Antibacterial Effect of Basil (*Ocimum basilicum*) and Table Salt as Alternative Disinfectants against *Staphylococcus aureus*

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Date received: June 22, 2020
Revision accepted: October 4, 2023

Abstract

This study was undertaken to evaluate the potency of basil (Ocimum basilicum) extract and table salt as alternative disinfectants based on their effect in inhibiting bacterial proliferation. A sample of bacteria (Staphylococcus aureus) with an initial mean density of 1.35 x 10^3 CFU/mL was used as the bacterial test culture. Treatments included basil extract (T1), table salt (T2), ethanol (T3, positive control) and distilled water (T4, negative control). The antibacterial effect of the treatments was evaluated in terms of the measurement of the zones of inhibition which was further validated by monitoring the mean recovery counts of bacteria post-application with the treatments given at different concentrations (100, 50, 25 and 10%). The results showed that the larger diameter of the zones of inhibition (with mean measurements that ranged from 17.65 to 20.98 mm) was related to the application of basil extract, table salt and ethanol applied at 100% concentration compared with the 50% (mean measurements ranged from 14.03 to 17.67 mm) underscoring the minimum inhibitory concentration of the candidate disinfectants at 50%. There was a comparable significant reduction of bacterial recovery counts associated with the application of basil extract and ethanol only at 3 h that were not sustained until 6 h. The results indicated that basil extract and table salt at the prescribed concentrations could be used and therefore recommended as disinfectants.

Keywords*:* basil, disinfectant, ethanol, *Staphylococcus aureus*, table salt

1. Introduction

In nature, pathogenic bacteria exist primarily by adhering to living tissues and body surfaces. Accumulation of live bacteria poses a serious problem for public health because of the potential of these organisms to cause infections

in patients and serve as a source of infection for healthy human beings (Gundran *et al*., 2018). Bacteria are the culprits of many disease outbreaks and the difficulty in eliminating pathogens and microbial contamination is hard to address. The elimination of microbes is the critical role of disinfectants, and this is related to essential components that destroy the osmotic barrier function of the bacterial membrane, destroy utilization of energy after microbial metabolism and destabilize many processes in bacteria, while others serve as chelating agents which act as metal binders for bacterial aggregates (Roy *et al.*, 2020; United States Environmental Protection Agency, 2020). As bacterial populations reportedly exhibit different degrees of tolerance against a broad spectrum of antimicrobial agents, research and screening of candidate products that can alternatively function as disinfectants in eliminating potential pathogens like *Staphylococcus aureus* are necessary.

One species of bacterium found on the human mucous membrane and skin is *S. aureus* (Taylor and Unakal, 2021). *S. aureus*, a gram-positive bacterium that also causes food poisoning, is one of the humans' most prevalent bacterial illnesses (Hennekinne *et al.*, 2012; Natividad *et al.*, 2014; Natividad and Rafael, 2014). Human infections such as scalded skin syndrome, cellulitis, carbuncles, furuncles, folliculitis, impetigo, infective endocarditis, bacteremia and urinary tract infections can be caused by *S. aureus* (Gnanamani *et al.*, 2017; Guo *et al.*, 2020). Even though infection control approaches such as methicillin-resistant *S. aureus* (MRSA) transmission prevention recommendations, handwashing techniques and hospital decontamination processes have proven effective, preventing *S. aureus* infections remains challenging (Taylor and Unakal, 2021).

Destruction of bacterial contaminants as a function of potential disinfectant agents has not been explored widely. There are naturally acquired products with reported health benefits like basil and table salt which can be evaluated as disinfectants based on their properties. Basil (*Ocimum basilicum*) is reported to contain natural products such as essential oils, phenolic acids, flavonoids, phenolics and polyphenols that exert positive effects against bacterial, fungal, viral, and some infections in addition to its pharmacological uses as anti-cancer, radio-protective, anti-inflammatory immune-modulatory, anti-diabetic activity, antipyretic and prophylactic activities against cardiovascular disease (Shahrajabian *et al*., 2020).

Araújo-Silva *et al.* (2016) described the antibacterial activity of *O. basilicum* against *S. aureus* relative to its essential oil – linalool. Das *et al.* (2020)

determined the possible metabolic compounds in *O. basilicum* and *Ocimum sanctum.* They found out that *O. basilicum* possesses phenolic acids like ursolic, vanillic, coumaric and syringic acid which reportedly decreased the propagation of pathogenic bacteria such as *Bacillus* sp., *Salmonella* sp., *Staphylococcus* sp. and *Listeria monocytogenes.* Further, damage in the cellular membranes of fungi, particularly *Candida albicans*, after treatment with basil essential oil was reported by Miao *et al.* (2020). Analyses showed that basil metabolic components, namely phenylpropanoids and terpenoids, were responsible for suppressing *C. albicans*.

