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The Influence of Sweet Soy Sauce or Honey Addition Toward Antibacterial Activity of Lime (*Citrus aurantifolia* Swingle) Juice

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Abstract: In Indonesia, lime (*Citrus aurantifolia* Swingle) juice is frequently used as a medicine for acute respiratory tract infection by mixing it with sweet soy sauce or honey. Therefore, a study aiming to know the influence of sweet soy sauce or honey addition toward antibacterial activity of lime juice at *Salmonella aureus* and *S. pyogenes* bacteria is conducted. At the beginning, lime juice is measured at its resistivity toward *S. aureus* and *S. pyogenes* bacteria. Then, the chosen concentration is mixed with sweet soy sauce or honey in different concentration. The result shows that lime juice concentration of 50% has a strong antibacterial activity with each value of MIC and MBC for *S. aureus* is 0.48 and 1.93% and for *S. pyogenes* is 0.59 and 2.97%. Sweet soy sauce or honey addition does not affect the antibacterial activity while the optimum concentration which can be added is 50%. Antibacterial activity of lime juice is indicated by cell wall damage which gives effect on lysis and morphological changes on bacterial cell.

Key words: Antibacterial activity, honey, lime juice, sweet soy sauce, wall

INTRODUCTION

Citrus has been used for food and medicinal herbs for years. One of the most used citrus for food and drug is lime. As a foodstuff, lime can be brewed for beverage as food acidulant (Meliono-Budianto, 2004), to eliminate fishy scent (Poemorno *et al*., 2004) to alleviate heavy metal in foodstuff (Haryani, 2011) as an antioxidant (Ghafar *et al*., 2010; Khuwijitjaru *et al*., 2008) to preserve the food or as antimicrobial agent (Nogueira *et al*., 2003; Tomotake *et al*., 2006; Razak *et al*., 2013; Matan, 2012; Taiwo *et al*., 2007; Jafari *et al*., 2011; Jayana *et al*., 2010), etc. As medicine, lime can be utilized for cough medicine, plaque on teeth medicine (Owhe-Ureghe *et al*., 2010), nefrocalcinosis medicine (Penniston *et al*., 2008), etc.

Parts of utilized lime for food and medicine is vary from the leaves (Chaisawadi *et al*., 2005; Thanaboripat *et al*., 2006), lime rind (Aibinu *et al*., 2007; Matan, 2012; Chanthaphon *et al*., 2008; Bosquez-Molina *et al*., 2010) and lime juice (Nogueira *et al*., 2003; Taiwo *et al*., 2007; Adedeji *et al*., 2007). Contributing components for preservatives or medicines are essential oil in the lime rind and organic acid in lime pulp. Lime essential oil and organic acid are antimicrobial characteristic which can be functioned as preservatives or medicines. Organic acid in lime is not only being an antimicrobial agent but also being a heavy metal solvent agent (Haryani, 2011), fishy scent solvent (Poemorno *et al*., 2004) and prevention of nefrocalcinosis shaping (Penniston *et al*., 2008).

Traditionally in Indonesia, lime has been used for cough medicine. This is related to the content of organic acid and essential oil in the lime with its antimicrobial characteristic against the causal bacteria of acute respiratory tract infection, they are *Staphylococcus aureus* and *Streptococcus pyogenes* (Haq *et al*., 2010). Utilization of lime juice for cough medicine in Indonesia is frequently mixed with sweet soy sauce or honey to alleviate its lime acid flavor. Hence, it needs to be analyzed the influence of sweet soy sauce or honey addition into lime juice toward antibacterial activity. It is expected from this study that it will reduce the antibiotic use for acute respiratory tract infection under its risk on health (Bayarski, 2006).

MATERIALS AND METHODS

Used materials in this study are lime (*Citrus aurantifolia* Swingle) and bacteria culture as well as some materials for microbiology analysis and chemical analysis. Used lime are bought from hypermart of Karawaci, oval-shaped lime with diameter of 3-4 cm with green color. Used bacteria culture are *Staphylococcus aureus* and *Streptococcus pyogenes* which are the collection of Microbiology Department of University of Indonesia.

