CREATIVE SCIENCE

Journal home page: csj.snru.ac.th

Comparative effects of graphene oxide and sodium alginate on filter paper against *S. aureus* and *E. coli*

Wachiraporn Choopan, Navapadol Kittiamornkul*

Biomedical Engineering Department, College of Health Sciences, Christian University of Thailand, Nakhon Pathom, 73000 Thailand.

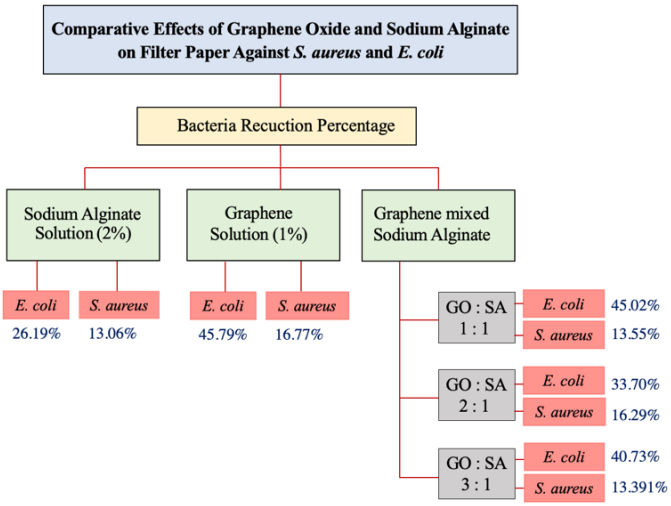
***Corresponding Author:** metalicaed@hotmail.com

DOI: 10.55674/cs.v17i2.258702

Received: 8 October 2024; **Revised:** 3 February 2025; **Accepted:** 19 February 2025; **Available online:** 25 February 2025

Abstract

This research investigated the efficacy of graphene oxide (GO) and sodium alginate (SA) solutions in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria coated on filter papers. The study compared the antibacterial properties of GO solution alone, GO mixed with SA at different ratios, and a control group. In the experiment, *S. aureus* and *E. coli* bacteria were cultured. The coated filter papers were soaked in the aforementioned bacteria and dried in a sterile cabinet. Finally, the dilutions were performed, sequentially. The results show that filter paper coated with 1% GO solution gave the best antibacterial activity against *E. coli* which had 45.79% reduction, but it did not significantly better than the paper coated with GO mixed with SA (ratio 1:1) which had 45.02% reduction. All coated paper samples had low antibacterial activity against *S. aureus* significantly (p-value < 0.05). GO solution normally lacks adhesion properties but mixing it with SA provided adhesion while maintaining antibacterial activity. This research can be applied to antimicrobial paper, textile industries, prosthesis organs and canvas printing. Further research is needed to explore human applications' long-term effectiveness and safety.



Keyword: properties; graphene oxide; sodium alginate; *Staphylococcus aureus*; *Escherichia coli*; bacterial culture

1. Introduction

Paper is a highly popular material for packaging due to its ease of biodegradation compared to other synthetic materials. More than 2.5 million tons of paper [1 - 2] are used worldwide to create packaging for food. Additionally, various forms of paper packaging have been employed for materials, equipment, and daily-life items to reduce direct manual contact, which can be a source of various contaminants. Developing paper with antimicrobial properties has led to the concept

of active packaging, a future-oriented packaging trend. This has become a significant area of scientific research and development as it adds value to various industries, such as the food industry, by preserving food quality, extending shelf life, maintaining color, flavor, and nutritional value [3 - 4], and enhancing convenience during transportation and distribution. It's particularly suitable for businesses with shorter food shelf lives, reducing costs related to food loss and returns caused by product spoilage before reaching the consumer.