Feriotto *et al.* (2018) described the therapeutic activity of essential oil and terpenes extracted from *O. basilicum* L. leaves in activating hemoglobin biosynthesis in a particular type of cell. On the other hand, Takeuchi *et al.* (2020) studied the therapeutic potential of sweet basil on obesity accompanied by a state of chronic inflammation. The result showed that basil extracts have an anti-inflammatory activity on inflammation from adipocytes. Meanwhile, Carocho *et al.* (2016) highlighted the antitumor, antimicrobial and antioxidant activities of basil leaves when prepared in soluble sugars, organic acids and phenolic compounds. In this study, the basil in dried or decoction form was reportedly added to the cheese before the cheese was subjected to nutritional analysis. The incorporation of basil preserved the proteins and fatty acids in the cheese, reduced its moisture and provided an antioxidant activity that underscored basil's importance in conserving and fortifying food products. Moreover, basil is known for its significant health properties as a medicinal plant and its antibacterial activity that matches the effects of antibiotics against bacterial strains of clinical importance (Araújo-Silva *et al.*, 2016).

Sea salt is another naturally acquired product with an antibacterial effect due to its anti-inflammatory action and ability to act as a wound cleanser or as a natural antiseptic, known for a thousand years. The expression "throwing salt on a wound" used to be an old expression that meant that people of many generations cleaned out infected cuts and scraps. It reportedly sounds painful, but it can anecdotally aid in getting an area free of infection quickly (Wasik and Tuuminen, 2021). Salt reportedly acts as a bacteriostatic because of its capability to lessen the accessibility of water in foods; hence, depriving microorganisms of utilizing available water as a nutrient and diminishing its enzymatic activities. Further, salt also inhibits the development of pathogens such as *Clostridium botulinum* and *Clostridium perfringens*. Some mechanisms were attributed to the growth-inhibition activity of salt in the microorganism. These mechanisms include interference with enzymes,

limiting oxygen solubility, glucose utilization, inhibited respiration and cellular plasmolysis. Furthermore, salt is proven to diminish enzymatic activities by deactivating bacterial enzymes and reducing and altering bacterial cofactors (Elias *et al.*, 2019).

The pieces of literature indicate that sea salt and basil, with their given properties and activities, have the potential to become alternative disinfectants. The majority of chemical disinfectants have halogenated component concentrations over the 0.01% environmental safety threshold. Because of this, chemical disinfectants are difficult to dispose of as regular garbage and are considered hazardous waste. In fact, certain disinfectants are regarded as poisonous as well as harmful. Certain disinfectants can be irritating to people and even dangerous in high amounts (Curran *et al.*, 2019).

Exploring the potential of naturally acquired products as alternative disinfectants to a pathogenic agent like *S. aureus* is necessary. With the present problems of antimicrobial resistance, this study aimed to investigate the potential of basil and table salt as disinfectants against pathogenic bacteria like the test organism *S. aureus*.

2. Methodology

2.1 Cultivation of the test bacterium

A pure culture of *S. aureus* (MK 629958, NCBI annotation) was used as the bacterial test culture. The culture was provided by the Veterinary Microbiology Laboratory, College of Veterinary Science and Medicine, Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija, Philippines, from a previous case of swine vulval infection. This sample was used to inoculate 50-mL nutrient broth (NB), incubated at 37 °C for 24 h and used in the study.

2.2 Extraction and Collection of Dehydrated Basil Extract

A total of 500 g of freshly collected basil leaves were washed with water in the laboratory. The leaves were allowed to drain in a clean plastic basin for about 2 h. The leaves were placed in a clean plastic net bag then pounded in a sterile mortar and pestle. The pounded leaves inside the plastic bag were squeezed to release the basil extract and then poured into a round-bottomed

flask until half-full with the extract to be evaporated in the machine (Rotary Evaporator R-100, United States). The flask was connected to the evaporator's "bump trap" using a plastic clip to prevent foaming or splashing solutions ("bumping") to the condenser. The joystick knob was used to lower the flask into the water partially submerged before the vacuum source was turned on. The basil extract was allowed to evaporate as the solvent collected in the large round-bottomed flask reservoir and until a solid or thin-film appeared after a day. As the sample had completed evaporation, it was allowed to remain in the reduced pressure system for a few minutes to remove any final solvent residue. The dry mass was obtained and transferred in a sterile test tube for use in the test.

