Antimicrobial Label from Lemongrass Oil Incorporated with Chitosan/Ascorbic Acid

Retno Yunilawati [1], Windri Handayani [2], Agustina Arianita C.[3], Bunda Amalia [3] and Cuk Imawan [*1]
[1] *Departemen Fisika, Fakultas Matematika dan Ilmu Pengetahuan Alam (FMIPA), Universitas Indonesia, Depok 16424, Indonesia*
[2] *Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam (FMIPA), Universitas Indonesia, Depok 16424, Indonesia*
[3] *Badan Penelitian dan Pengembangan Industri, Kementerian Perindustrian, Indonesia*

Keywords: Antimicrobial Label, Lemongrass Oil, Chitosan, Ascorbic Acid

Abstract: Lemongrass oil is one of the essential oil which potential to be used as an antimicrobial agent in active packaging. The aim of this research is to prepare antimicrobial labels and assess their activity. Antimicrobial labels are made from a matrix of chitosan/ascorbic acid and lemongrass oil as active ingredients with various concentrations ranging from 1% to 10%. Lemongrass oil was characterized using Gas Chromatography-Mass Spectrometry (GC-MS) to determine compounds suspected of having antimicrobial activity. The GCMS chromatograms have shown that lemongrass oil contains 73.21% citral compounds composed of 29% neral (beta-citral) and 44.21% geranial (alpha-citral) as antimicrobial agents. Lemongrass oil was tested on Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli using direct method and vapor method. This test has shown that lemongrass oil has antimicrobial activity in both bacteria. The labels provide optimum antimicrobial activity for the lemongrass oil concentration of 10%. These results conclude that the lemongrass oil incorporated with chitosan/ascorbic acid has the potential to be an active packaging. The abstract should summarize the contents of the paper and should contain at least 70 and at most 200 words. It should be set in 9-point font size, justified and should have a hanging indent of 2-centimenter. There should be a space before of 12-point and after of 30-point.

1 INTRODUCTION

Antimicrobial label is one form of active packaging application, where the packaging made with the aim to maintain the quality of the material it is packaged. Antimicrobial labels are made by combining antimicrobial materials into a polymer. Essential oil has been widely used as an antimicrobial agent considering its safe, natural, environmentally friendly, and has a broad spectrum. One of the essential oils is lemongrass oil. Lemongrass oil contains several compounds such as neral and geranial which can function as antimicrobials (Argyropoulou et al., 2007).

In this research lemongrass oil is incorporated with chitosan which is a biodegradable polymer forming an antimicrobial label. Chitosan is a polymer that insoluble in neutral pH, but soluble in acidic environment, such as acetic acid, formic acid, and hydrochloric acid. Acetic acid has an unpleasant and pungent odor that can later affect food products in the label application. Likewise, formic acid and hydrochloric acid which has a pungent odor and can penetrate (Ozdemir Kubra S, 2017). Therefore, this research uses ascorbic acid as an alternative to chitosan, which has safer than acetic acid and hydrochloric acid.

This research aims to prepare antimicrobial label using lemongrass oil incorporate with chitosan/ascorbic acid and investigate their antimicrobial activity

2 MATERIALS AND METHODS

2.1 Materials

Lemongrass oil was used in this experiment obtained from Nusaroma, a local essential oils company in Indonesia. The chemical materials used in this

Yunilawati, R., Handayani, W., Arianita C., A., Amalia, B. and Imawan, C.
Antimicrobial Label from Lemongrass Oil Incorporated with Chitosan/Ascorbic Acid.
DOI: 10.5220/0009968501470152
In *Proceedings of the 2nd International Conference of Essential Oils (ICEO 2019)*, pages 147-152
ISBN: 978-989-758-456-5

experiment were ascorbic acid (Merck), chitosan in powder form (PT. Biokitosan Indonesia), and tween 80.

2.2 Methods

2.2.1 Lemongrass Oil Characterization

Lemongrass oil compounds were identified by gas chromatography with a mass spectrometer detector (GC-MS) Agilent 6890 series with capillary column HP-5MS, 30 m x 0.25 mm id x 0.25 µm film thickness. Helium gas was used as the carrier gas at constant pressure of 65 kPa. The lemongrass oil was injected with a volume of 1 µL in split ratio of 1:25. The increasing of oven temperature was programmed from 60-240°C with step of 3°C per minute until reaching 240°C.