Research on developing antimicrobial properties in paper dates back to 1985 [5], primarily for medical applications, using various antimicrobial agents like halogenated aromatic nitriles, dichlorophene, vanillin, vanillin mixed with cationic surfactants, clove oil, and guava leaf extracts. From the study, there were research works related to the development of paper with antibacterial properties. In 2016, the antibacterial effectiveness of vanillin-coated rigid paper against three types of bacteria: *Bacillus* cereus, *Staphylococcus aureus*, and *Escherichia coli* was proposed. They experimented with different concentrations (15%, 20%, 25%, 30%, 35%, and 40% w/v) of vanillin in ethanol to coat the paper. The antibacterial effectiveness was evaluated using the agar-disc diffusion method. The study found that as the concentration of vanillin increased, the antibacterial effectiveness also increased. The paper with the highest effectiveness in inhibiting *B. cereus* was followed by *S. aureus* and *E. coli* in that order [6]. In addition, the effectiveness of coated paper in inhibiting the growth of *Colletotrichum gloeosporioides*, the fungus responsible for anthracnose disease in post-harvest mangoes was presented. The evaluation of the coating paper's effectiveness was done using 1.5% (w/v) concentrated chitosan-coated paper and chitosan-coated paper mixed with varying concentrations (0.5%, 1%, 2%, and 4% w/v) of vanillin. A dual culture technique was used to assess the inhibition of *C. gloeosporioides* growth. The study found that chitosan-coated paper mixed with 1% (w/v) vanillin had the best effectiveness in inhibiting the growth of the fungus [7]. Furthermore, a sturdy paper revealing resistance against microbial growth by using a hydrophobic starch coating at concentrations of 5% and 8% (w/v) was proposed. This hydrophobic starch acted as a carrier substance, and canola oil was used as a component at concentrations of 5%, 10%, and 15% (w/v) relative to the weight of the starch. When tested for effectiveness against the growth of three types of bacteria, namely *B. cereus*, *E. coli*, and *S. aureus*, it was found that paper coated with 8% hydrophobic starch and 15% canola oil exhibited good inhibitory properties against all three types of bacteria [8]. Besides, paper combined with extracts from guava leaves that inhibited the *S. aureus* bacteria was presented. This paper was intended for use in food packaging [9]. In 2020, silver nanoparticles mixed with a coating agent to coat sugarcane bagasse paper were presented. The inhibitory effects on the growth of *Bacillus subtilis*, *S. aureus,* and *Staphylococcus epidermidis* were found to be similar between coated and uncoated sugarcane bagasse paper [10].

Many researchers are interested in exploring the potential of graphene oxide (GO), a new type of material and allotrope of carbon because of its favorable properties such as strength, good electrical conductivity, high flexibility, and the ability to be twisted and bent. It possesses antibacterial properties and is non-toxic to the human body. Examples of using graphene for antibacterial purposes were presented in 2023. A GO solution with calcium lactate that had antimicrobial properties to create antimicrobial filter paper was presented. The results show that the GO solution had antibacterial properties, but the antimicrobial properties weakened when GO was mixed with calcium lactate solution [11].

GO is currently being utilized in various technological fields worldwide. It finds applications in disease detection devices such as sensors, nanoscale drug delivery systems in medicine, and electronics circuits, solar panels, and batteries. Many regions, including the United States, Europe, and Asia, are actively

researching graphene to adapt it to diverse industries. The continuous research efforts are directed towards making graphene more accessible and cost-effective, to cater to a wide range of industries and thereby promoting its widespread usage.

Graphene, a single-atom-thick sheet of carbon arranged in a honeycomb lattice shown in Fig. 1, was discovered in 2004 [12]. This groundbreaking discovery earned them the Nobel Prize in Physics in 2010 and sparked significant global interest in this new material. Each carbon atom in graphene bonds to three neighbors, resulting in antibacterial properties, strength and flexibility, high electrical conductivity, non-toxicity, and biodegradability. This makes graphene distinct from other carbon allotropes.

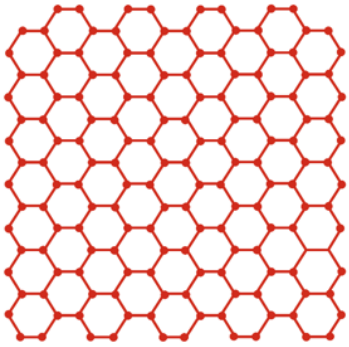


Fig. 1 Structure of carbon atoms of single-layered graphene

If multiple layers of graphene are stacked on top of each other, the resulting structure resembles a pencil's lead and is known as graphite, as shown in Fig. 2. The initial separation of graphene layers can be achieved using a simple technique called micromechanical cleavage or the "Scotch tape method." This involves placing a piece of adhesion tape onto a graphene sample and then peeling it off. The process is repeated by placing another piece of tape onto the peeled graphene layer and peeling that off as well. With each iteration, the thickness of the graphene layers on the tape thins gradually (Fig. 3), eventually yielding a single-atom-thick layer.