2.3 Treatments

The study utilized a dehydrated form of basil extract as described in the previous section. Reagents such as ethanol (analytical grade) (Sigma-Aldrich, Singapore) were obtained in the laboratory, while table salt was obtained commercially. Treatments included basil (for 100% concentration, 10 g of the extract was dissolved in 10-mL water, treatment 1), table salt (for 100% concentration, 10 g dissolved in 10-mL water, treatment 2), ethanol (absolute ethanol, positive control) and sterile distilled water (negative control). Other concentrations were prepared using similar procedures (for 75% concentration, 7.5 g was dissolved in 10-mL water; for 50% concentration, 5 g was dissolved in 10-mL water, and so on). The dehydrated basil extract and table salt were wrapped separately in an aluminum foil and autoclaved (ES-215, Tomy Seiko Co., Ltd, Japan) at 121 °C for 15 m. These were allowed to dry before preparing the suspensions in distilled water contained in sterile screw-capped glass jars. The distilled water and ethanol were dispensed separately in separate clean bottles and kept under refrigeration (TRYY-200, SurgicoPhil, Inc., Philippines) during the study.

2.4 Qualitative In-Vitro Evaluation of the Inhibitory Effects of the Basil and Table Salt

The protocol for evaluating the inhibitory effects of the basil and table salt involved a modification of the agar well diffusion method (Garcia, 2013). Three replicated plates of nutrient agar were prepared to test the disinfectant effect of basil extract on *S. aureus*. The test bacterium was seeded to a molten nutrient agar and agitated gently to make a homogenous bacterial culture before plating in plates. Six holes were made on the surface of the agar in each plate based on the methods described by Garcia (2013) to accommodate four different concentrations of each disinfectant (100, 50, 25 and 10%) and one

for the positive and negative control. The previous procedures were repeated in testing the inhibitory effect of table salt on the test bacterium. All plates were incubated at 37 °C for 24 h. The zones of inhibition produced on the test bacterium in interaction with the test disinfectants were measured using a Vernier caliper (Mitutoyo, MESCO, Philippines) after the 24-h incubation. The sizes of the zones of inhibition (diameter measurements expressed in mm) produced in response to each concentration of treatment applied were computed and expressed as a mean of three data (three replicates). The minimum inhibitory concentration (MIC) of each treatment was determined and served as the basis for making the disinfectant substrate or medium for the bacterial recovery test.

2.5 Evaluation of Bacterial Counts before and after Exposure to the Disinfectants

Serial dilutions of the bacterial test samples (100 µL) were obtained from designated wells before (counts varied from 0.62 to 1.88 x 10^3 CFU/mL, mean 1.35 x 10^3 CFU/mL) and after exposure to the disinfectants which were previously laid in a 96-well flat-bottom microtiter plate (Nunclon, United States). Each sample was prepared in a series of seven tubes containing 0.9 mL 0.85% saline. A volume of 20 µL from each diluted solution (in -5, -6 and -7) was deposited separately into Petri plates containing nutrient agar. The three plates were marked; each with three divisions designated as -5, -6 and -7 which were set as three replicated plates. Bacteria were spread evenly in each division with the use of a sterile glass rod. This procedure was repeated with the other replicates. The plates were incubated at 37 °C for 24 h. Colonies growing on the surface of the nutrient agar after incubation were counted. The number of bacteria (expressed as CFU/mL) before and after the application of the treatments (basil and table salt) was computed as average count x 50 x 10^7 (the 50 is a multiplier which is expressed as the reciprocal of the ratio of the volume of bacteria plated [20 µL] per 1,000 µL).

2.6 Quantitative Evaluation of the Inhibitory Effects of the Different Treatments against the Test Bacterium

Sixty wells of a 96-well flat-bottom microtiter plate were filled with 250 µL of each of the concentrations of the basil extract (100, 50, 25, 10 and 0%) before placing a 50-µL bacterial suspension. Fifteen holes were used to represent treatment with basil. The microtiter plate was incubated at 37 °C for 24 h. Samples inside the three holes (replicates) were obtained and pooled per collection intervals (set at 0, 3 and 6 h of exposure to each treatment). These

were subjected to serial dilution as described above (evaluation of bacterial counts) before determining the colony-forming units of test bacterium recovered after exposure to basil at each collection time. The preceding procedures were repeated to determine the test bacteria's recovery counts post-exposure to the different concentrations of table salt (100, 50, 25, 10 and 0%).