Other materials are Nutrient Agar Merck media, Nutrient Broth Merck media, Blood Agar Base Oxoid media, defibrination of sheep blood from Microbiology Department of University of Indonesia, sweet soy sauce ABC, pure honey Madurasa and distilled water.

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Used equipments in this study are bench scale, analytical scale, micro pipette, tip, petri dish, measuring cylinder, screw tube, incubator, beaker glass, Erlenmeyer flash Pyrex, Vernier calipers, autoclave, refrigerator, oven, thermometer, laminar air flow, test tube, heater, pH meter and Scanning Electron Microscope (SEM) Jeol JSM-6360LA.

Lime juice preparation: Washed fresh lime is cut in half and liquidized using citrus juicer manually in order to get its juice or citrus juice. Then, it is strained using tea strainer.

Antibacterial activity testing of lime juice: Antibacterial activity testing is conducted by Well Diffusion Methods of Bloomfield (1991). A number of 1 mL bacterial suspension (*S. aureus* and *S. pyogenes*) is inoculated into the media which will be poured into petri dish to make it dense. Next, there will be made a well in it with diameter of 0.6 cm aseptically. A number of 60 μL lime juice with concentration of 10, 20, 30, 40 and 50% (v/v) are put into the well which was made aseptically. Then, the petri dish is incubated at temperature of 37°C for 24 h. Diameter of inhibition zone which is marked by clear area is measured by ruler.

MIC (Minimum Inhibition Concentration) value is determined by Bloomfield (1991) Method that is by creating linear regression curve between X-axis (ln Mo = ln Mt) and Y-axis (Z^2 = quadrat score of inhibition zone). Linear regression curve which cut the X-axis is called Mt value. MIC value is 0.25 Mt. MBC (Minimum Bactericidal Concentration) value is 4 times of MIC. MIC value is an extract minimum concentration which can hamper bacterial growth. MBC value is an extract minimum concentration which can kill the bacteria.

Antibacterial activity testing of lime juice with sweet soy sauce and honey: Antibacterial activity testing from lime juice in different concentration and mixing material are conducted by well diffusion method of Bloomfield (1991). The best three concentration of lime juice from the previous research phases are used as the treatments in this phase. Used mixing materials are sweet soy sauce and honey. If the chosen lime juice concentration is x%, the mixing concentration is (100-x)%. After lime mixture making, antibacterial activity testing is conducted by Well Diffusion Method.

RESULTS AND DISCUSSION

Antibacterial activity of lime juice: Antibacterial activity of lime juice toward the causal bacteria of acute Respiratory tract infection *S. aureus* and *S. pyogenes* which are measured based on inhibition zone diameter can be seen in Table 1.

Table 1: Lime juice inhibition toward *Staphylococcus*

	Inhibition zone (mm)	
Concentration/Inhibition (%)	*S. aureus*	*S. pyogenes*
10	9.81±1.01^{a}	3.02±0.17^{e}
20	13.67±0.60^{b}	5.28±0.54^{f}
30	16.14±0.60^{c}	8.01±1.02^{g}
40	17.55±0.76^{cd}	9.6±1.02^{h}
50	18.63±1.00^{d}	11.35±1.11^{i}
MIC	0.48	0.59
MBC	1.93	2.37

Superscript letter notation shows tangible difference at $\alpha = 0.05$

Table 2: Phytochemistry components in qualitative manner within lime juice

Phytochemistry components	Testing results
Saponin	+
Alkaloid	+
Tannin	+
Phenolic	+
Flavonoid	+
Triterpenoid	+
Steroid	+
Glycoside	+

+ = positive; - = negative

In Table 1, it shows that lime juice is able to hamper *S. aureus* and *S. pyogenes* bacteria growth where the higher concentration of the lime juice is the wider of its inhibition zone diameter. Lime juice at concentration of 40 and 50% have the biggest inhibition zone diameter and so does for *S. aureus* bacteria while for *S. pyogenes*, the biggest resistivity is at concentration of 50%. Antibacterial activity division uses David Stout Method based on measurement of inhibition zone diameter (Suryawiria, 1978) that is <5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong) and >20 mm (very strong). Thereby, inhibition which is resulted by lime juice at concentration of 50% toward *S. aureus* and *S. pyogenes* is strong.