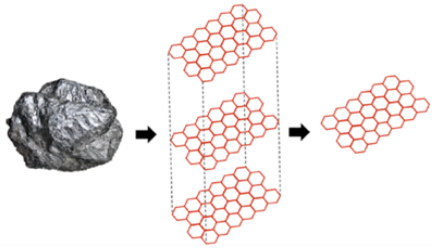


Fig. 2 Structure of graphene crystal

Fig. 3 Scotch tape method of graphene synthesis from graphite block [13]

Initially, the obtained graphene sheets were small, irregular flakes measuring less than 1 square millimeter. Scientists sought methods to produce larger graphene with controlled properties, optimizing structure and synthesis techniques for various industrial applications.

Graphene Oxide (GO) exhibits significant antibacterial properties due to its unique physicochemical characteristics. The inhibition of bacterial growth by GO can be attributed by physical disruption. o GO has sharp edges and a high surface area, which allow it to physically penetrate and damage bacterial cell membranes. This process, often referred to as "nanoknives," leads to cell lysis and ultimately bacterial death [14 - 15].

It is not only physical disruption, but also oxidative stress. GO generates reactive oxygen species (ROS) when in contact with bacterial cells. ROS can damage critical biomolecules such as DNA, proteins, and lipids, disrupting vital cellular processes and causing cell death [16 - 18].

The electrostatic interaction is one of the antibacterial mechanisms of GO. The negatively charged GO interacts with the positively charged components of bacterial cell walls, particularly in Gram-negative bacteria

like *E. coli*. This interaction leads to membrane destabilization, leakage of intracellular contents, and bacterial death [19 – 21].

In addition to graphene, certain substances also possess antibacterial properties. Sodium alginate ($NaC_6H_7O_6$) or SA, a hydrocolloid, thickens and gels food upon contact with calcium. Sodium alginate also has excellent adhesion properties due to its chemical structure containing hydroxyl and carboxyl groups. These properties allow it to adhere well to various surfaces. SA can be used in food stabilization, water treatment and energy storage components [22 - 24]. Because of its adhesion properties, sodium alginate is often used as a binder or to link different substances, particularly in systems requiring flexibility.

Since SA has antimicrobial and adhesion properties, it is suitable for blending with GO, which has low adhesion to flexible materials, in paper coating to create antimicrobial paper.

This article utilizes *S. aureus* and *E. coli*, commonly encountered bacteria, to test the effectiveness of the proposed antibacterial paper. *S. aureus*, a gram-positive bacterium, commonly found on the skin and mucous membranes. It can cause infections like skin abscesses, respiratory tract infections, and food poisoning [25 - 26].

E. coli, is a Gram-negative bacterium typically residing in the gut. While most strains are harmless, some can cause infections like diarrhea and urinary tract infections. Transmission often occurs through contaminated food or poor hygiene [27 - 28].

This research aims to compare the efficacy of GO and sodium alginate (SA) solutions that have antimicrobial properties in adhering to paper for studying its effectiveness in inhibiting *S. aureus* and *E. coli* bacteria. The filter paper was selected as a model in this study because of its simplicity, uniform structure, and wide use in scientific research for testing antimicrobial coatings. The uniformity of filter paper ensures consistent results when evaluating the efficacy of antimicrobial agents like the GO-SA coating. Additionally, its porous nature allows for effective absorption and interaction with bacterial cultures, making it an ideal substrate for this experiment. This approach allows for accurate measurement and analysis of the antibacterial properties of the coating, paving the way for further research on its application to other types of packaging materials in industrial settings. Moreover, it assesses the effectiveness of inhibition against *S. aureus* and *E. coli* bacteria on paper coated with various concentrations of GO solution.

