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Phytochemical and Microbial Analysis of Calamansi peel extract Against Staphylococcus aureus and E. coli

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Abstract
Calamansi (Citrus microcarpa) peel, an abundant agro-waste was investigated as a source of bioactive compounds and as a topical antibacterial agent. Preliminary phytochemical screening revealed pronounced reactions for tannins/polyphenols in the ferric chloride test, abundant flavonoids in the alkaline reagent test, and appreciable amounts of carbohydrates, reducing and non-reducing sugars via Molisch, Benedict's, Fehling's and lead acetate assays.

These metabolite classes are well documented for antioxidant, anti-inflammatory and antimicrobial activities. The agar-well diffusion method was used to assess the antibacterial efficacy. Three concentrations-100% crude extract (T1), 50% extract ointment (T2) and a commercial antibiotic ointment control (T3) were tested in triplicate for 18 hours and 24 hours. Zones of inhibition increased with exposure time; all 24 hour readings exceeded their corresponding 18 hour values. Against S. aureus, T1 and T3 produced very strong inhibition, classifying T1 as bactericidal and comparable to the reference drug. E. coli displayed moderate susceptibility, though T1 and T3 again yielded the widest clearings.

Statistical analysis confirmed concentration-dependent activity. Thus, calamansi peel extract exhibits a rich repertoire of tannins and flavonoids and demonstrates potent anti-staphylococcal action, endorsing the 100% extract as the optimum concentration for ointment formulation. Valorization of calamansi peel could therefore convert waste to a cost-effective plant-based topical antibacterial preparation for broader community healthcare applications.

Keywords: Phytochemical, Antibacterial, Bioactive Substance

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Introduction
One of the concerns of people now a days is to find alternative solutions using natural treatments. In the Philippines, Calamansi is known to have it's sour taste and mainly used as part of the condiments, others make use of it as a source of juice and even vinegar. Calamansi or calamondin (citrofortunella microcarpa) belongs to the citrus family. It is a fruit tree native in the Philippines. The tree is low set, spreading and well branched. The leaves are broad and oval and dark green to pale green on the upper and lower surfaces, respectively. The fruit is usually small and round, ranging from 3.0 to 3.0 inches in diameter. The rind may be thin or thick. It's size is about 10-20 ft and considered as a small shrub. The plant is characterized by wing-like appendages on the leaf petioles and white or purplish flowers. The size of the Calamansi ranges to 25-35 mm in diameter or even reaches 45 mm. When riped, the juice and pulp usually appears to be orange in color. Each fruit contains 8 to 12 seeds. Lime and orange becomes the unique flavor of the Calamansi pulp, giving it's flesh a juicy and acidic nature. Calamansi is also a good source of Vitamin C and can blend well with other fruits like banana, apple, papaya, mango and even coconut water. Calamansi also provide some health benefits like helping proper blood circulation and normal digestion. It is also beneficial in the food industry as it is used as flavoring, can also be used in beverages and syrups and even the Calamansi peel was also used in making jams, candies and marmalade.

Calamansi was known to be the most abundant and popular product in the locality of Tagudin, famously called as its "OTOP"- The One Town, One Product" where the municipality was able to produce its own Calamansi juice out from its extracts. Many products that are out in the market utilized the pulp extract as substitute to other commercialized products which pose beneficial effects to humans and its helpful effect in the community. According to Marilla Mulwane, citrus fruits also contain ingredients that kill or repel pests. An example of this citrus fruit as insecticide is calamansi. Calamansi is chosen for that research as it is found to contain phenolic compounds for example caffeic, coumaric acid and sinapic acid which play a role as natural antimicrobial agents and are fat free, sodium free and cholesterol free. They contain carbohydrates, fibers, vitamin C, potassium, folicacid, calcium,riboflavin, thiamine, niacin, vitamin B6, copper, phosphorous, magnesium, riboflavin,pantothenic acid and other varieties of phytochemicals. Also, the pulp of lime tastes sour and the fruit contains twice the amount of juice as the yellow, larger lemon. Calamansi has several alternative uses. It lightens your skin, good as mouth wash and can cure coughs. It is also helpful upon dealing with hangover, it maintains kidney health and lowers body cholesterol and the like. (Benedicte et al., 2014).