In order to know an active component in lime juice in qualitative manner which may play a role to hamper bacterial growth there will be phytochemistry testing. Result of phytochemistry testing can be seen in Table 2.

Table 2 shows that almost all phytochemistry are exist in lime juice. Components of phenolic, flavonoid, alkaloid and triterpenoid have antibacterial activity toward *B. subtilis*, *Salmonella* sp., *E. coli* and *S. aureus* (Sohn *et al*., 2004). According to Cooper *et al.* (1999) the existence of active components such as phenolic, flavonoid and tannin can function as antibacterial components toward patogenic bacteria like *E. faecalis*, *S. pyogenes* and *B. cereus*. Saponin, tannin, steroid and glycoside compounds also have antibacterial activity toward gram-positive bacterium and gram-negative bacterium such as *S. aureus*, *E. coli* and *B. cereus* (Seotan *et al*., 2006).

Table 3: Inhibition of lime mixture toward *Staphylococcus bacteria*

	Inhibition diameter (mm) of *S. pyogenes*		Inhibition diameter (mm) of *S. aureus*	
Concentration of lime juice (%)	Sweet soy sauce	Honey	Sweet soy sauce	Honey
40	8.53±0.26^{a}	9.54±0.72^{ab}	14.46±0.29^{a}	15.03±0.13^{a}
50	10.85±0.35^{b}	13.47±1.19^{c}	14.98±0.23^{ab}	15.73±0.18^{ab}
60	13.53±1.93^{c}	14.51±1.15^{c}	15.69±0.44^{bc}	16.43±0.82^{b}
MIC (%)	0.86	0.85	0.51	0.49
MBC (%)	3.46	3.39	2.04	1.96

Superscript letter notation shows a tangible difference at $\alpha = 0.05$

According to Haq *et al.* (2010) the existing phenolic components in lime such as limonene, carvacrol, citral and geraniol have activity to hamper the growth of *S. aureus*. This phenolic component hampers bacterial growth by destroying phospholipid layer from the cell membrane which causes permeability rise and cell composing constituents loss (Parhusip *et al*., 2006). Grounded on conducted study by Razzaghi-Abyaneh *et al.* (2009), the bigger of used antimicrobial compound concentration is the bigger of resulted microorganism growth inhibition ability.

Minimum concentration or dosis of bacterial inhibition is shown by MIC value while the minimum dosis to kill the bacteria is shown by MBC value. From Table 1, it can be seen that MIC and MBC value of lime juice toward *S. aureus* bacteria is smaller than *S. pyogenes* bacteria. It means that antibacterial activity of lime juice toward *S. aureus* is bigger than antibacterial activity toward *S. pyogenes*. It also can be seen by virtue of the similar lime juice concentration which the resulted inhibition diameter at *S. aureus* is bigger than at *S. pyogenes*. The large of inhibition diameter shows that bacteria is susceptible to antimicrobial component (Nuraini, 2007). The more susceptible of bacteria toward antimicrobial compound is the smaller of required antimicrobial concentration to hamper or to kill this bacteria. In other words, *S. aureus* is more sensitive toward antibacterial component containing in lime juice than in *S. pyogenes*.

Determination of lime juice concentration selection: Lime juice concentration selection to be mixed with honey or sweet soy sauce is conducted by selecting the best lime juice concentration in hampering bacterial growth from Table 1. Based on Table 1, the best lime juice concentration in hampering the growth of *S. aureus* is the concentration of 40 and 50% while in hampering *S. pyogenes*, concentration needs is 50%. Hence, the chosen concentration which will be used in the future research by increasing sweet soy sauce or honey is lime juice with concentration of 50%.

Antibacterial activity of lime juice with sweet soy sauce and honey: In this phase, lime juice will be combined by sweet soy sauce or honey with concentration of 100-x% thus if the concentration of lime juice is 50%, the concentration of sweet soy sauce or honey is 50%. As a comparison treatment, it uses the lime juice concentration of 40 and 60%. The used bacterial test is still *S. aureus* and *S. pyogenes*.

