

ANTIBACTERIAL ACTIVITY OF USED COOKING OIL-BASED LIQUID SOAP AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

Rokhmad Fajar Setiyo^{1*}, Zahra Anggita Pratiwi¹, Arindra Nirbaya¹, Puji Putri Isnaini²

¹Department of Biology, Universitas Negeri Malang

²Department of Applied Science, Universitas Negeri Malang

E-mail: fajar.setiyo@um.ac.id^{1*}, zahra.anggita.fmipa@um.ac.id¹, arindra.nirbaya.fmipa@um.ac.id¹,
puji.putri.isnaini@um.ac.id²

*Author Correspondence : fajar.setiyo@um.ac.id

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Abstract

Used cooking oil can be turned into liquid soap by saponification, but soap made only from oil and KOH contains no added antibacterial agent. This study aimed to analyze the antibacterial activity of used cooking oil-based liquid soap with added lemongrass (*Cymbopogon citratus*) against *Escherichia coli* and *Staphylococcus aureus*. A laboratory experiment with a completely randomized design used four formulations: F0 (no lemongrass), F1 (1%), F2 (2%), and F3 (3%). Activity was tested by well diffusion, with chloramphenicol and distilled water as positive and negative controls and pure lemongrass essential oil as a comparison; inhibition zones (mm) were measured in triplicate. All formulations inhibited *E. coli*, with mean zones increasing from 4.95 ± 0.80 mm (F0, weak) to 6.13 ± 0.5 mm (F3, moderate), indicating a dose-response pattern. Against *S. aureus*, all formulations showed only weak activity (3.03–3.83 mm) without a clear concentration trend. The soap base alone showed some inhibition, and responses differed between bacteria, possibly because of interactions between surfactants and lemongrass essential oil in the soap matrix.

Keywords: antibacterial activity, *Escherichia coli*, lemongrass, liquid soap, used cooking oil

INTRODUCTION

Practicum activities at the Dietetics and Culinary Laboratory, S1 Nutrition Study Program, State University of Malang, routinely produce used cooking oil (used cooking oil) in large quantities. So far, the management has not been optimal, even though the oil has the potential to be processed into raw materials for liquid dish soap through the saponification process. Although commercial dish soap products are available on the market with antibacterial claims, liquid soap formulations made from recycled cooking oil, which are generally only based on the reaction of KOH with used oil, do not necessarily have the same ability to inhibit microbial growth, because they are not accompanied by the addition of antibacterial active ingredients. In fact, this recycled soap is projected to be a substitute for the need for daily dish soap in the laboratory, so its antibacterial function needs to be ensured to be equal to or close to commercial products that are already in circulation.

This need is relevant considering that cookware and tableware are one of the media that are at risk of becoming a source of microbiological contamination if the washing and sanitation process is inadequate. *Escherichia coli* and *Staphylococcus aureus* bacteria are widely known as indicator bacteria used to assess the success of the implementation of food sanitation, as stipulated in the provisions of sanitary hygiene for restaurants and restaurants which require tableware and food to be free of both bacteria (Saadah & Hadi, 2026). According to the Ministry of Health Regulation (2011) No. 1096, the equipment must be free of *Escherichia coli* (*E. coli*) and other germs. Various field studies also show that *E. coli* is often used as the main indicator of fecal contamination and poor hygiene practices in tableware, while washing aids such as dishwashing sponges have the potential to become breeding grounds for pathogenic bacteria including *E. coli* if the cleaning and storage conditions are not appropriate (Pribadi & Khusna, 2026). These findings indicate that cleanliness alone is not enough to ensure the microbiological safety of equipment in the dietetic and culinary laboratory environment, which in fact demands high sanitary standards because it is directly related to food processing and nutrition.

This condition opens up opportunities for the development of liquid soap based on used cooking oil not only as a solution for waste utilization, but also as a cleaning product with an additional function in the form of antibacterial resistance. One of the natural ingredients that has the potential to be added to provide antibacterial properties to soap is lemongrass (*Cymbopogon citratus*), a spice plant that is easy to obtain and has been widely studied for its essential oil content. Several studies have specifically tested the effectiveness of lemongrass extracts and essential oils against *E. coli* and *S. aureus*. (Rita et al., 2018), for example, reported that a solid soap formulation made from kitchen lemongrass essential oil has been proven to have antibacterial activity against both bacterial species. Other studies have also shown that citronella essential oil is able to produce a strong category of barrier zone, ranging from 10–20 mm, against *E. coli* and *S. aureus* at an optimum concentration of 30% (Hermawati et al., 2023). This antibacterial effect is generally associated with the content of active compounds such as citronelal and geraniol in lemongrass essential oil, which are able to damage the structure of bacterial cell membranes.