2.2.2 Antimicrobial Assay of Lemongrass Oil

Direct Contact Agar Diffusion Tests. This method used the method carried out by (Handayani *et al.*, 2019). The antimicrobial activities determined by the paper disc diffusion method using type strain of Staphylococcus aureus NBRC 100910 and Escherichia coli NBRC 3301 in The Mueller Hinton Agar. 10 ml of molten media poured into sterile Petri plates (d=90 mm) and allowed to solidify for 5 minutes. After that, in a tube, 10 µl of bacteria culture 10-6 CFU/mL added with 10 ml of medium and mixed gently with the inoculate before poured on the top of molten media before and allowed to dry for 5 minutes. The negative control (sterile distilled water), positive control (tetracycline 15 µg/mL), lemongrass oil with concentration 1000 µg/mL loaded on 6 mm disc, whereas the volume for each disc was 10 µl. The loaded disc placed on the surface of the medium then incubates at 35°C for 18 hours. After the end of incubation, a clear zone formed around the disc was measured.

Vapor Phase Agar Diffusion Test. This vapor method used the method carried out by (Wang *et al.*, 2016). The vapor phase agar diffusion test was technically similar to the direct contact diffusion test. However, the filter discs were placed at the top in centre of the inner side of the Petri dish cover. The dishes were then sealed using laboratory parafilm to avoid evaporation of the test compounds, followed by incubation at 37°C for 24 h. The diameter of the inhibition zone was recorded.

2.2.3 Antimicrobial Labels Preparation

The chitosan solution was prepared by dissolving 2 g of chitosan powder into 100 mL of 1% (w/v) ascorbic acid and stirring at 200 rpm for 2 h at 50 °C using a magnetic stirrer. The antimicrobial label was prepared by mixing lemongrass oil with 30 mL of the chitosan solution in four different concentrations (1 % v/v, 3%v/v, 5% v/v and 10% v/v) with the added of tween 80 as surfactant (0.2% v/v) and stirring the resultant mixture for 10 min at room temperature using a magnetic stirrer. The label solution was poured onto a 10 × 15 cm acrylic board and left for 48 h at room temperature to form the film.

2.2.4 Antimicrobial Labels Characterization

A uv vis spectrophotometer (Shimadzu UV-2450) was used to measure the reflectance of the chitosan label and lemongrass-chitosan labels. A Fourier Transform Infrared (FTIR) spectra were collected for the chitosan label dan the lemongrass-chitosan labels using a double-beam spectrophotometer (Thermo Nicolet iS5) to determine the functional group

2.2.5 Antimicrobial Assay of Labels

The antimicrobial activities of labels were tested in direct contact agar diffusion test and vapor phase agar diffusion test. Labels are cut in a circle with a diameter of 6 mm and then placed in a petri dish to test antimicrobial activity with the technique as described previously.

3 RESULTS AND DISCUSSION

3.1 Chemical Compounds of Lemongrass Oil

Characterization using GC-MS showed the chromatogram profile detected 6 peaks in lemongrass oil (Figure 1) which indicated there were 6 compounds in lemongrass oil. The compounds were identified based on comparison of mass spectrum