This research is made with the aim to investigate potentials of plant materials in order to come up and provide appropriate concentration for alternative products out from natural wastes which have beneficial uses to eliminate the huge number of microbial growths that causes illnesses.

Therefore, the goal of the study is to determine the phytochemical and microbial analysis of calamansi peel as basis for a potential ointment. This study specifically aims to: a. Determine the phytochemistry of the calamansi peel extract, b. Assess the microbial properties of the calamansi peel extract against Staphylococcus aureus and E. coli. And, c. Determine the potential of calamansi peel extract along: Zone of inhibition and Best concentration for the ointment.

Staphylococcus Aureus

Staphylococcus aureus is a type of bacteria. It stains Gram positive and is non-moving small round shaped or non-motile cocci. It is found in grape-like (staphylo-) clusters. This is why it is called Staphylococcus. *Staphylococcus aureus* or "staph" is a type of bacteria that resides on human skin, in the nose, armpit, groin, and other areas. While these microorganisms are not all the time harmful, it can still cause sickness or can be caught anytime at any circumstances. Infections in tissues and the epidermis was recorded as the leading effect of being exposed to *S. aureus*. Moreover, it can severely affect the lungs, the bloodstream, the bones and joints and even the heart. Body fluids, infected blood or bodily fluids can be the major source of infection. *It resides most of the time in an individual* noses, pharynx and on their skin. In normal healthy and immunocompentent person, *S. aureus* colonization of the skin, intestinal tract, or nasopharynx does not lead to any symptoms or disease. According to Mansour, et.al (2021), Staphylococcus aureus continues to be one of the most involved bacteria in human diseases. Even in lower forms of organisms like plants and animals, the bacterium still exists at about 30%. Antibiotic resistance develops quickly in S. aureus, and the rise of multidrug-resistant forms is a major problem. It has been reported that the annual mortality toll from antibiotic-resistant diseases has surpassed 10 million and that by 2050, it will outnumber cancer deaths. The morbidity and mortality consequences reinforce the need to urgently discover new effective solutions due to the inefficiency of traditional antibiotics. Therefore, alternative treatments represent a challenging yet exciting area of investigation due to the lack of new antibiotic classes. Different strategies have been conducted, notably based on drug design that could reduce virulence factors. However, these studies have not yet generated remarkable outcomes due to toxicity and/or low bioavailability. New options are now under study with a focus on biological molecules or compounds to interfere with toxins or toxin-regulator genes, constituting a new generation of promising anti-staphylococcal treatments.

Escherichia Coli

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, facultative anaerobic bacterium. This microorganism was first described by Theodor Escherich in 1885. Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora. However, there are some strains that have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands. This pathogenic E. coli can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors.(Lim,et.al 2010). Also, according to the Pathobiology of Human Disease (2014), *Escherichia coli* are nearly ubiquitous in the human gastrointestinal tract; they most often exist in this setting without compromising host health. Yet, *E. coli* are capable of expressing virulence traits that allow them to cause a variety of diarrheal disease syndromes which are also frequent throughout the world. It is estimated that on any single day, 200 million individuals are affected by diarrheal illness. While the majority of diarrheal disease caused by *E. coli* in the resource-sufficient world are minor illnesses that are more of a nuisance, they still have a public health impact, for example, in days of work or school lost. In the resource-limited world and in other selected settings, *E. coli* diarrheal illnesses may result in a significant impact on health and are a significant public health burden. Using stool culture, *E. coli* that cause diarrheal disease are typically indistinguishable from *E. coli* that are part of normal flora, thus complicating precise clinical diagnosis of the illnesses caused by *E. coli*.

