



HERBAL DISINFECT PRODUCTION AGAINST BACTERIA SPECIES FOUND IN THE SCHOOL ENVIRONMENT

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ABSTRACT:

In our project, it is aimed to identify the bacteria species found in the environments in which students spend most of their time in school and to produce herbal disinfectant against these bacteria types. During the research phase, microorganism samples were taken from the school environment and some bacteria species that could threaten health were identified. In the school environment, as a result of the cultivation of microorganisms in the living spaces of the students (window handle, stair railing, classroom door handle, cabinet interior, handle, bottom of the desks, siphon, etc.), 5 different types of bacteria have been identified and a herbal antiseptic mixture has been created against these bacteria types.

Bacteria samples were taken from the school environment with the swabs at the experimental stage of the project, inoculated in petri dishes and bacteria species were determined by Gram staining method. After detecting bacteria species, disinfectant material was obtained by extraction method from herbal substances showing antibacterial properties. Inhibition diameters of plant extracts on bacteria were observed by disc diffusion method. According to the findings; Gram (+) bacteria were detected on the bottom of the desk, classroom window handle, corridor handles, computer keyboard-mouse and classroom smartboard. *S. pyogenes*

bacteria were detected on the bottom of the desk and *S. epidermidis* bacteria in other areas including window handle, handrail, computer mouse and keyboard, class smart board were detected.

Extracts of chamomile, clove, cinnamon, tea tree, orange peel and rose plants, which have antibacterial properties, were applied to bacteria mediums. While both species of bacteria form the greatest inhibition diameters against clove and chamomile plants; they created a small amount of zones in the cinnamon and tea tree. No inhibition diameter was observed in rose and orange peel extracts. According to the overall results obtained from the project, we recommend to analyze the possibility of shifting the research area to different areas due to the capture of pathogenic features in bacteria in the environment, and since the extracts of chamomile and cloves are effective alone, they can also be effective in their combination.

Key words: *S.pyogenes*, *S.epidermidis*, herbal, disinfectant, inhibition

1. INTRODUCTION

Bacteria are single-celled microscopic prokaryotic organisms. There are many bacteria species in nature, soil, air, water, plants, animals and even humans. Bacteria are chlorophyll-free and without nucleus creatures, 1-6 microns in size that are too small to be seen with the naked eye and multiply by dividing. In addition to the harmful bacteria, there are also beneficial types of bacteria. For example; Many types of bacteria species have great importance in making cheese, butter, yoghurt, vinegar and obtaining some antibiotics. In addition, acetone and butyl alcohol in the industry are obtained by bacterial fermentation. Bacteria in general classified as; disease-causing bacteria (pathogenic), yeast bacteria, corrosive bacteria and nitrogen bacteria. They can live in extremely hot environments (thermophiles) where other living things cannot survive or even under very high pressure. By decomposing the structure of dead organisms, they decompose and ensure that organic substances become available to other organisms. Bacteriologists report that there are more than 100 types of bacteria and classify

bacteria according to their form, structure, growth, culture medium and staining types (cell wall structure). Some bacteria species are identified by finding the disease caused by them. These bacteria are inoculated to animals because of this.

Bacteria are divided into six main groups in terms of their morphology: cocci(*coccus*), bacilli (*bacillus*), spirals, spirochetes, filamentous, and budding and extending bacteria (Madigan, Martinko, Brock & Collins, 2012). Streptococcus, staphylococcus and pneumococcal species, which are round shaped, are included in the first group. Streptococci and Staphylococci, consisting of consecutive coccus bacteria, and community-forming coccus bacteria. Including the species *Salmonella*, *colibacille* etc. the second group, contains rod-shaped bacteria. The spirals are curved like commas; *Leptospira*, *treponema* etc. are included in this type. Bacteria can also be classified according to gram staining, which is widely used in the detection of bacterial species: These are gram positive and gram negative bacteria. Bacteria that have extensions like whips at the ends and stained pink are gram negative bacteria

whereas Gram positive bacteria appear purple. Examples of Gram positive bacteria are *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*. Examples of Gram negative bacteria are *E. Coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*.

One of the Gram (+) bacteria, *Staphylococcus*-genus are aerobic bacteria with typical respiratory metabolism and they are catalase-positive. This test allows this bacterium to be distinguished from *Streptococcus* and some other gram-positive cocci. Gram-positive cocci are relatively resistant to low water potential and show good resistance to drying and high salt concentrations. Their ability to grow in very salty environments provides a selectivity for isolation. *Staphylococci* are commensal and parasites commonly found in humans and animals, can cause serious infections. Two main *Staphylococcus* species have been identified; *Staphylococcus epidermidis* and *Staphylococcus aureus*. *Staphylococcus epidermidis* is a non-pathogenic organism that does not form pigment and is found on the skin or mucosal membranes.