2. Materials and Methods

This research aims to assess the antibacterial effectiveness and adhesion properties of hard paper coated with a solution of GO and SA, all known for their inhibitory properties against bacterial growth. This research used *S. aureus* and *E. coli* for antibacterial tests. The method of this research could be divided into 3 parts which are bacterial culture, preparation of the coated filter paper and analysis of the coated filter paper with different conditions and sample dilutions. The details of these methods are shown as follows:

GO and SA sources

The source of GO in this research using the Hummer method was carried out by researchers from the graphene laboratory at the Department of Physics, Faculty of Sciences, King's Mongkut University of Technology Thonburi (KMUTT) as part of an academic collaboration.

The SA used in this research was bought from Chemipan Corporation Co., Ltd, Bangkok. Thailand.

Bacterial culture

This research used *E. coli* and *S. aureus* to test the antimicrobial properties of GO and GO mixed with SA solutions. The microorganism to be tested was inoculated into 10 mL of tryptic soy broth (TSB) liquid medium, which is a general-purpose culture medium that is used to grow a wide variety of microorganisms to provide the nutrients that the microorganism needs to grow, and incubated at 37°C for 24 hours. The first two transfers of the inoculum were incubated for 24 hours each, but the last transfer was incubated for 18 hours because the microorganism was already in the exponential

growth phase. The number of microorganisms was counted, then the 18-hour-old microbial solution was diluted to an appropriate concentration to be used as the starting inoculum for further testing. This step is done to ensure that the concentration of the inoculum is appropriate for the subsequent tests. The desired concentration varies depending on the specific test being performed. (In this test, the initial inoculum concentration was 8 Log CFU) [29].

Preparation of the coated filter paper

In this research, the 0.5 x 0.5 cm filter papers were coated with varying concentrations of graphene and a fixed concentration of sodium alginate. The filter paper without coating was used as a control. The filter papers were coated with 1% w/v of GO and 2% w/v of SA, which are commonly used in research and industry [30], to evaluate their antibacterial properties.

The GO and SA mixtures were prepared by dissolving 1 % (w/v) of graphene oxide in distilled water and 2% (w/v) of sodium alginate in a separate distilled water solution. These solutions were then mixed at specific ratios (1:1, 2:1, 3:1) using a magnetic stirrer at 500 rpm for 30 minutes to ensure complete homogenization and uniform dispersion of GO and SA.

The GO ratios (1 : 1 , 2 : 1 , and 3 : 1) were selected based on preliminary trials to evaluate the balance between the antibacterial efficacy of GO and the adhesive properties provided by SA. These ratios were chosen to systematically study the effect of increasing GO concentration relative to SA on both adhesion and antibacterial performance.

To ensure a uniform coating, 0.5 × 0.5 cm filter paper squares were immersed in the prepared GO-SA mixtures for 2 minutes, as shown in Fig. 5. Excess solution was carefully removed by gently blotting the coated paper against sterilized filter paper. The coated filter papers were then dried in a sterile cabinet at 37°C for 15 minutes, as illustrated in Fig. 6.

The filter paper used in this study had a basis weight of 80 g/m² and a thickness of 0.18 mm, which provided a consistent surface for coating. These characteristics ensured that the coating adhered evenly and allowed for reproducible results.

Analysis of the coated filter paper with different conditions and sample dilutions

This test was used to evaluate the antibacterial properties of GO and its mixtures with SA against *S. aureus* and *E. coli* [29].

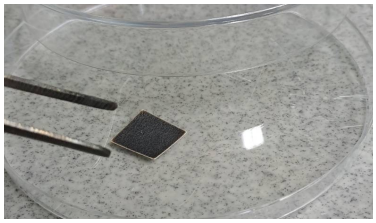


Fig. 4 A 0.5 x 0.5 cm filter paper coated by graphene oxide

To ensure a uniform coating, 0.5 × 0.5 cm filter paper shown in Fig. 4 was immersed in the prepared GO-SA mixtures for 2 minutes, as shown in Fig. 5. Excess solution was carefully removed by gently blotting the coated paper against sterilized filter paper. The coated filter papers were then dried in a sterile cabinet at 37°C for 15 minutes, as illustrated in Fig. 6.