2.7 Data Gathering and Statistical Analysis

All data on measurements of the zones of inhibition related to applying the different concentrations of basil and table salt were gathered and expressed as means. Mean data on recovery counts of bacteria were also computed and expressed as means. Mean data on the zones of inhibition and bacterial recovery counts were compared statistically using the least significant difference. P-values that are less than 0.05 were considered significant.

3. Results and Discussion

Evaluation of the antibacterial effect of basil extract and table salt was undertaken as a basis for their selection as alternative disinfectants. In testing the efficacy of candidate disinfectants, their ability to inhibit the growth of bacteria was an important parameter.

Table 1 summarizes the diameters of the zones of inhibition produced by the disinfectants to the test bacterium (*S. aureus*). Results showed that the candidate disinfectants (basil extract and table salt) and ethanol (positive control) induced a larger diameter of the inhibition zones at the test bacterium when applied at 100%, compared with the 50% concentration.

The data also showed that at 100 and 50% concentrations, basil extract and ethanol were comparably better than the table salt at the same concentrations as a disinfectant ($p = 0.036$). Statistical analysis showed significant differences ($p < 0.05$) in the diameters of the zones of inhibition caused by the candidate disinfectants on the test bacterium as an effect of disinfectant concentration. Statistical analysis also demonstrated significant differences ($p < 0.05$) in the diameters of the zones of inhibition as an effect of disinfectant treatment. Based on the results of the study, it was demonstrated that the MICs of table salt and basil extract were 50%.

Table 1. Measurements of the zones of inhibition (mm) on test bacterium (*S. aureus)* related to the application of different treatments

Treatments	Concentrations (%)				
	100	50	25	10	0
Table salt	17.65 (±0.05)$^{a, y}$	14.03 (±0.04)$^{b, y}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$
Basil extract	20.48 (±0.02)$^{a, x}$	17.67 (±0.03)$^{b, x}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$
Ethanol (positive control)	20.98 (±0.07)$^{a, x}$	15.87 (±0.12)$^{b, x}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$
Distilled water (negative control)	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$	0.00 (±0.00)$^{c, z}$

Values represent mean (±SD) diameter of the zones of inhibition (mm) on test bacterium (*S. aureus*) related to the application of different treatments; 0 means no zone of inhibition. a, b, c (significant differences within treatments at specified concentration ($p < 0.05$); x, y, z (significant differences across treatments at specified concentrations ($p < 0.05$).

The efficacy of table salt and basil may be attributed to their inherent characteristic. Sodium chloride, also known as table salt, belongs to the group of crystalline minerals. It is an ionic compound formed from the reaction of the metallic element sodium and the well-known toxic element chlorine. Salt is one of the oldest and explicitly one of the most commonly used substances for food preservation, treating wounds, or even for many industrial purposes. Some of the earliest evidence of salt processing dates back to around 8,000 years ago (Hall, 2016). Relative to bacterial formation and aggregation, bacteria require a water-rich environment in order to survive and multiply. In turn, the addition of salt penetrates intracellular components of bacterial cells. A high salt concentration within bacteria induces dehydration or desiccation and prevents bacteria from reproducing. By creating a hypertonic high solute concentration outside the cell, compared with the inside environment, the cell shrinks as the water flows out of the bacterial cell (dehydration). Deprivation of sufficient moisture by the action of salt kills the bacteria (Hall, 2016).

The antibacterial effect of the candidate disinfectants was further evaluated in terms of the recovery counts of the bacteria post-exposure from each of the disinfectants. Table 2 shows that the application of table salt had no effect in reducing bacterial count as early as 3 h. Still, its antibacterial effect was enhanced at 6 h, as shown by the significant reduction in the number of bacteria. Conversely, this was not the trend in the treatment with basil extract, where a substantial reduction in bacterial number was only noted at 3 h that was not sustained thereafter. Ethanol exerted a significant reduction in bacterial count noted only in the first 3 h, contrary to the continuous

proliferation of bacteria in distilled water (negative control) as exposure time was extended.

Table 2. Recovery counts of bacteria as a measure of the antimicrobial effect of the potential disinfectants

Treatments	Time interval (h) 0 (x 10^3)	3 (x 10^3)	6 (x 10^3)
Table salt (50% MIC)		1.50 (±0.10)$^{a, xy}$	1.30 (±0.02)$^{b, z}$
Basil extract (50% MIC)		0.70 (±0.02)$^{b, z}$	1.58 (±0.02)$^{a, yz}$
Ethanol (positive control, 50% MIC)	1.35 (±0.53)	1.00 (±0.10)^{b,y}	2.37 (±0.02)$^{a, y}$
Distilled water (negative control)		1.67 (±0.02)$^{b, x}$	3,420.0(±10.0)$^{a, x}$

Values represent mean (±SD) recovery counts of *S. aureus* (CFU/mL) as test organism to evaluate the antibacterial effect of the potential disinfectants at a minimum inhibitory concentration of 50%; a, b, c (significant differences in recovery counts of bacteria with reference to count at the start of the experiment as an effect of the duration of bacterial exposure to the disinfectants ($p < 0.05$); x, y, z (significant differences in recovery counts of bacteria as an effect of the application of the different disinfectants ($p < 0.05$).