Based on this description, the addition of lemongrass extract to used cooking oil-based liquid soap is suspected to provide added value in the form of antibacterial activity, as well as answer the need for more functional cleaning for Dietetic and Culinary Laboratory equipment. However, the effectiveness of the antibacterial activity of liquid soap products specifically against *E. coli* and *S. aureus* is not known empirically, so it is necessary to conduct separate tests to ensure that the final formulation of the product really has an inhibition against the two sanitary indicator bacteria. This study aims to analyze the antibacterial activity of liquid soap made from used cooking oil with the addition of lemongrass extract against the growth of *E. coli* and *S. aureus*.

LITERATURE REVIEW

Used Cooking Oil: Degradation and Management Problems

Cooking oil that is used repeatedly for deep-fat frying undergoes a series of chemical changes. Exposure to high temperature, moisture, and oxygen triggers hydrolysis, oxidation, and polymerization of the triacylglycerols. Hydrolysis releases free fatty acids, whereas oxidation and polymerization generate polar and high-molecular-weight products that lower oil quality (Choe & Min, 2007). Repeated heating also alters the fatty acid composition of plant-based cooking oils (Szabo et al., 2022). Once the oil is no longer acceptable for cooking, it becomes a waste stream. Used cooking oil is regarded as potentially carcinogenic when it continues to be consumed after repeated use, and when it is discharged into the environment it can damage soil structure by impeding the movement of water through soil pores (Setiawan et al., 2025). These problems make non-food valorization routes, including soap production, a practical option for institutions that generate used oil routinely, such as food-processing and culinary laboratories.

Liquid Soap from Used Cooking Oil

Liquid soap is produced by saponification, in which oils or fats react with an alkali; potassium hydroxide (KOH) is the alkali used for liquid formulations (Tutik et al., 2022). Used cooking oil has been explored as a raw material for this purpose (Bakhri et al., 2023). The physical and chemical quality of liquid soap is commonly evaluated against the Indonesian national standard using parameters such as pH (8–11), foam height, and density (Tutik et al., 2022; Widyaningsih et al., 2018).

Reports on the intrinsic antibacterial activity of soap made from used cooking oil are inconsistent. Bakhri et al. (2023) reported that liquid soaps made from charcoal-purified used cooking oil and KOH inhibited *Staphylococcus aureus* strongly, with the best result at an oil-to-KOH ratio of 375:125 (g), without any plant-derived additive. In contrast, a used-oil soap containing butterfly pea flower (*Clitoria ternatea* L.) did not inhibit *S. aureus* at all (Putri et al., 2024), and work on used-oil antiseptic soaps enriched with aloe vera and betel leaf indicated that the most active formulation differs among test bacteria (Nisa, 2023). A soap base made from olive oil without any plant extract likewise produced no inhibition zone against *S. aureus* or *Escherichia coli* (Tutik et al., 2022). Differences in oil purification, oil-to-alkali ratio, and additives across these studies make direct comparison difficult.

Two lines of evidence suggest why a soap base might, or might not, show activity. Free fatty acids can inhibit or kill bacteria, mainly by disrupting the cell membrane, the electron transport chain, and oxidative phosphorylation (Desbois & Smith, 2010), so residual fatty acids in a soap base could contribute to its activity, although this has not been demonstrated for used-oil soaps in the studies reviewed here. On the other hand, adding an antibacterial component to a soap formulated according to the national standard can raise activity markedly: a nyamplung (*Calophyllum inophyllum* L.) oil liquid soap containing 0.2% *Curcuma heyneana* fraction and 2.5% fragrance produced a 14.92 mm inhibition zone against *S. aureus* (Widyaningsih et al., 2018). Interest in natural antibacterial additives is also driven by concern over synthetic agents such as triclosan, for which reduced bacterial susceptibility,

including possible cross-resistance to clinically important antimicrobials, has been documented (Tutik et al., 2022; Yazdankhah et al., 2006).