Antibacterial activity of Fruit Peel Extract

Compounds that kill or inhibit the development of bacteria locally without being very harmful to adjacent tissues are known as antibacterial active molecules (Mohapatra et al., 2018). Results showed that methanolic extract of satkora peel had the highest antibacterial activity against *Bacillus spp.* and *E. coli, respectively.* On the other hand, adajamir had the lowest antibacterial activity while citron had moderate activity against both *Bacillus spp.* and *E. coli.* Citrus fruits' non-edible components such as peels and seeds may also be quite beneficial if used properly. Peel waste is very perishable and seasonal, posing a difficulty for processing companies and pollution control authorities. Appropriate procedures are used to convert them into value-added goods (Kumar et al., 2011). Citrus peels are used for a variety of purposes, including fish feed, activated carbon, and conventional paper raw materials (Arias and Ramón-Laca, 2005). Citrus fruit products are recognized to possess antibacterial and antifungal activity (Viuda-Martos et al., 2008). Citrus fruits' peels contain an ample quantity of flavanones and polymethoxylated flavones, which are uncommon in other plants. These chemicals are not only vital for human health and the environment, but they also have a wide range of economic uses in the food and pharmaceutical sectors. With the desire of this study to utilize the waste products of Calamansi, the researchers made use of the peel to investigate and determine its functional properties. By this, the researchers aims to find answers and to help each and every one of us to get rid of possible inflammations that can be caused by insect bites and other related illnesses, also, to ensure that it doesn't contain chemicals that can harm the people in the community.

Review of Related Literature

The nutritional benefit and flavorful nature of Citrus juices caused it to be consumed by majority of individuals. Along with its nutritional value, the fruit juice can help maintain good health and reduce diseases. This was supported by the study of Boudries, et.al and Rekha, et.al that the health benefits of citrus juices have been attributed to Vitamin C. Also, Aruoma, et.al and Karimi, et.al believed that Citrus fruits possess antioxidative, anti-inflammatory, antitumor and antimicrobial potentials which were all attributed to the presence of bioactive compounds such as phenolics, flavonoids, vitamins, and essential oils. The usual dilemma in the world of medicine now a days is focused on the microorganisms resistance to antimicrobial drugs. On the other hand, plants with antimicrobial potentials are becoming more known for its less side effects compared to the synthetic ones. Therefore, Parekh, et.al emphasized that the continuous investigation for new antimicrobial compounds from natural sources is, thus, an ongoing one. The peptidoglycan-containing periplasmic space and the lipopolysaccharide layer in the outer membrane caused the gram-negative bacteria to have a lower zones of inhibition as compared to the gram-positive bacteria. In addition, Holetz, et.al and Cheruiyot, et.al, described the outer membrane of gram-negative bacteria as barriers which prevents entry of various environmental substances. Also, the enzymes that has the ability to break down foreign materials has been observed in periplasmic spaces that attempted to enter the microorganism.
The susceptibility of *C. albicans*, *A. Niger* and *Penicillium spp.* were revealed that citrus juice concentrates have the potential antifungal activities. *C. albicans* being the most susceptible, had an observed zones of inhibition that ranges from 8 mm for grape juice and 24 mm for lemon juice. However, *A. Niger* and *Penicillium spp.* were less susceptible to juice concentrates. A notable observation was seen on A. Niger having a 2 mm zone of inhibition in tangerine juice while 6 mm for the grape juice. The Penicillium spp only showed a 10 mm zone of inhibition for lemon juice but not susceptible to grape juice. Also, from the study of Venkatachalam, et.al. (2023) Calamondin fruits are an incredibly versatile and beneficial citrus fruit that offer a range of nutritional and economic advantages. These small fruits can be processed into various economically valuable products, such as juices, jams, and flavorings, and are a popular substitute for limes in many cuisines. Furthermore, calamondin cultivation is suitable for both small-holder farmers and larger-sized farmers, making it a profitable option for agriculture. The juice, pulp, seeds, peel, and fruit residue of calamondin fruits contain a rich chemical composition and bioactive compounds, offering a range of potential applications in several industries. These compounds have been shown to possess antioxidant, anti-inflammatory, and antimicrobial properties, among other health benefits, making them a valuable resource for food and beverage production, pharmaceuticals, and cosmetics. Additionally, utilizing different parts of the fruit, such as the peel and seeds, can minimize agricultural waste and add value, supporting the Bio-Circular-Green (BCG) economic model. By promoting a circular economy, we can reduce waste and environmental impacts, while also creating economic opportunities for communities.

METHODOLOGY

The study utilized the Experimental Research design. According to the Science and the Global Environment (2017), conclusions can be drawn in an Experimental design if the process of carrying out research is in an objective and controlled fashion that allows precision to be maximized. Generally, the purpose is to establish the effect that a factor or independent variable has on a dependent variable. Experimental design methods allow the experimenter to understand better and evaluate the factors that influence a particular system by means of statistical approaches. Such approaches combine theoretical knowledge of experimental designs and a working knowledge of the factors to be studied. Although the choice of an experimental design ultimately depends on the objectives of the experiment and the number of factors to be investigated. Further, the study protocol was reviewed and approved by the College of Veterinary Medicine, at DMMMSU, North La Union Campus, Bacnotan, La Union.