As a result of this project, there are studies that show *Staphylococcus epidermidis*, which is one of the bacterial species detected in the school environment, has a protective role against pathogenic microorganisms in the nose. However (Liu, et al., 2020), there are supporting opinions that it is the main source of acne problem in adolescents. (Claudel, et al., 2019). An opportunistic type of bacteria *Staphylococcus epidermidis*, can easily become a harmful microorganism by creating a biofilm on different surfaces and skin. It has been a type of bacteria that can infect humans and

cause hospital sepsis, especially by creating biofilms on devices in hospitals. (Nguyen, Park, & Otto, 2017).

Bacterial habitats of the genus *Streptococcus* contain quite different homofermentative species. Some species are pathogenic to animals and humans. Other streptococci are lactic acid, they play an important role in animal feed, the production of buttermilk and other fermented factors. Some genera are the main factor of tooth decay. Three different genera have been identified to distinguish non-pathogenic streptococci from the ones pathogenic to humans; *Lactococcus* type is important for dairy production, *Enterococcus* type includes fecal origin streptococci. *Streptococcus pyogenes*, a second type of bacteria found as a result of this project, is the most disease-causing streptococcus in humans. In the blood agar, small grayish, slightly blurred appearance, large colonies with full hemolysis zones are formed around them. Cellular building materials; *lipoteic acid*, protein M, capsule polysaccharide, streptokinase, streptolysin, nuclease, hyaluronidase, erythrogenic toxins are the most common substances. In addition, streptococci produce enzyme-like substances such as proteinase, phosphatase, esterase, amylase, N-acetyl glucose-aminidase, neuromycinase, lipoproteinase, ribonuclease, diphosphopyridine nucleotidase, esterase. Among the diseases caused by this group of streptococci; serpentine (erysipelas), sepsis, endocarditis, postpartum fever (puerperal sepsis), fever like toxic shock, skin and subcutaneous infections, streptococcal angina (pharyngitis), scarlet, acute rheumatic fever, acute glomerulonephritis. (Madigan, Martinko, Brock, & Crashed, 2012).

Many researches have been done to find out different types of microorganisms in school environment. Kõljalg, Mändar, Sõber, Rööp and Mändar (2017) found different bacteria species in high school students' cell phones. They encountered many potential pathogenic microorganism species (Staphylococcus aureus, Acinetobacter spp., Pseudomonas spp., Bacillus cereus and Neisseria flavescens), among which E. faecalis species were more dominant. No relationship was found between the found microorganism species (s) with the gender of the students mobile phone and the phone brands of students mobile phones. According to the results of the research, high number of median microbial colonies [median 10.5 (IQR 3-16.8) colony building units (CFU) / cm²] were determined. A high level of bacterial contamination in secondary school students' smartphones has emerged with the contact plate (ø 60 mm) method. In total, more than 20 different dominant microbial species have been identified. Gram negative bacteria were detected in 41% (n = 11) of cultured telephone surfaces. The most common pollutants of the phones were *Micrococcus luteus* (n = 15; 63%), *Acinetobacter lwoffii* (n = 9; 33%), *Staphylococcus epidermidis* (n = 8; 30%) and *Staphylococcus hominis* (n = 5; 19%). Other microbes such as *Staphylococcus aureus*, *Pseudomonas luteola* and *Neisseria flavescens* have also been found among dominant species in one phone and *Bacillus cereus* in two phones (Kõljalg, Mändar, Sõber, Rööp, & Mändar, 2017). In another experimental article published by the University Hospitals Sports Medicine Institute, Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE) and influenza were detected in the

environment according to the experiments conducted by the high schools and universities in the sports and training rooms. The aim of the project is to reduce (increase) the detected bacteria with the cleaning methods they tried. MRSA and VRE were seen on 24% of surfaces prior intervention. *Influenza* was initially detected on 25% of the surfaces (Labelle, et al., 2019).

The reproduction of pathogenic bacteria can be brought under control if the hygiene rules are followed and certain substances are used. Controlling the reproduction of aerobic bacteria is also an issue that needs to be taken under consideration. According to Mayo Clinic (2006) 's report on children's health, families also reported that they should not send their children infected with pathogenic bacteria to the school environment.

Many methods are used to prevent microorganisms from causing disease. Disinfection is the killing or stopping the reproduction of pathogenic microorganisms other than bacterial endospores in the inanimate environment. Many disinfection methods are used to destroy microorganisms in the environment. Tools created to serve this purpose are called disinfectants. Ambient temperature, pH, hardness of water affect the disinfection process. Increasing heat accelerates chemical reactions, but too high temperature causes the active substances to break down and lose their activity. The factors that play a role in the different levels of disinfectants have different effects in different types of disinfectants. The optimal pH values differ. For example, although the effectiveness of glutaraldehyde increases at high pH, it shows negative effects in

some disinfectants. Due to these active factors, some disinfectants may not work in harmony with other disinfectants. An example of this phenomenon is that when bleach and quaternary ammonium compounds are used together, they can create a neutralization reaction and eliminate the activity of both substances. There is a possibility that disinfectant substances may blunt each other's properties; Therefore, the results of the mixture (herbal extract) produced in this project did not reveal different results compared to the use of herbal extracts alone; having a mixture did not cause it to be more effective.