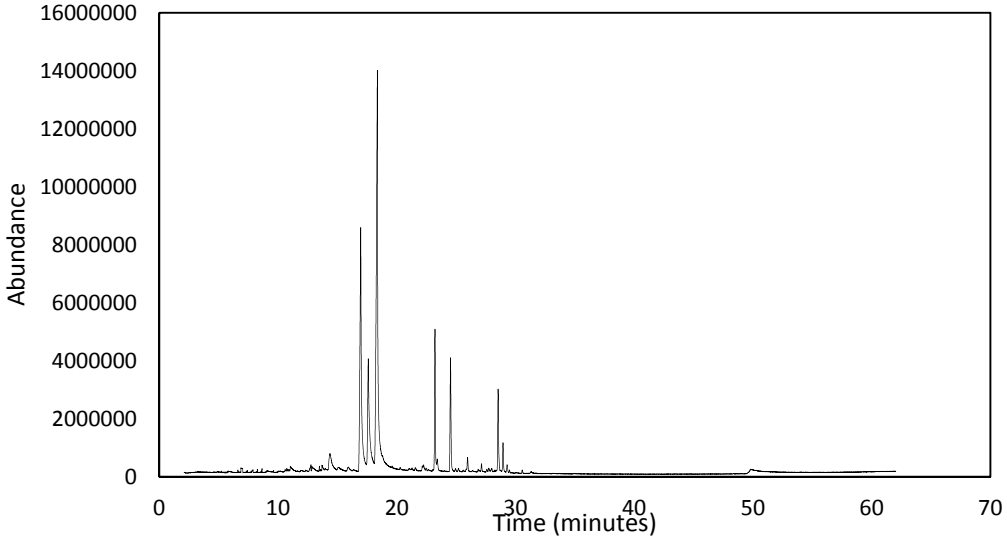


Figure 1: GCMS chromatogram of lemongrass oil

Table 1: Chemical compound identified of lemongrass oil with GC-MS

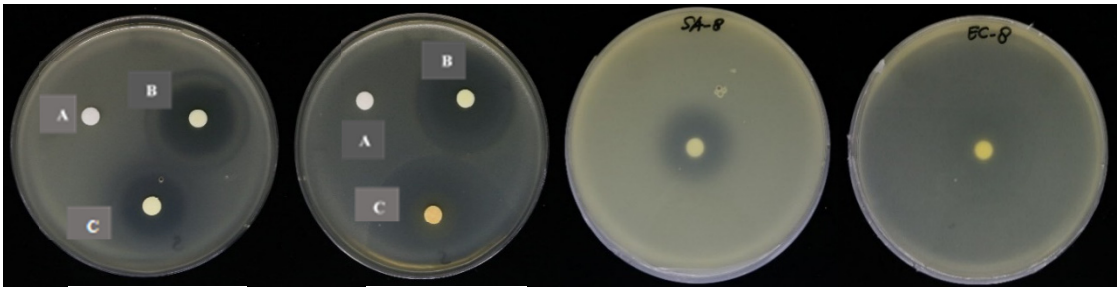
No	Retention time	Identified compound	Molecular formula	Relative percentage area (%)
1	17.101	Neral (beta-citral)	$C_{10}H_{16}O$	29.00
2	17.753	Geraniol	$C_{10}H_{18}O$	10.80
3	18.524	Geranial (Alpha citral)	$C_{10}H_{16}O$	44.21
4	23.302	Geranyl acetate	$C_{12}H_{20}O_2$	6.50
5	24.588	Beta-caryophyllene	$C_{15}H_{24}$	5.67
6	28.589	Gamma-cadinene	$C_{15}H_{24}$	3.83

with reference data from the database (Wiley 7). Based on this, lemongrass oil was known contain 6 compounds, namely neral (beta-citral), geraniol, geranial (alpha-citral), geranyl acetate, beta-caryophyllene and gamma cadinene (Table 1) with the main compounds being citral and geraniol. These results appropriated with previous finding reported in literature, citral and geraniol has been described as the main compounds of lemongrass oil (Ganjewala, 2009). Citral as the major component of lemongrass oil present at level of approximately 65%-85% (Saddiq and Khayyat, 2010). The content of citral in this research was 73.21%.

Citral (3,7 dimethyl-2-6-octadienal) is mixture of two isomers geometric, neral (beta-citral) and geranial (alpha-citral) which are monoterpene aldehyde. Citral has an activity antibacterial against Gram-positive bacteria and Gram-negative bacteria, both on oil form and vapor form (Argyropoulou *et al.*, 2007) Geraniol (3,7-dimethyl-octa-trans-2,6-dien-1-ol) is an acyclic monoterpene alcohol with the chemical formula $C_{10}H_{18}O$ (Ternus ZR, 2015). Geraniol is reported to have activity against several pathogenic bacteria (Ternus ZR, 2015). The aldehyde groups in citral and alcohol groups in geraniol that play a role in antibacterial activity. Aldehydes, phenols, esters, oxygenated terpenoids, ketones, and amines are the principle components responsible for the antimicrobial activity of essential oil (Ju *et al.*, 2019).