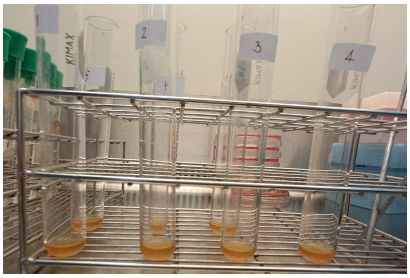


Fig. 5 The coated filter papers immersed in the suspension of tested bacteria in a sterilized container for 2 minutes

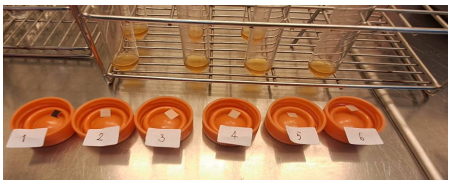
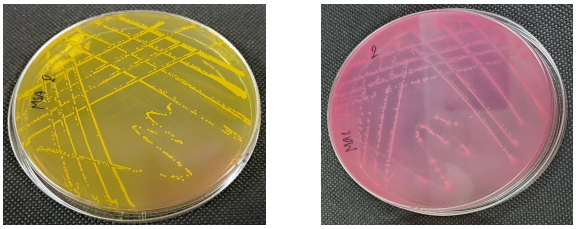


Fig. 6 Drying of coated filter papers in a sterile cabinet for 15 minutes

To quantify the initial bacterial concentration on the coated filter papers, 1 mL of the bacterial suspension, after contact with

the coated paper, was added to 9 mL of 0.1% peptone solution (1:10 dilution). Each dilution, tenfold, was then performed sequentially. Aliquots of 100 µL from appropriate dilutions were plated onto agar plates containing the selected media. Mannitol Salt Agar (MSA) was used to culture *S. aureus*, while MacConkey Agar was used for *E. coli*. According to Fig. 7, the images qualitatively illustrated bacterial survival, where colony density and color changes reflect the survival percentage presented in Table 1. The control group showed high colony density, corresponding to a high survival percentage. In contrast, the groups treated with GO or GO:SA exhibited a reduction in colony density, aligning with the percentage decrease indicated in Table 1.

Uncoated filter papers and sterile peptone solutions were used as negative controls. Plates were incubated for 24 to 48 hours at 35°C ± 2°C. Colonies on each plate were counted, and the initial bacterial concentrations on the paper discs were calculated based on the dilution factor and colony count.



a. b.

Fig. 7 Bacterial growth on selective media for *E. coli* and *S. aureus* after testing with different formulations.
(a) Growth of *S. aureus* on MSA
(b) Growth of *E. coli* on Mac

The surviving bacteria were analyzed using the spread plate technique, plating 100 µL of appropriately diluted samples onto the respective agar media. The colonies formed on the culture medium were then observed. The *S. aureus* on MSA caused the medium to change from pink to yellow, with yellow colonies. The *E. coli* on MacConkey agar resulted in dark pink colonies. Finally, the colonies on each plate were counted to quantify the number of surviving bacteria. If necessary, additional confirmation tests were performed to verify colony identity.

3. Results and Discussion

Comparing the antibacterial activity against *S. aureus* and *E. coli*, filter paper coated with various concentrations of graphene mixed with SA showed significant effectiveness compared to the control (uncoated paper). Table 1 summarizes both bacterial strains at different graphene concentrations.

According to Table 1, GO has high antibacterial properties against *E. coli*, but these properties are reduced with *S. aureus*. The antibacterial properties of GO were reduced when mixed with SA.

To evaluate the effectiveness of different treatments in reducing bacterial survival, the two-sample assuming unequal variances was conducted to compare the reduction percentage between *E. coli* and *S. aureus*. The analysis yielded a p-value of 0.0012 (one-tailed) and 0.0025 (two-tailed), both of which are below the significance threshold of 0.05. These results confirmed that the difference in reduction percentages between *E. coli* and *S. aureus* is statistically significant.

To get both antibacterial properties from GO and adhesion properties from SA, the ratio of GO and SA should be 1:1 to get the best antibacterial property for *E. coli*. For *S. aureus*, GO and SA had low antibacterial properties.