Chemical disinfectant compounds can be classified according to their spectrum of effect. Accordingly, they can be separated as those used for chemical sterilization (chlorine dioxide, etc.), high-level disinfectants (glutaraldehyde, hydrogen peroxide, etc.), medium-level disinfectants (alcohols, iodine compounds, phenol compounds, etc.) and low-level disinfectants (four-valued ammonium compounds, etc.). Effect level of disinfectant; It also relates to the type,

density and contact time of the used chemical ingredients. Different intensity use of the same substance or longer, shorter usage times may not provide the same effect.

Many different disinfectant substances are used in schools to prevent the reproduction of pathogenic microorganisms. Among them, bleach, surface cleaners (including different brands and arabic soap) are used and are inhaled by students. As an alternative to these disinfectants, there are herbal disinfectants. Disinfectant effects of substances such as essential orange oil, vinegar, rose water, coconut oil, herbal glycerin, carbonate, tea tree oil have been found. Many plants have been found to have disinfectant effects and are currently used in the treatment of many diseases (Polat, & Satil, 2012). *Aloe vera*, *Aloe barbadensis*, *Piper nigrum* (black pepper), *Matricaria chamomilla* (chamomile), *Cinnamomum verum* (cinnamon), *Syzygium aromaticum*, *Citrus paradisa* (grapefruit) and many more plant families have been found to be disinfectant. (Cowan, 1999)

Table 1. Herbal disinfectant substances and the types of microorganisms they affect (Cowan, 1999)

Common Name	Scientific Name	Compound Affected	Organism
Alfalfa	<i>Medicago sativa</i>		Gram Positive organisms
Allspice	<i>Pimenta dioica</i>	Eugenol	General
Blueberry	<i>Vaccinium</i> spp.	monosaccharide	<i>E. coli</i>
Cinnamon	<i>Matricaria chamomilla</i>	Phenolic acid	<i>M.tuberculosis</i> , <i>S.typhimurium</i> , <i>S.aureus</i> ,helminths

Eucalyptus	<i>Echinacea angustifolia</i>		General
Garlic	<i>Allium sativum</i>	sulfoxide Sulfated terpenoids	General
Ginseng	<i>Panax notoginseng</i>		<i>E.coli, Sporothrix schenckii, Staphylococcus, Trichophyton</i>
Green Tea	<i>Camellia sinensis</i>	Catechin	<i>Shigella, Vibrio, S. mutans, Viruses</i>
Henna	<i>Lawsonia inermis</i>	Gallic acid	<i>S. aureus</i>
Lavender-Cotton	<i>Santolina chamaecyparissus</i>		Gram-positive bacteria, <i>Candida</i>
Olive oil	<i>Olea europaea</i>	Hexanal	General
Licorice	<i>Glycyrrhiza glabra</i>	Glabrol	<i>S.aureus, M. tuberculosis</i>

Daisy as a disinfectant in this project, cloves, orange peel, tea tree (castor laurel), rose and cinnamon is used. Chamomile is generally known for its antioxidant, antimicrobial, antidepressant, anti-inflammatory, antidiarrheal activities, angiogenesis activity, anticarcinogenic, hepatoprotective and antidiabetic effects. In addition, chamomile is good for knee osteoarthritis, ulcerative colitis, premenstrual syndrome and gastrointestinal disorders. Various

combinations of the extract, oil and leaves of chamomile and its many medicinal properties make it subject to much more research. Chamomile affects gram negative and gram positive bacteria (Miraj, & Alesaeidi, 2016). In a study of the effect of the clove plant on bacteria, *Staphylococcus epidermidis* and many other Gram (+) bacteria inhibition zones against the clove plant have formed. (Okmen, Mammadhkanli & Vurkun, 2018)

Table 2. Effects of plants used in the project on different bacterial species

Common Name	Scientific Name	Organism
Chamomile	<i>Affected Matricaria chamomilla</i>	<i>Gram-positive and Gram-negative bacteria</i>
Carnation	<i>Syzygium aromaticum</i>	<i>Staphylococcus sp. S. aureus Staphylococcus epidermidis Staphylococcus and Gram positive bacterias</i>

