatest zone diameter in Clove plant extract (10mm) and subsequently chamomile (8mm), tea tree (3mm) and cinnamon (2mm) plants extract caused *Staphylococcus epidermidis* to form an inhibition zone diameter. Contrary to expectations, against rose and orange peel, which are known to be disinfectant, *Staphylococcus epidermidis* did not create any zone diameter. *Streptococcus pyogenes* bacteria species, on the other hand, created the same amount of zone diameter when chamomile (4mm) and clove (4mm) plant extracts were added; and subsequently formed inhibition zone diameters in cinnamon (2mm) and tea tree (2mm). Contrary to expectations and *Staphylococcus epidermidis* as seen in, rose and orange peel did not form any zone diameter, and many number of colonies was observed. The herbal extract mixture (cinnamon, clove, chamomile, tea tree, rose, orange peel) did not cause a remarkable zone diameter in both species of bacteria; therefore, application of these plant species gave an effective result when they are applied alone. As Ismail et al. (2013's) research about the cidal effect of daisy on *Staphylococcus*, in this project *Staphylococcus epidermidis* has constructed a diameter of inhibition zone against the camomile extract too.

Clove extract, as expected (Okmen, Mammadhkanli, & Vurkun, 2018), caused the *Staphylococcus epidermidis* and *Streptococcus pyogenes* to create inhibition zones. Unlike previous research (Kamijo, Kanazawa, Funaki, Nishizawa, &

Yamagishi, 2008; El-Dosoukey, Saleh, & Alhowamil, 2018); both species of bacteria did not create any inhibition zone diameter against orange peel and rose plant extracts; on the contrary, they formed many colonies.

The reason why orange peel and tea tree leaf plants did not affect both types of bacteria; may be because some important chemical components may be lost while obtaining the extracts; or these extracts can be tested on bacteria in a different agar and the results can be observed again.

The presence of *Streptococcus pyogenes* bacteria in the school environment poses a health hazard for students and school workers; and it requires disinfection of the school environment and belongings against this bacterium. *Staphylococcus epidermidis* which is not a pathogenic bacterium,; can become pathogenic under certain environmental conditions and therefore can pose a threat to health. It is known that inhalation of chemical disinfectants is harmful to health; and alternatively, the use of herbal disinfectants becomes a logical method for both health and environmental awareness. In this research; particularly, chamomile and clove plant extracts had a remarkable, noticeable effect on these bacteria and it was observed that they would contribute to the removal / destruction of these bacteria. For this reason, the use of these plant extracts (chamomile, clove and other inhibition diameter; tea tree and cinnamon plants) for cleaning purposes in the school environment would be a logical and healthy solution.

7. RECOMMENDATIONS

1) In our project, bacteria species in 5 different environments where students frequently spend their time have been identified and herbal extracts that are thought to affect these bacteria types have been tested; and observed their effects on bacterial reproduction. Clove and Chamomile plants (which may also contain cinnamon and tea tree plants), which had the most effect on bacteria, can be offered to students in a product such as a hand ointment, wet wipes or hand gel; thus, precautions can be taken against pathogenic or potentially pathogenic bacteria in schools.

2) In our project, only 5 different bacterial species were detected. For future research, bacteria samples can be taken from

different environments where students mostly spend their time in school and these bacteria species can be determined.

3) For further research, a substance can be developed to act as a sensor depending on the metabolism of the bacteria, so that it can help control bacterial reproduction in the school environment. With the addition of a substance that bacteria can react to the reproductive environment; it will be useful to show the sensor feature of that substance and to measure the bacterial density in that environment whichever medium is to be added. Thus, it will be possible to prevent potential disease-causing bacteria in the school environment.

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