The results show that SA exhibits significant antibacterial effects against *E. coli*, aligning with the findings of Muzammil Talib [31], who tested the effectiveness of SA combined with nanoparticles, such as silver and magnesium oxide, against *E. coli*. Moreover, Fatma Mohamed [32] also found that SA had antibacterial properties to *E. coli* when combined with silver nanoparticles. It is because of cell wall structure of *E. coli* is a Gram-negative bacterium with a thinner peptidoglycan layer surrounded by an outer membrane composed of lipopolysaccharides. This structural composition makes it more susceptible to the antibacterial effects of GO, which disrupts the bacterial membrane through oxidative stress and direct physical interactions.

Table 1 Comparative study on the antibacterial efficacy of GO and SA-coated filter paper against *S. aureus* and *E. coli* at various concentrations compared to the control.

Treatment	Survival of bacteria tested (log CFU)		Bacteria Reduction Percentage (%)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
Filter paper	6.53	6.20	-	-
SA (2%)	4.83	5.39	26.19	13.06
GO (1%)	3.54	5.16	45.79	16.77
GO:SA (1:1)	3.59	5.36	45.02	13.55
GO:SA (2:1)	4.33	5.19	33.70	16.29
GO:SA (3:1)	3.87	5.37	40.73	13.39

In contrast, *S. aureus*, a Gram-positive bacterium, has a thicker peptidoglycan layer that acts as a barrier, reducing the penetration and effectiveness of GO. This structural difference limits the interaction of GO-SA with the bacterial membrane, leading to lower antibacterial efficacy.

In addition, the mechanism of GO interaction also affects this result. GO's sharp edges and high surface area facilitate physical damage to bacterial membranes. The thinner and less robust outer membrane of *E. coli* allows GO to penetrate more easily, causing cell lysis. Conversely, the thicker cell wall of *S. aureus* offers greater protection, reducing the effectiveness of GO.

Furthermore, SA enhances adhesion and uniform coating on the filter paper, improving the overall antibacterial performance. However, its role is primarily supportive in providing adhesion rather than directly enhancing GO's antibacterial properties, which may contribute to the greater efficacy observed against *E. coli* compared to *S. aureus*.

Finally, GO generates reactive oxygen species (ROS), the highly reactive molecules containing oxygen [16], which can damage bacterial DNA, proteins, and membranes. *E. coli* is less equipped to handle oxidative stress compared to *S. aureus*, which has stronger antioxidative defense mechanisms. This contributes to the differential susceptibility observed between the two bacterial strains.

These findings suggest practical applications in multiple industries. In the textile industry, GO-SA coatings could be applied to fabrics, enhancing antimicrobial properties for medical textiles such as hospital gowns, masks, and wound dressings. In food packaging, antimicrobial paper coated with this mixture could help prevent bacterial contamination, improving food safety. Additionally, its adhesive and antibacterial properties could make it suitable for biomedical applications, such as prosthetics or antimicrobial coatings for medical devices. Finally, in printing, this material might be used for specialized canvas prints or functional coatings, providing durability along with bacterial resistance.

Future work should explore more bacterial strains, including those for specific applications, and investigate long-term effectiveness, safety, cytotoxicity, and in vivo studies for human suitability.

4. Conclusion

This research investigated the potential of GO and SA solutions for coating filter papers to inhibit the growth of *S. aureus* and *E. coli*. The experiment compared the antibacterial properties of GO solution alone, GO mixed with SA at different ratios, and a control group.

The result shows that filter paper coated with GO showed the best antibacterial activity against *E. coli* (45.70% reduction), which is close to paper coated with GO mixed with SA (ratio 1:1) (45.02% reduction). On the other hand, all coated paper samples had low antibacterial activity against *S. aureus* significantly (p-value < 0.05).

This research has potential applications in antimicrobial paper, textiles, and prosthetics. Further studies are needed to confirm its long-term effectiveness and safety, as well as test

GO-SA composites on various materials to evaluate adhesion durability.

5. Acknowledgement

The authors thank the Christian University of Thailand for their support through the 2021 research grant.

6. References

[1] I. Suominen, M.L. Suihko, M.S. Salonen, Microscopic study of migration of microbes in food-packaging paper and board, JIMB. 19(1997) 104 – 113. 10.1038/sj.jim.2900424.

[2] R. Bandara, G.M. Indunil, Food packaging from recycled papers: chemical, physical, optical properties and heavy metal migration, Heliyon. 8(10) (2022) 1 – 10. 10.1016/j.heliyon.2022.e10959.