Materials and Methods

The first part of the experiment started with the collection of the plant materials. The Calamansi fruits were collected from one of the largest plantations of Calamansi in Tagudin which is situated at Pudoc West. The researchers gathered 4 kilograms of calamansi peel from the locale which will be used for the sample extracts that will be subjected to the phytochemical and microbial analysis.

Preparation of peels

Peels were removed from fruits and rinsed in sterile distilled water after being washed in tap water. After that, it was sun dried for 24 h at 40 °C. The result of the sun drying led to a fine powder through the use of the blender. It was stored at the Science laboratory maintaining a room temperature.

Preparation of peel extracts

Ten (10) grams of each sample were weighed into a disinfected, clean conical flask, and 50 ml of extraction solvent (methanol) were added in a 1:5 ratio (g/ml) and left to extract for 2–3 days at room temperature by covering the flask with foil paper. Thereafter, filter paper was used to remove the impurities and supernatant was preserved.

Concentration of the peel extract

The filtrates were concentrated by oven drier at 60 °C. The concentrated filtrate was then mixed with the sterile DMSO (Dimethyl sulfoxide) in 1:2 proportion. These were kept at a temperature of 4 °C in the refrigerator for antibacterial sensitivity testing.

Preparation of nutrient agar (NA) for Antibacterial Test:

Twenty-eight grams of NA will be mixed in a beaker with 1L of distilled water. The mixture will be boiled using microwave oven until agar will completely be dissolved. Then the solution will be transferred into a sterilized Erlenmeyer flask and will be covered with a cotton plug. The prepared nutrient agar will be sterilized in a pressure cooker at 15 psi for 30 minutes. The sterilized NA will be cooled and will be poured into each of the sterilized Petri dishes.

Preparation of Microorganisms used

The test organisms were collected from the Laboratory of the College of Veterinary Medicine, situated at DMMMSU-NLUC, Bacnotan, La Union to investigate the potential activity of the extracts. The organisms involved the gram-positive bacteria *Staphylococcus aureus* and the gram-negative *Escherichia coli*. The organisms used, are all human pathogenic organisms of clinical origin.

Inoculum preparation

Each bacterium was initially sub cultured in a nutrient agar medium for 24 hours at normal room temperature. To achieve consistency, a standardized inoculum of each bacterium was distributed on a nutrient agar plate using a sterilized cotton bar.

Inoculation of test plates

After dipping a sterile cotton swab into the solution, surplus fluid was collected by pressing and spinning the swab against the wall. A proper streaking technique was done to the nutrient agar Plates for about twice or thrice. As a result, the inoculum is evenly distributed throughout the whole surface. The aseptic condition was maintained in the process.

The Kirby – Bauer Technique

The Kirby- Bauer Method or filter disc diffusion method will be used in the bioassay. Using some sterile forceps, the sterilized 6mm filter paper disc will be soaked into the prepared treatments. The moistened disc will be laid gently on the seeded agar plates.
Treatments will be assigned as follows:
Treatment 1: 100 % calamansi extract
Treatment 2: 50 % calamansi extract and 50 % Normal saline solution
Treatment 3: Control Drug (Ciprofloxacin)

Antibacterial activity

The Disc diffusion method adopted from Bonev, et.al was used to estimate the antibacterial activity of different calamansi extracts. Blank discs were soaked into the sample in a Petri plate for about 2 hours. Then the discs were ready to use. Discs were placed into the inoculated nutrient agar plates. It was left for an hour to let diffusion take place before being incubated at normal room temperature for 24 hours. A standard Vernier caliper was used to measure the zone of inhibition (ZOI). The size of ZOI was calculated in millimeters.

Determination of zone of inhibition (ZOI)

Each plate was analyzed after 24 hours of incubation. On the surface, there existed a circular inhibitory zone. A Vernier caliper was used to calculate the diameters of the zones to the closest full millimeters.