Lemongrass (*Cymbopogon citratus*): Constituents and Antibacterial Activity

Lemongrass (*C. citratus*) is a herb widely used in tropical countries. Its essential oil contains terpenes, alcohols, ketones, aldehydes, and esters, notably citral α and β (geranial and neral), nerol, geraniol, citronellal, myrcene, and geranyl acetate, while the plant material also contains flavonoids and phenolic compounds (Shah et al., 2011). Citral-type aldehydes dominate: in one GC-MS analysis, citral (26.73%), neral (18.57%), and β -myrcene (9.30%) were the main components (Yang et al., 2025), and composition varies with geographical origin (Shah et al., 2011). Citronellal, geraniol, and citronellol, in contrast, are the characteristic constituents of citronella oil from *C. nardus* (Olivisvelos et al., 2023), so the two species should not be treated interchangeably when interpreting antibacterial data.

Antibacterial activity of lemongrass oil has been reported against both of the sanitary indicator bacteria used in this study. The essential oil of *C. citratus* showed antibacterial and antibiotic-modulating activity against standard and multidrug-resistant strains of *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Tintino et al., 2015), and a GC-MS-characterized oil produced inhibition zones of 25.43 ± 0.24 mm against *S. aureus* and 23.54 ± 0.67 mm against *E. coli* (Yang et al., 2025). Lemongrass oil has also been reported to be effective against aerobic and anaerobic organisms (Ajjai et al., 2021), and Hermawati et al., (2023) reported inhibition zones of 10–20 mm against *E. coli* and *S. aureus* at an optimum concentration of 30%. Activity varies with the plant source, harvest, and extraction method (Shendurse et al., 2021), and between plant fractions such as crude extract and essential oil.

Activity has also been demonstrated in soap formulations. A solid soap containing kitchen lemongrass essential oil inhibited both *E. coli* and *S. aureus* (Rita et al., 2018). In an olive-oil-based liquid soap, ethanolic lemongrass extract at 10% gave inhibition zones of 7.25 mm against *S. aureus* and 8.25 mm against *E. coli*, both in the moderate category, and 9.00 mm and 9.50 mm at 15%, whereas the extract-free base gave no zone (Tutik et al., 2022). The same study showed a concentration-dependent response for the extract alone, with zones of 11.41 mm (*S. aureus*) and 16.66 mm (*E. coli*) at 100%. Notably, the zones of the 10% extract alone (7.25 and 8.33 mm) were almost identical to those of the soap containing 10% extract, which led the authors to conclude that the soap base did not measurably attenuate the extract's activity (Tutik et al., 2022). This contrasts with the view that surfactant and micellar components of a soap matrix may reduce the release of active compounds, and it shows that the matrix effect has not been settled.

METHOD

This study is a laboratory experimental research with a Complete Random Design design. The research treatment was in the form of variations in the concentration of lemongrass extract added to the formulation of used cooking oil-based liquid soap. The lemongrass extract added to the soap is lemongrass essential oil. The study consisted of several treatment groups, namely formulations without the addition of lemongrass extract as treatment control and formulations with the addition of lemongrass extract at concentrations of 1%, 2%, and 3%. Each formulation was tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using the well diffusion method. Each treatment was carried out with several repetitions. The observed parameter was the diameter of the inhibition zone formed around the wells after incubation, expressed in millimeters (mm). The measurement data were then statistically analyzed to determine the effect of variations in the concentration of lemongrass extract on the antibacterial activity of liquid soap preparations.

Time and Place

The research was carried out in July-August 2026. The antibacterial activity testing activity was carried out at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Malang.