3.2 Antimicrobial Activity of Lemongrass Oil

The result of antimicrobial assay showed that the clear zone was formed in positive control and sample (lemongrass oil) both in Gram-positive Bacteria *S. aureus* and Gram-negative bacteria *E. coli* (Figure 2). The diameter of clear zone/inhibition zone in *S. aureus* is lower than in *E. coli* (Table 2). Generally, essential oils are more active in Gram-positive bacteria than in Gram-negative bacteria (Bhavaniramya *et al.*, 2019) (Huang *et al.*, 2014). Gram-negative bacteria have a rigid outer membrane, composed of a double layer of phospholipids (lipopolysaccharide),



S. aureus *E. coli* *S. aureus* *E. coli*

direct vapor

Figure 2: Antimicrobial activities of lemongrass oil against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*; A = negative control; B=positive control; C=sample

Tabel 2: Diameter of Inhibition zone of lemongrass oil

Samples	*S. aureus* (mm)	*E. coli* (mm)
Lemongrass oil	25	47
Lemongrass oil (vapor)	22	36

thereby limiting the diffusion of hydrophobic compounds through it. In this experiment, lemongrass oil is more active in Gram-negative bacteria *E. coli*, contrary to that statement. The antimicrobial activity of essential oil is influenced by many factors, such as the respective composition of the essential oils, the structural configuration of the constituent components, their functional groups and possible synergistic interactions between components (Dorman and Deans, 2000). The lemongrass oil has two functional groups (aldehydes and alcohol) which expected have synergistic interactions in antimicrobial activity.

The antimicrobial activity of lemongrass oil in vapor form was lower compare with in oil form. Some experiments have indicate that lemongrass oil in vapor phase is more effective than in the liquid phase (Tyagi and Malik, 2010) (Hyun *et al.*, 2015), contrary with this experiment. It can be explained that the antimicrobial activity in vapor contact was influence by the concentration of vapor, and the major constituent (Inouye, Takizawa and Yamaguchi, 2001).

3.3 Labels Characterization

The antimicrobial labels made of lemongrass oil and chitosan/ascorbic acid were shown at Figure 3. The colour of control label (chitosan/ascorbic acid without lemongrass oil) was yellowish and the label was transparent. When lemongrass incorporated in matrix, the more lemongrass oil was added, the label was opaquer and less transparent. The transparency of the label was optically expressed as a reflectance and determined using a UV spectrophotometer. The reflectance of each label was shown in Figure 4. The reflectance value decreases with increasing concentration of lemongrass oil.

FTIR spectroscopy was performed to explore the intermolecular interaction between lemongrass oil and chitosan. The FTIR spectra of the control (chitosan/ascorbic acid matrix) along with the lemongrass oil incorporated chitosan/ascorbic acid are shown in Fig.5.

control LO LO 3% LO 5% LO 10%

Figure 3: Antimicrobial label from lemongrass oil incorporated with chitosan/ascorbic acid

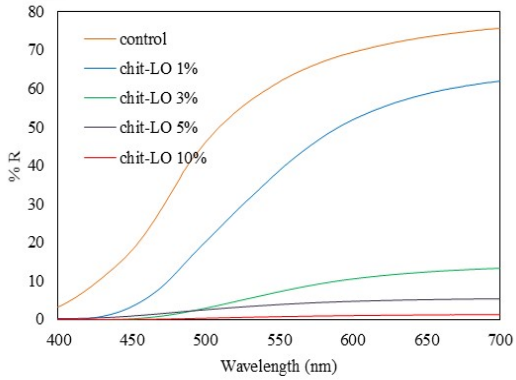


Figure 4: Reflectance spectra of antimicrobial labels

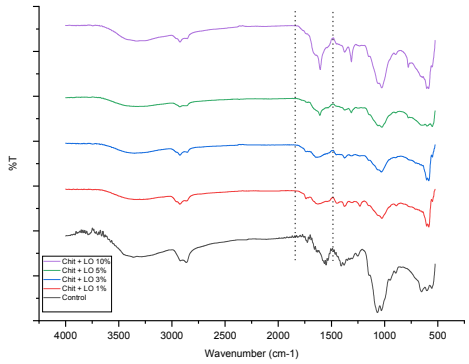
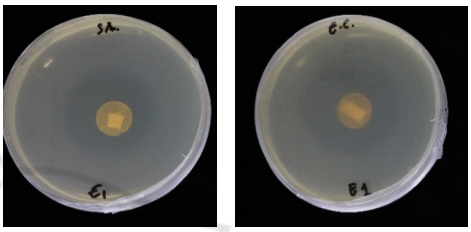