Data also highlighted bacterial recovery counts as a significant measure of the effect of treatments. The disinfectant effect of basil extract on the test bacterium was instantaneous as shown by the significant reduction of recovery counts as early as 3 h compared with the effects of table salt and ethanol. Contrary to the short-lived basil extract-related effect on reduced bacterial recovery count, a table salt-related disinfectant effect marked by the significant reduction of bacterial recovery counts was demonstrated at 6 h. There was also an apparent increase in the recovery counts of the test organism relative to the application of ethanol (positive control) observed at 6 h.

Basil is a leafy herb that is commonly found everywhere and its therapeutic application has been widespread in Asia. It is used as a medicine to treat different ailments. Basil has the properties of preventing bacteria from adhering to surfaces. Further, studies have shown that basil exhibits antibacterial properties against *Salmonella sp.*, *Clostridium perfringens*, *Escherichia coli* and *Campylobacter jejunii* (Wannissorn *et al.*, 2005). Furthermore, essential oils of basil also showed effects against *Staphylococcus*, *Enterococcus* and *Pseudomonas* genera (Opalchenova and Obreshkova, 2003). The antibacterial property of basil is reportedly attributed to linalool, a chemical compound that gives the pleasant scent of herbs and the active compound of basil (Araújo-Silva *et al.*, 2016). The role of linalool in basil as an antibacterial was reportedly undertaken in *Pseudomonas aeruginosa* by Liu *et al.* (2020), who evaluated the minimum inhibitory concentration (431 μg/mL MIC) and minimum

bactericide concentration (862 μg/mL MBC) of linalool. Other parameters such as respiratory chain dehydrogenase, membrane potential, cell membrane permeability, and monitoring of the growth curve in the bacterium were also tested. The growth curve assay revealed the inhibition of *P. aeruginosa* growth, while SEM showed that linalool can disrupt bacterial cell normal morphology. The respiratory chain was reportedly damaged by respiratory chain dehydrogenase determined at an absorbance of 490 nm, also supporting the potential of linalool as a food preservative, reducing bacterial contamination in foods.

Defining the antibacterial activity of commonly used preparations as potential disinfectants could effectively minimize, if not eliminate bacteria, in infected tissues. Identifying the preparations as alternative disinfectants would be a practical method of lessening tissue destruction and preventing contamination that can complicate existing infections. An assessment of the potency of the test preparations based on their concentrations could provide a substantial basis for their selection and application as potential disinfectants.

The efficacy of candidate disinfectants required an in-vitro evaluation of the reaction of a test bacterium to different concentrations of disinfectants. One of the findings of this experiment showed that the disinfectant effects of basil extract and ethanol were dependent on disinfectant concentrations which were best exerted at 100 and 50% concentrations. Another observation drawn from the results of this experiment demonstrated the influence of exposure time on disinfectant action. It has to be emphasized that potential disinfectants such as table salt and basil extracts possess inhibitory effects distinct from their chemical compositions. This was shown by the sustained inhibitory effects of table salt on the test organism *S. aureus* that contributed to lower recovery counts after exposure to salt solution for 6 h which were not exerted by the basil extract on the test organism given the same duration of exposure. The rapid vaporization of ethanol that can be linked to its chemical composition also had a different effect on the reduced inhibition of bacterial growth as a disinfectant action of ethanol.

4. Conclusion and Recommendation

Pathogens found in the environment are essentially used in screening the potential of naturally occurring substances such as table salt and basil extract as alternative disinfectants. In this study, basic microbiological and biochemical techniques are applied in the cultivation and recovery of a test

organism and preparation of required concentrations of treatments. The results demonstrated the effectiveness of using naturally obtained products such as basil and table salt as alternative disinfectants against common pathogens that can be found and acquired in the environment. Data generated from this study have provided a basis for evaluating these products as alternative disinfectants that can be used in remote areas where immediate medical intervention is limited. Refining the protocols for basil and table salt preparation as disinfectants may be considered for future applications.

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