Data Collection and Interpretation

After 18-24 hours of incubation, the plates were taken out from the incubator and examined for a clearing around each hole, known as the zone of inhibition. The widest part of each clear zone was measured using a Vernier caliper. For unequal zone of inhibition, the average diameter of x and y axes will be measured. The results of the zones of inhibitions will be interpreted based from Moraleta, 2007 and as follows;

a. Susceptible (Very Active, >19 mm) – implies that an infection due to an organism can be appropriately treated with the recommended dosage of antimicrobial agent for that type of infection and infecting organism, unless otherwise contraindicated.

b. Moderately susceptible (Active, 14-19 mm) – implies that the amount of the antimicrobial agent given can be increased beyond standard or recommended dosages without serious risk of adverse reaction to obtain higher levels in the infected site.

c. Intermediate (Partially Active, 10-13 mm) – indicates that the test result is equivocal, and that susceptibility of that particular organism cannot be predicted. This is general as applied to antimicrobial agent with a narrow toxic therapeutic ratio in which higher dosages are likely to be associated with adverse side effects.
d. Resistant (Inactive, <10 mm) – implies that an infection due to an organism is unlikely to respond and will not reliably respond to the antimicrobial agent.

RESULTS AND DISCUSSIONS

Table 1. Phytochemical Testing Results

Types of Test	Results
Mayer's test	(-)
Hager's test	(-)
Wagner's test	(-)
Dragendorff's test	(-)
Molisch test	(++)
Benedict's test	(++)
Fehling's test	(++)
Lead Acetate test	(++)
Alkaline Reagent test	(++)
Ferric Chloride test	(+++)
Froth test	(-)
Filter Paper test	(++)

Table 1 above showed the results of the phytochemical testing of the calamansi peel extract. As seen on the table, the different types of tests were conducted to determine the significant presence of bioactive compounds. The presence of variety of compounds were indicated by the 2 positive signs (++) and three positive signs (+++). The Ferric Chloride test showed a very significant presence of tannins/ polyphenolic compounds. Flavonoids were also found significantly present through the Alkaline reagent test. On the other hand, the significant presence of carbohydrates, reducing and non-reducing sugars were also determined through the Molisch, Benedicts, Fehling's, and Lead acetate test respectively. According to Singh and Kumar (2019), Tannins are the secondary metabolites present in a substantial amount in plant-based food products. Due to their positive effects on the food as antibacterial and antioxidants, they are the major constituent of food products. Furthermore, it was also emphasized by Singh and Kumar (2019) that tannins play a very significant role as a raw material for sustainable green industries. Therefore, they are mainly used in diverse types of industries such as leather, feed, fisheries, beverages, etc. They also find application as potential medicinal agents, antioxidants, metal chelators; and cater as inhibitors of harmful pro-oxidative enzymes and of lipid peroxidation process. Recently, several important properties like antiseptics, anticarcinogenic, and anti-inflammatory of tannins have been documented in the human that make them suitable candidates for pharmaceuticals and nutraceutical industries. Because of current concerns related to synthetic compounds used in the human health and food industries, which leave highly adverse effects on the human body and environment, tannins can offer an alternative to these harmful chemicals in recently emerging industries.

Flavonoids were also seen significantly present in the peel extract, according to the National Library of Medicine, flavonoids are secondary metabolites, which mainly consists of a benzopyrone ring bearing a phenolic or poly-phenolic groups at different positions. They are most found in fruits, herbs, stems, cereals, nuts, vegetables, flowers, and seeds. The presence of bioactive phytochemical constituents present in these different plants parts gives them their medicinal value and biological activities. Hence, flavonoids possess several medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects. These biological activities depend upon the type of flavonoid, its (possible) mode of action, and its bioavailability. In addition, the study of Khatri, et.al (2020) showed that sugar plays a pivotal role in plants as both nutrient and central signaling or regulatory molecules that modulate gene expression related to plant growth, development, metabolism, stress response, and disease resistance. Reducing and nonreducing sugar play an important role in the central metabolic pathways and help in the production of secondary metabolites that enhance the medicinal properties of plants.

ANTI-MICROBIAL ASSAY

Antimicrobial efficacy is usually determined by examining minimum inhibitory concentration, bactericidal effects and other test that commonly utilize various microbial culture techniques.