[3] S. Mangalassary, Antimicrobial Food Packaging to Enhance Food Safety: Current Developments and Future Challenges, JFPT. 3(5) (2012) 1 – 2. 10.4172/2157-7110.1000e103.

[4] C. Pérez-Pérez, C. Regalado-González, C. A. Rodríguez-Rodríguez, J.R. Barbosa-Rodríguez, F. Villaseñor-Ortega, Incorporation of antimicrobial agents in food packaging films and coatings, in: R. G. Guevara-González, I. Torres-Pacheco (Eds.), Advances in agricultural and food biotechnology, Research Signpost, Trivandrum, 2006, pp. 193 – 216.

[5] H.S. Intili, Antimicrobial Paper, U.S. patent. 4533435. 6 August 1985.

[6] P. Somboon, T. Jinkarn, R. Yoksan, Efficacy of vanillin-coated paperboard on microbial inhibition, The 54th Kasetsart University Annual Conference: Science, Genetic Engineering, Architecture and Engineering, Agro-Industry, Natural Resources and Environment, Bangkok. 2 – 5 February 2016, 887 – 894.

[7] R. Jaimun, J. Sangsuwan, Efficacy of chitosan-coated paper incorporated with vanillin and ethylene adsorbents on the control of anthracnose and the quality of Nam Dok Mai mango fruit, Packag. Technol. Sci. 32(8) (2019) 383 – 394. 10.1002/pts.2446.

[8] S. Vanit, P. Suppakul, T. Jinkarn, Efficacy of antimicrobial paperboard coated with clove oil in modified starch carriers, The 48th Kasetsart University Annual Conference: Agro-Industry, Bangkok. 3 – 5 February 2010, 108 – 116.

[9] T. Intachang, S. Phooyam, P. Limpaporn, Prevention of Bacterial Growth in Extract Guava Leaves Paper for Food Packaging. Faculty of Architecture and Design, Rajamangala University of Technology Phra Nakhon, Bangkok, 2014.

[10] S. Ritthisorn, S. Chamdit, Production of Bagasse Paper with Antibacterial Activity against Foot-Odor Causing Bacteria, Sci & Tech RMUTT J. 10(1) (2020) 10 – 18. 10.14456/10.14456/stj.2020.2.

[11] W. Choopan, N. Kittiamornkul, The Antimicrobial Efficiency Test for Solution of Graphene Oxide and Calcium Lactate using Pixel Counting Method, IJABME. 16(2) (2023) 21 – 27.

[12] K.S. Novoselov, A.K. Geim, S.V. Morozov, D. Jiang, Y. Zhang, S.V. Dubonos, I.V. Grigorieva, A.A. Firsov, Electric Field Effect in Atomically Thin Carbon Films, Science. 306(5696) (2004) 666 – 669. 10.1126 /science.110289.

[13] R.V. Van Noorden, Production: Beyond Sticky Tape, Nature. 483 (2012) 32 – 33. 10.1038/483S32a.

[14] S. Liu, T.H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, Y. Chen, Antibacterial Activity of Graphite, Graphite Oxide, Graphene Oxide, and Reduced Graphene Oxide: Membrane and Oxidative Stress, ACS Nano. 5(9) (2011) 6971 – 6980. 10.1021/ nn202451x.

[15] D. Reza, A. Hamed, K. Amir, J. Behrooz, B. Hossein, Antimicrobial properties of Graphene sheets embedded with Titanium Oxide and Calcium Oxide nanoparticles for industrial wastewater treatment, Nat. Sci. Rep. 15(2025) 1 – 12. 10.1038/ s41598-024-84335-x.

[16] S. Helmut, P.J. Dean, Reactive oxygen species (ROS) as pleiotropic physiological

signaling agents, Nat. Rev. Mol. Cell Biol. 21(2020) 363 – 383. 10.1038/s41580-020-0230-3.

[17] C.A. Juan, J.M. Pérez de la Lastra, F.J. Plou, E. Pérez-Lebeña, The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies, Int. J. Mol. Sci. 22(9) (2021) 1 – 21. 10.3390/ ijms22094642.