Tools and Materials

The tools used include autoclaves, laminar air flow, analytical scales, measuring cups, measuring flasks, Erlenmeyer flasks, test tubes, test tube racks, petri dishes, mixing rods, magnetic stirrer hotplates, mortars and pestles, micropipettes, yellow tips, dropper pipettes, vortex mixers, ose needles, bunsen burners, tweezers, disc paper, calipers, incubators, and refrigerators. Materials used are *Escherichia coli* bacteria, *Staphylococcus aureus* bacteria, Aquades, NA media, Cloramphenicol

Prodedur

The antibacterial activity test was carried out using the well diffusion method. Suspension of *Escherichia coli* and *Staphylococcus aureus* bacteria with a standardized density was inoculated evenly on the surface of the media to use the dispersion method. Next, the medium was made with a mixture of uniform diameter. Each mixture was then filled with a sample of liquid soap according to the treatment formulation, while the negative control was filled with aquades and the positive control used Cloramphenicol as a comparative antibacterial. The media was then incubated under conditions suitable for bacterial growth. After the incubation period was completed, the antibacterial activity was determined based on the diameter of the inhibition zone formed around the wells by measuring them using calipers in millimeters (mm). The test is carried out with several iterations to obtain data that can be used in the analysis of the antibacterial activity of each formulation.

RESULTS AND DISCUSSION

Antibacterial Activity of Liquid Soap against *Escherichia coli*

The test results showed that all used cooking oil-based liquid soap formulas (F0–F3) were able to inhibit the growth of *E. coli*, with the diameter of the inhibition zone gradually increasing from F0 (4.95 ± 0.80 mm; weak category) to F3 (6.13 ± 0.5 mm; moderate category). The results of the activity of used cooking soap against *E. coli* bacteria are presented in table 1.1. This consistent pattern of increase with the addition of lemongrass essential concentrations (0%, 1%, 2%, 3%) indicates a dose-to-response relationship, in line with the findings of Shendurse et al., (2021) who reported that lemongrass essential oil increases resistance to pathogens as the concentration is used.

Tabel 1.1 Antibacterial activity of used cooking soap against *E. coli* bacteria

No	Treatment	Repetition (mm)			Mean	STD Dev	Description
		P1	P2	P3			
1	Cloramphenicol (K+)	4.08	7.28	8.82	6.73 ± 1.97	1.97	Moderate
2	Lemongrass essential	9.75	10.20	10.55	10.17 ± 0.33	0.33	Strong
3	F0 (0%)	6.00	4.80	4.05	4.95 ± 0.80	0.80	Weak
4	F1 (1%)	4.78	5.60	5.30	5.23 ± 0.34	0.34	Moderate
5	F2 (2%)	6.38	6.00	4.50	5.63 ± 0.81	0.81	Moderate
6	F3 (3%)	5.60	6.00	6.80	6.13 ± 0.5	0.50	Moderate
7	Aquades (K-)	0.00	0.00	0.00	0.00	0.00	None

Source : Research Results Data

When compared to the positive control of pure lemongrass essential oil which resulted in an inhibition zone of 10.17 ± 0.33 mm (strong category), the entire soap formula showed lower inhibition. This is suspected because the concentration of lemongrass essential oil in the soap formula was much lower compared to the positive control. In addition, the interaction of active compounds with soap-based components, such as surfactants, water, and other saponification products, could potentially affect its antibacterial activity. These conditions allow some of the antibacterial potential of lemongrass essential oil not to be optimally detected through in vitro testing.

The antibacterial activity of lemongrass essential is generally associated with the content of monoterpenoid compounds such as citral (a mixture of neral and geranial), geraniol, and citronelal sitronelal (Shendurse et al., 2021). These lipophilic compounds work by partitioning into the lipid layer of the cell membrane, increasing membrane permeability, causing the leakage of ions and intracellular components, and disrupting the enzyme system involved in cell respiration (Burt, 2004). Some studies have shown that lemongrass essential oil tends to be more effective against gram-positive bacteria than gram-negative ones (Naik et al., 2010; Shendurse et al., 2021). So in contrast to the results of this study, where the barrier zone against *E. coli* (Gram negative) is actually greater than against *S. aureus* (Gram positive). This is suspected to be caused by the interaction between lemongrass essential and the soap surfactant matrix. Saponified fatty acids are suspected to act as permeabilizer agents that weaken the lipopolysaccharide layer on the outer membrane of *E. coli*, thereby facilitating the penetration of active compounds of lemongrass essential which under normal conditions tend to be inhibited by the membrane (Burt, 2004).