Figure 5: FTIR spectra of chitosan and all of the labels

FTIR spectroscopy was performed to explore the intermolecular interaction between lemongrass oil and chitosan. The FTIR spectra of the control (chitosan/ascorbic acid matrix) along with the lemongrass oil incorporated chitosan/ascorbic acid are shown in Fig.5. The FTIR spectra of chitosan and all of the labels gave a broad peak in the range of 3200–3500 cm^{-1} indicate the stretching vibration of hydroxyl group (O-H) (Zhang *et al.*, 2018). When the lemongrass oil was incorporated into chitosan/ascorbic acid, the major peak of the infrared spectrum did not change very much, suggested that there was no significant change in the chitosan/ascorbic acid. There were no significant changes was due the lemongrass oil didn't form covalent bonding with chitosan. These results were appropriate with several previous studies that used chitosan as a matrix for essential oils (Gursoy *et al.*, 2018) (Li *et al.*, 2019). However, there was the peak in 1722 cm^{-1} in the labels indicating the presence of citral, the major component of lemongrass oil (Natrajan et al., 2015). The intensity of this peak was greater when more lemongrass oil was added.

3.4 Antimicrobial Activity of the Labels

The antimicrobial test results from the label showed that the label has antimicrobial activity on the lemongrass oil concentration was 10% as summarized in the diameter of inhibition zone were shown in Tabel 5. The clear zone/inhibition zone was formed both in Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* (Figure 6).



(a) (b)

Figure 6: Antimicrobial activities of antimicrobial labels with lemongrass oil concentration 10% against Gram-positive bacteria *S. aureus* (a) and Gram-negative bacteria *E. coli* (b)

Tabel 3: Diameter of inhibition zone of the antimicrobial labels

No.	% lemongrass oil (v/v)	Direct contact test		Vapor test	
		S. aureus (cm)	*E. coli* (cm)	*S. aureus*(cm)	*E. coli* (cm)
1.	1	-	-	-	-
2.	3	-	-	-	-
3.	5	-	-	-	-
4.	10	2.6	3.0	2.5	2.7

Labels with lemongrass oil concentrations above 10% have been tried in this experiment but the results show that the labels are not compatible. There was a separation between lemongrass oil and chitosan matrix when the concentration of lemongrass was above 10%. Therefore, the optimal concentration of lemongrass oil on the label is 10%. Previous studies that have been conducted have shown that lemongrass concentrations below 10% have had antimicrobial activity (Ali, Noh and Mustafa, 2014) but in difference of the solvent of chitosan and difference of microbe.

4 CONCLUSIONS

The lemongrass oil was used in this study contained 73.21% of citral as the major compound which is an antimicrobial agent. The lemongrass oil has the antimicrobial activity in Gram-positive bacteria *S. aureus*) and Gram-negative bacteria E. coli. The labels from lemongrass oil incorporated with chitosan/lemongrass oil shown the antimicrobial activity in Gram-positive bacteria *S. aureus* and Gram-negative bacteria E. coli with the optimal lemongrass oil concentration of 10% (v/v).

ACKNOWLEDGEMENT

This research is supported by PSNI (Penelitian Strategis Nasional Institusi) from Kementerian Riset, Teknologi, dan Perguruan Tinggi Republik Indonesia No NKB-1798/UN2.R3.1/HKP.05.00/2019. We also thank the Center of Excellence Biology Resources Genome Study (CoE IBR-GS) FMIPA UI and the Center for Chemical and Packaging (CCP) for the facilities and equipment to support this research.

REFERENCES

Ali, A., Noh, N. M. and Mustafa, M. A. (2014) 'Antimicrobial activity of chitosan enriched with lemongrass oil against anthracnose of bell pepper', *Food Packaging and Shelf Life*. Elsevier Ltd., 3, pp. 56–61. doi: 10.1016/j.fpsl.2014.10.003.

Argyropoulou, C. *et al.* (2007) 'Chemical composition of the essential oil from leaves of Lippia citriodora H.B.K. (Verbenaceae) at two developmental stages', *Biochemical Systematics and Ecology*, 35(12), pp. 831–837. doi: 10.1016/j.bse.2007.07.001.

Bhavaniramya, S. *et al.* (2019) 'Role of essential oils in food safety: antimicrobial and antioxidant applications', *Grain & Oil Science and Technology*. doi: 10.1016/j.gaost.2019.03.001.