Antibacterial activity of lime and sweet soy sauce or honey mixture toward *S. aureus* and *S. pyogenes* can be seen in Table 3. From the statistical testing result, it is gained that mixing materials, i.e., sweet soy sauce and honey, do not give a tangible effect on the resistivity toward *S. aureus* or *S. pyogenes* ($p>0.05$) bacteria. It gives an effect if the concentration is $p<0.05$ (Table 3). For the lime juice mixed by sweet soy sauce or honey, lime juice concentration of 40% is not really different from its concentration of 50%. Yet, lime juice concentration of 50% gives a tangible different from a lime concentration of 60%. Thus, the chosen concentration is the mixing lime juice with honey or sweet soy sauce with concentration of 50%.

If this antibacterial activity mixture (Table 3) is distinguished from antibacterial activity of lime juice (Table 1), it is seen that sweet soy sauce or honey addition does not give any effect on antibacterial activity toward *S. aureus* but it slightly weakens antibacterial activity of the lime juice toward *S. pyogenes*. However, it is reported that there is an antibacterial components in honey and sweet soy sauce. According to Maddocks *et al.* (2012), antibacterial components in honey can be used to hamper *S. pyogenes* growth which is an infection causal bacteria on injured skin. Components of hydrogen peroxide and phytochemistry in honey can hamper *S. pyogenes* growth by destroying the bond between *S. pyogenes* and fibronectin found on human skin tissue. Other than hydrogen peroxide, honey has bioactive components such as polyphenol, flavonoid, tannin and glycoside which can hamper the growth of *E. coli*, *P. mirabilis*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans* (Mboto *et al*., 2009). The existing antibacterial component in sweet soy sauce which is called nicotinamide can hamper bacterial growth of *S. aureus*, *S. flexneri*, *V. cholera*, *S. enteridis* and *E. coli* (Kataoka, 2005). Thereby, it can be said that mixed sweet soy sauce or honey addition only plays a role on

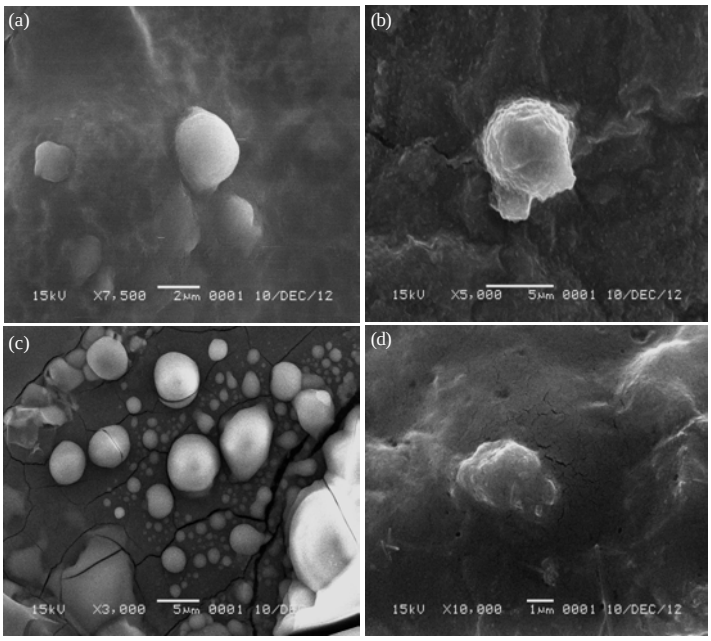

Fig. 1: a) Morphological observation by SEM, control over *S. aureus* (7500x), b) shows an exposure of lime (5000x), c) control over *S. pyogenes* (3000x) and d) shows an exposure of lime (10000x)

masking taste due to the quite acid of lime taste. This is in accordance with one of the opinion stated by Nattadiputra (2009). He says that the use of two combined antimicrobial components can give an equal effect or a stronger effect; synergistic effect, stronger effect resulted by effect from two kinds of additive medicine or antagonist effect, a weaker effect which can be reached from one of the two components in individual use.