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

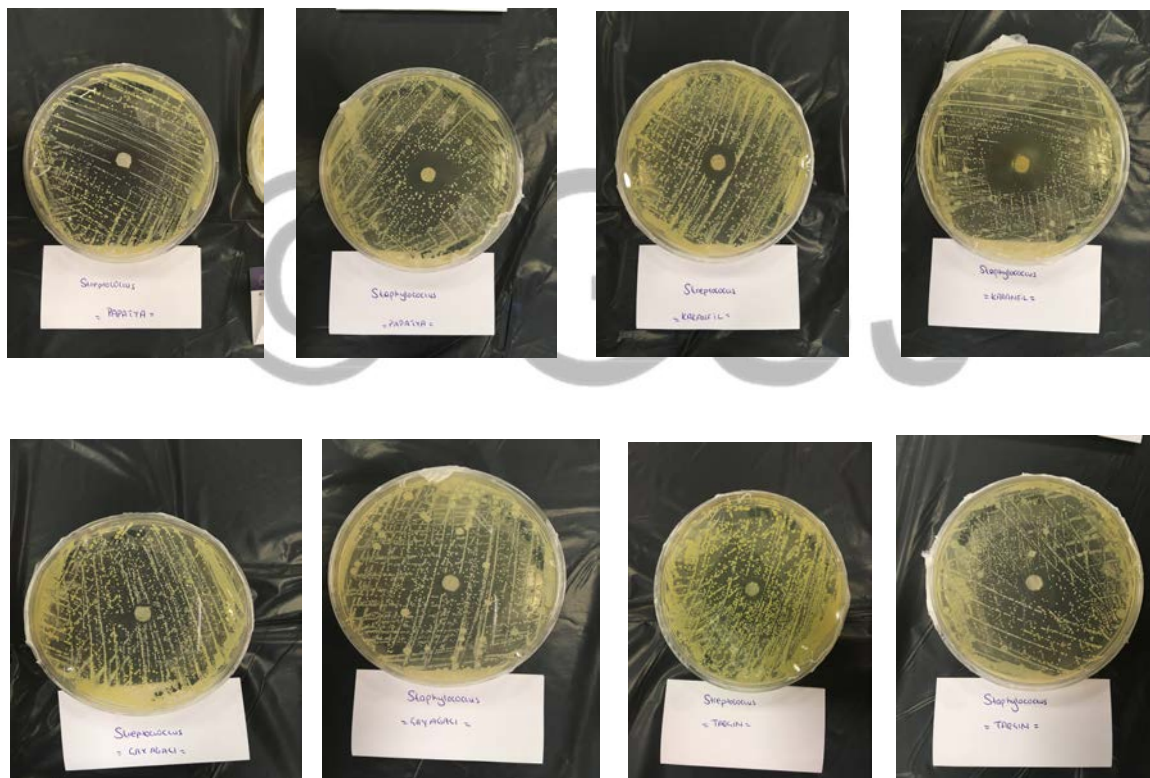
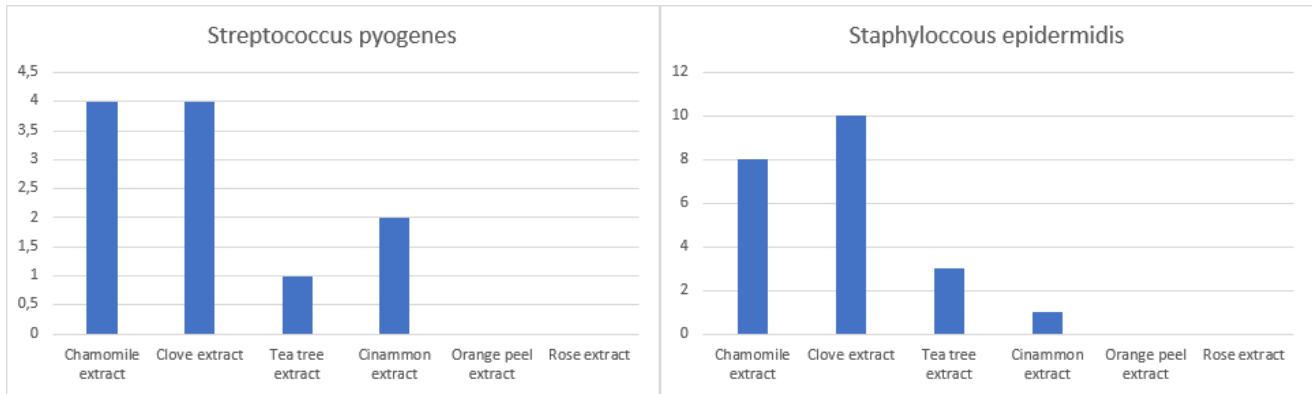


Figure 8. Inhibition diameters obtained by the disc diffusion method

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 (Kõljalg, Mändar, Sõber, 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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