Dorman, H. J. D. and Deans, S. G. (2000) 'Antimicrobial agents from plants: Antibacterial activity of plant volatile oils', *Journal of Applied Microbiology*, 88(2), pp. 308–316. doi: 10.1046/j.1365-2672.2000.00969.x.

Ganjewala, D. (2009) 'Cymbopogon essential oils : Chemical compositions and bioactivities', *International Journal of Essential Oil Therapeutics*, 3, pp. 56–65.

Gursoy, M. *et al.* (2018) 'False flax (Camelina sativa) seed oil as suitable ingredient for the enhancement of physicochemical and biological properties of chitosan films', *International Journal of Biological Macromolecules*. Elsevier B.V., 114, pp. 1224–1232. doi: 10.1016/j.ijbiomac.2018.04.029.

Handayani, W. *et al.* (2019) 'Coriandrum sativum l . (apiaceae) and elettaria cardamomum (l .) maton (zingiberaceae) for antioxidant and antimicrobial protection Coriandrum sativum l . (apiaceae) and elettaria cardamomum (l .) maton (zingiberaceae) for antioxidant and antimi', *Journal of Physiscs: Conference Series*. doi: 10.1088/1742-6596/1317/1/012092.

Huang, D. F. *et al.* (2014) 'Chemical constituents, antibacterial activity and mechanism of action of the essential oil from Cinnamomum cassia bark against four food-related bacteria', *Microbiology (Russian Federation)*, 83(4), pp. 357–365. doi: 10.1134/S0026261714040067.

Hyun, J. E. *et al.* (2015) 'Preservative effectiveness of essential oils in vapor phase combined with modified atmosphere packaging against spoilage bacteria on fresh cabbage', *Food Control*. Elsevier Ltd, 51, pp. 307–313. doi: 10.1016/j.foodcont.2014.11.030.

Inouye, S., Takizawa, T. and Yamaguchi, H. (2001) 'Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact', *Journal of Antimicrobial Chemotherapy*, 47(5), pp. 565–573. doi: 10.1093/jac/47.5.565.

Ju, J. *et al.* (2019) 'Application of essential oil as a sustained release preparation in food packaging', *Trends in Food Science and Technology*, 92(1800), pp. 22–32. doi: 10.1016/j.tifs.2019.08.005.

Li, Z. *et al.* (2019) 'Preparation, characterization and anti-aflatoxigenic activity of chitosan packaging films incorporated with turmeric essential oil', *International Journal of Biological Macromolecules*. Elsevier B.V., 131, pp. 420–434. doi: 10.1016/j.ijbiomac.2019.02.169.

Ozdemir Kubra S, G. V. (2017) 'Extending the shelf-life of pomegranate arils with chitosan-ascorbic acid coating', *Food Science and Tachnology 76 (2017) 172-180*, 76, pp. 172–180. doi: 10.1016/j.lwt.2016.10.057.

Saddiq, A. A. and Khayyat, S. A. (2010) 'Chemical and antimicrobial studies of monoterpene: Citral', *Pesticide Biochemistry and Physiology*. Elsevier Inc., 98(1), pp. 89–93. doi: 10.1016/j.pestbp.2010.05.004.

Ternus ZR, Z. M. (2015) 'Microbiological Characterization of Pure Geraniol and Comparison with Bactericidal Activity of the Cinnamic Acid in Gram-Positive and Gram-Negative Bacteria', *Journal of Microbial & Biochemical Technology*, 07(04), pp. 186–193. doi: 10.4172/1948-5948.1000203.

Tyagi, A. K. and Malik, A. (2010) 'In situ SEM, TEM and AFM studies of the antimicrobial activity of lemon grass oil in liquid and vapour phase against Candida albicans', *Micron*, pp. 797–805. doi: 10.1016/j.micron.2010.05.007.

Wang, T. H. *et al.* (2016) 'Evaluation of the antibacterial potential of liquid and vapor phase phenolic essential oil compounds against oral microorganisms', *PLoS ONE*, 11(9), pp. 1–17. doi: 10.1371/journal.pone.0163147.

Zhang, Z. *et al.* (2018) 'Preparation and characterization of biocomposite chitosan fi lm containing Perilla frutescens (L .) Britt . essential oil', *Industrial Crops & Products*, 112(December 2017), pp. 660–667. doi: 10.1016/j.indcrop.2017.12.073.