Antibacterial Activity of Liquid Soap against *Staphylococcus aureus*

The test results showed that the entire formula (F0–F3) showed only weak category activity against *S. aureus* bacteria with an average of 3.03–3.83 mm. In contrast to the results in *E. coli*, the results for *S. aureus* did not indicate a clear dose-to-response relationship. The results of the activity of used cooking soap against *S. aureus* bacteria are presented in table 1.2.

Table 1.2 Antibacterial activity of used cooking soap against *S. aureus*

No	Treatment	Repetition(mm)			Mean	Std Dev	Keterangan
		P1	P2	P3			
1	Cloramphenicol (K+)	10.10	10.40	10.50	10.33 ± 0.17	0.17	Strong
2	Atsiri Sereh	4.90	6.10	5.50	5.50 ± 0.49	0.49	Moderate
3	F0 (0%)	3.90	3.50	4.10	3.83 ± 0.25	0.25	Weak
4	F1 (1%)	3.00	2.60	3.50	3.03 ± 0.37	0.37	Weak
5	F2 (2%)	3.30	3.00	3.20	3.17 ± 0.12	0.12	Weak
6	F3 (3%)	4.20	3.50	3.50	3.73 ± 0.33	0.33	Weak
7	Aquades (K-)	0.00	0.00	0.00	0.00	0.00	None

Source : Research Results Data

Based on the results in table 1.2. shows an irlinear pattern. This is suspected because the addition of lemongrass essential in the range of 1–3% has not had a large enough effect to be detected consistently in the agar diffusion method against this bacteria. This is supported by the findings of other research on used cooking oil-based soaps, research on used cooking soap with telang flowers. Putri et al., (2024) reported that the soap was completely unable to inhibit *S. aureus* and was included in the resistant category, this defines that the inhibition of used cooking oil-based soaps against *S. aureus* tends to be low and unstable compared to Gram-negative bacteria.

Comparisons with controls of pure lemongrass essential also support this interpretation. Pure lemongrass essential against *S. aureus* produced only a moderate category inhibition zone (5.50 ± 0.49 mm), much lower than its activity against *E. coli* (10.17 mm; strong category) in this study. This condition actually contradicts the general consensus in the lemongrass essential literature, which tends to report stronger activity against Gram-positive bacteria (Ajjai et al., 2021; Naik et al., 2010; Shendurse et al., 2021). This difference is thought to indicate that there are variations in the chemical composition of lemongrass essential between plant sources and extraction methods, in addition to that the content of citral, geraniol, and citronelal can vary greatly depending on geographical origin, harvest age, and distillation method so that the antibacterial activity of lemongrass essential cannot be generalized without considering the characteristics of the specific sample used (Shendurse et al., 2021).

Based on the characteristics of the bacteria, the low and inconsistent response of *S. aureus* is thought to be related to the structure of its cell wall. Although the general literature states that Gram-positive bacteria are generally more susceptible to hydrophobic compounds because they do not have a lipopolysaccharide outer membrane (Burt, 2004), the thick, cross-linked peptidoglycan layer of *S. aureus* can still be a diffusion barrier for larger molecule-sized active compounds or for micellar complexes formed from surfactant-essential interactions in soap formulas. Thus, it can be interpreted that the limitation of diffusion of active compounds into the media in testing for *S. aureus* is thought to be more influenced by the characteristics of the soap matrix used, rather than solely by the activity of the lemongrass essential oil itself.

Differences in Antibacterial Activity against *E. coli* and *S. aureus*

The overall soap formula and control of lemongrass essential oils in this study showed a larger inhibitory zone against *E. coli* than *S. aureus*. This result is in contrast to the pattern shown by the positive control of chloramphenicol, which resulted in a larger inhibitory zone against *S. aureus* (10.33 mm) than *E. coli* (6.73 mm). These findings are also not in line with the general trend of antibacterial activity of essential oils reported in the literature (Burt, 2004; Naik et al., 2010). This difference is one of the important findings of this study because it shows that the antibacterial activity of soap formulas containing lemongrass essential oil cannot always be estimated based on the characteristics of each component separately.