[18] D.R. Paul, H. Bo-Wen, T. Yoshiaki, Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling, Cell. Signal. 24(5) (2012) 981 – 990. 10.1016/j.cellsig. 2012.01.008.

[19] A. Olborska, A. Janas-Naze, Ł. Kaczmarek, T. Warga, D.S.C. Halin, Antibacterial Effect of Graphene and Graphene Oxide as a Potential Material for Fiber Finishes, Autex Res. J. 20(4) (2020) 06 – 516. 10.2478/aut-2020-0009.

[20] J. Chen, H. Peng, X. Wang, F. Shao, Z. Yuana, H. Han, Graphene oxide exhibits broad-spectrum antimicrobial activity against bacterial phytopathogens and fungal conidia by intertwining and membrane perturbation, Nanoscale. 6(3) (2014) 1879 – 1889. 10.1039/ C3NR04941H.

[21] S.N. Sitansu, K.Y. Dong, K. Kwangmeyung, Study of antibacterial mechanism of graphene oxide using Raman spectroscopy, Nat. Sci. Rep. 6 (2016) 1 – 12. 10.1038/srep28443.

[22] J. Smith, B. Johnson. The Use of Sodium Alginate in Food Stabilization, J. Food Sci. 35(4) (2019) 567 – 580. 10.1234/jfs.2019. 567.

[23] R. Abka-Khajouei, L. Tounsi, N. Shahabi, A.K. Patel, S. Abdelkafi, P. Michaud, Structures, properties and applications of alginates, Mar Drugs. 20(6) (2022) 1 – 18. 10.3390/ md20060364.

[24] Y. Wang, Y. Lu, Sodium Alginate-Based Functional Materials toward Sustainable Applications: Water Treatment and Energy Storage, Ind. Eng. Chem. Res. 62(29) (2023) 11279 – 11304. 10.1021/acs.iecr. 3c01082.

[25] M.T. Chomnawang, S. Surassmo, K. Wongsariya, N. Bunyapraphatsara, Antibacterial Activity of Thai Medicinal Plants against Methicillin-resistant *Staphylococcus aureus*, Fitoterapia. 80(2) (2009) 102 – 104. 10.1016/j.fitote.2008. 10.007.

[26] B.P. Howden, S.G. Giulieri, T.W.F. Lung, S.L. Baines, L.K. Sharkey, J.Y.H. Lee, A. Hachani, I.R. Monk, T.P. Stinear, *Staphylococcus aureus* host interactions and adaptation, Nat. Rev. Microbiol. (21) (2023) 380–395. 10.1038/s41579-023-00852-y.

[27] R. Vogt, L. Dippold. *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June–July 2002. PHR. 120(2) (2005) 174 – 178. 10.1177/003335490512000211.

[28] J.D. McCue, Gram-negative bacillary bacteremia in the elderly: incidence, ecology, etiology, and mortality, JAGS. 35(3) (1987). 213 – 218. 10.1111/j.1532-5415.1987.tb02311.x.

[29] W. Hu, C. Peng, W. Luo, M. Lv, X. Li, D. Li, Q. Huang, C. Fan, Graphene-Based Antibacterial Paper, ACS Nano. 4(7) (2010) 4317 – 4323. 10.1021/nn101097v.

[30] G. Ievgenii, Study of Structural and Mechanical Properties of Sodium Alginate Gels, EUREKA Health Sci. 2 (2021) 82 – 89. 10.21303/2504-5679.2021.001732.

[31] M. Talib, M.A. Nabeel, S.U. Haq, M.S. Waqas, H. Jamil, A.I. Aqib, A. Muneer, D. Fouad, F.S. Ataya, Recent Trends in *S. aureus* and *E. coli*-Based Endometritis, and the Therapeutic Evaluation of Sodium Alginate-Based Antibiotics and Nanoparticles, Gels. 9(12) (2023) 955 – 971. 10.3390/gels9120955.

[32] F. Mohamed, A. Ahmed, O.F. Abdel-Gawad, Preparation and evaluation of the antimicrobial activity of sodium alginate-grafted diphenylamine embedded with silver nanoparticles, Polym. Bull. 80 (2023) 9797 – 9810. 10.1007/s00289-022-04505-7.