Table 2. Antimicrobial Assay (Staphylococcus aureus)

Treatment	Time			Time		
	18 hours			24 hours		
	R1	R2	R3	R1	R2	R3
T1	18.5mm	24.7 mm	8.8 mm	27.6mm	32.6mm	10.2mm
T2	12.5 mm	16.8 mm	2 mm	14.2mm	17.4mm	19.1mm
T3	15.3mm	17.7 mm	21.4 mm	16.3mm	27.8mm	23.1mm

Above is the result of the antimicrobial assay of the three different treatments of calamansi peel extract with three (3) replicates against staphylococcus aureus between eighteen (18) and twenty-four (24) hours. It can be gleaned from the table that Treatment 1 and Treatment 3 exposed within 24 hours had the greatest diameter (in mm) of inhibition against Staphylococcus. This is supported by research conducted by Ouchari et al (2019), compounds with a strong inhibitory power have an inhibitory diameter of 10-20 mm, whereas compounds with a very strong inhibitory diameter have an inhibitory diameter of more than 20 mm. It can be deduced that Treatment 1 under the 24-hour time period has antibacterial potential against the said microbe.

Table 3. Antimicrobial Assay (Escherichia Coli)

Treatment	Time			Time		
	18 hours			24 hours		
	R1	R2	R3	R1	R2	R3
T1	9.2 mm	8.3 mm	5.2 mm	20.3mm	12.7mm	10.2mm
T2	8.7 mm	9.1 mm	23.4 mm	19.1mm	17.4mm	9.1mm
T3	21 mm	8.5 mm	23.5 mm	21mm	27.8mm	8.5mm

As gleaned above, the antimicrobial assay of three different treatments of calamansi peel extract with three (3) replicates against Escherichia coli between eighteen (18) and twenty-four (24) hours. Treatment 1 and 3 under exposed to the 24-hour period exhibited the greatest diameter of its zone of inhibition. It simply implies that pure calamansi extract and commercialized drug are comparable in terms of its potential as antibacterial but a good agent to make the microbe susceptible to it.

Table 4. Mean zone of inhibition of all calamansi peel extracts against Staphylococcus aureus and E. coli

Treatments	Mean (18 hours) Staphylococcus Aureus	Mean (18 hours) Escherichia Coli	Mean (24 hours) Staphylococcus Aureus	Mean (24 hours) Escherichia Coli
T1	17.3mm	7.57 mm	23.47mm	14.4mm
T2	10.43mm	13.73mm	16.9 mm	15.2mm
T3	18.13mm	17.67mm	22.4 mm	19.1 mm

It can be gleaned from the table that the means of the different treatments within 24 hours showed a higher result compared to the treatments exposed only for 18 hours. And among the three treatments exposed within a day, treatment 1 appeared the highest. It exhibited the greatest mean diameter of its zone of inhibition which explains that the recorded mean diameter which is 23.47 mm is greater than 19mm from the given standard of the zone of inhibition of Moraleta 2007. Therefore, staphylococcus aureus is susceptible to treatment 1 of the said calamansi peel extract and exhibited comparable potential as that of Treatment 3. Among the treatments exposed in the 24-hour period, Treatment 1 was recorded to be the best concentration to inhibit the growth of microbes. On the other hand, treatments exposed to 24-hour time period against E.coli also showed moderate susceptibility which implies that the treatment can also be an effective antibacterial agent. It can be deduced that the pure calamansi peel extract showed an efficient potential against staphylococcus aureus.

CONCLUSIONS AND RECOMMENDATIONS

This study came up with the conclusions that the phytochemistry of the calamansi peel extract showed a very significant presence of bioactive compounds which gives the extract the potential to become a useful product in the locality. In terms of the concentration, the pure calamansi extract exhibited an antibacterial property against Staphylococcus aureus and Escherichia coli. To deduce further, the Staphylococcus aureus were more susceptible than the E.coli under the pure calamansi extract exposed in a 24 hour period and was considered the best treatment to inhibit the growth of microbes.

To further improve the study, it has been highly recommended that in order to make the calamansi extract more susceptible to Escherichia coli, there must be an increase in the dosage or concentration of peel extracts. It is also suggested to use combinations of calamansi parts and be subjected to phytochemical and anti-microbial tests to be able to come up with alternatives products. Lastly, the production of possible health products out from the said potential of the calamansi extract is highly recommended.

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