Theoretically, *E. coli* has an outer membrane containing lipopolysaccharides (LPS) and serves as a barrier to the entry of various compounds, especially hydrophobic compounds such as lemongrass essential oil components (Burt, 2004). However, in the soap formula used in this study, the presence of surfactants is thought to affect the structure of the outer membrane. The interaction of surfactants with the lipopolysaccharide layer has the potential to

increase membrane permeability, thereby facilitating the entry of active compounds into bacterial cells. This mechanism may be one of the explanations why *E. coli* exhibits a greater inhibitory response to soap formulas than *S. aureus*. Although *S. aureus* does not have an outer membrane and only has a thick layer of peptidoglycan, these conditions do not necessarily make the active compound easier to enter cells. Thus, the effect of surfactants on the two bacteria is likely to be different and may contribute to the differences in inhibition produced.

However, the explanation is still hypothetical and cannot be ascertained based on the results of the inhibition zone test. Further research, for example through observations using electron microscopy or intracellular component leak testing, is needed to find out whether surfactants actually play a role in increasing the permeability of bacterial membranes. This hypothesis may be one of the bases to explain the difference in the pattern of antibacterial activity of soap formulas in this study compared to the reported tendency in single-cellum essential oil testing.

The Effect of Adding Lemongrass Essential in Soap Formulations

The F0 formula as a basic control showed that the soap base of used cooking oil had antibacterial activity against both test bacteria, even without the addition of lemongrass essential oil. These results are in line with previous studies on used cooking oil-based liquid soaps which also reported bacterial inhibition without the addition of active ingredients from plants (Bakhri et al., 2025). The addition of lemongrass essential oil to formulas F1–F3 further gave different responses to the two bacteria. Resistance to *E. coli* increased consistently with the addition of lemongrass essential, while in *S. aureus* the increase did not show a clear pattern.

These differences in responses suggest that the effectiveness of adding natural active ingredients to the soap base can be influenced by the type of bacteria being tested (bacteria-dependent). This is also in line with research on used cooking oil-based antiseptic soaps with the addition of various natural extracts, such as betel leaves, aloe vera, and telang flowers, which suggests that formulas with optimal antibacterial activity may differ depending on the type of test bacteria (Nisa, 2023). In this study, the concentration of lemongrass essential oil used, which was 1–3%, was relatively lower than the concentration of pure essential in the positive control. This concentration was thought to be sufficient to produce a measurable inhibitory effect against *E. coli*, but it did not provide the same response to *S. aureus*. Therefore, the use of lemongrass essential concentrations higher than 3% can be considered in future studies to find out whether increased concentrations can increase resistance to *S. aureus*.

In addition to the concentration of active ingredients, the characteristics of the soap matrix are also thought to affect the results of antibacterial tests. Volatile compounds in lemongrass essential oils, such as citral, can undergo changes in solubility and stability when in surfactant-based systems. In soap formulas, essential compounds have the potential to disperse or become trapped in the micellar structure, so their release into the agar medium may be different compared to essential oils in their pure form. These differences in release and diffusion rates can affect the size of the barrier zones formed. Thus, the results of tests using the diffusion method do not always directly describe the amount of active compounds contained in the formula as well as their overall antibacterial potential.

CONCLUSION

Liquid soap made from used cooking oil with added lemongrass showed antibacterial activity against both *E. coli* and *S. aureus*, but only at weak to moderate levels. Against *E. coli*, inhibition rose with lemongrass concentration, from 4.95 mm (0%) to 6.13 mm (3%). Against *S. aureus*, all formulations stayed in the weak category (3.03–3.83 mm) with no consistent dose-response. Unlike most of the literature, the soap formulas and pure lemongrass essential oil were more active against Gram-negative *E. coli* than Gram-positive *S. aureus*. This is tentatively explained by surfactants increasing outer membrane permeability in *E. coli* and by the soap matrix limiting diffusion of active compounds, and it remains a hypothesis. The soap base (F0) also showed some inhibition on its own, so the benefit of adding lemongrass depends on the bacterial species. No formulation matched pure lemongrass essential oil against *E. coli* (10.17 mm) or chloramphenicol against *S. aureus* (10.33 mm). The product therefore cannot yet be considered equivalent to a strong antibacterial cleaner. Future work should test lemongrass concentrations above 3%, characterize the essential oil composition, evaluate the physical and chemical quality of the soap (pH, foam height, density), compare it with commercial dish soap, and verify the surfactant-permeability hypothesis using electron microscopy or cellular leakage assays.

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