Grounded on MIC and MBC value, *S. aureus* bacteria is smaller than *S. pyogenes*. It proves that *S. aureus* is more sensitive toward antimicrobial components in lime and honey mixture than *S. pyogenes* bacteria. The same result is also shown by a research conducted by Moussa showing that MIC and MBC value from honey for *S. aureus* bacteria is smaller than *S. pyogenes*.

Cell damage resulted by Scanning Electron Microscope (SEM): Occurred damage in *S. aureus* and *S. pyogenes* bacterial cell as the effect of lime juice exposure can be observed by using SEM. Result of SEM can be seen in Fig. 1.

Result of SEM shows that there is a cell morphological changes of *S. aureus* and *S. pyogenes* which were added by lime juice. Morphological damage in *S. aureus* and *S. pyogenes* cell is visible on cell form which had not been round intact and on the cavity formation in the cell as an indication of cell wall and lysis damage. This is in accordance with a statement of Hartmann *et al*. (2010), the occurrence of cell damage in bacteria by giving antimicrobial addition is proved by cell bacterial form which had not been round intact, perforated wall and lysis.

CONCLUSION

Lime juice concentration of 50% indicates a strong antibacterial activity toward *S. aureus* and *S. pyogeness* with each value of MIC and MBC is 0.48 and 1.93, 0.59 and 2.37%. Honey or sweet soy sauce added to lime juice does not affect antibacterial activity toward acute respiratory tract infection bacteria of S. *aureus* and *S. pyogenes*. Optimum concentration of sweet soy sauce or honey which can be added to the lime juice is 50%. Antibacterial activity of lime juice grounded on SEM observation is the occurrence of cell wall damage thus lysis and cell morphology is not formed.

REFERENCES

Adedeji, G.B., O.E. Fagade and A.A. Oyelade, 2007. Prevalence of *Pseudomonas aeruginosa* in clinical samples and its sensitivity to citrus extract. Afr. J. Biomed. Res., 10: 183-187.

Aibinu, I., T. Adenipekun, T. Adelowotan, T. Ogunsanya and T. Odugbemi, 2007. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr. J. Trad. Compl. Altern. Med., 4: 185-190.

Bayarski, Y., 2006. Antibiotics and their side effects. http://hamiltoncountypreppers.org/Antibiotics_and_Their_Types.pdf.

Bloomfield, S.F., 1991. Methods for Assessing Antimicrobial Activity. In: Mechanism of Action of Chemical Biocides Thesis Study and Explanation, Denyer, S.P. and W.B. Hugo (Eds.). Blackwell Scientific Publication, London, pp: 1-22.

Bosquez-Molina, E., E. Ronquillo-de Jesus, S. Bautista-Banos, J.R. Verde-Calvo and J. Morales-Lopez, 2010. Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. Postharvest Biol. Technol., 57: 132-137.

Chaisawadi, S., D. Thongbute, W. Methawiriyasilp, N. Pitakworarat and A. Chaisawadi *et al*., 2005. Preliminary study of antimicrobial activities on medicinal herbs of thai food ingredients. Acta Hort., 675: 111-114.

Chanthaphon, S., S. Chanthachum and T. Hongpattarakere, 2008. Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. Songkhlanakarin J. Sci. Technol., 30: 125-131.

Cooper, R.A., P.C. Molan and K.G. Harding, 1999. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J. R. Soc. Med., 92: 283-285.

Ghafar, M.F.A., K.N. Prasad, K.K. Weng and A. Ismail, 2010. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. Afr. J. Biotechnol., 9: 326-330.

Haq, G.I., A. Permanasari and H. Sholihin, 2010. Efektivitas penggunaan sari buah jeruk nipis terhadap ketahanan nasi. J. Sains dan Teknologi Kimia, 1: 44-58.

Hartmann, M., M. Berditsch, J. Hawecker, M.F. Ardakani, D. Gerthsen and A.S. Ulrich, 2010. Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S and PGLa as revealed by transmission and scanning electron microscopy. Antimicrob. Agents Chemother., 54: 3132-3142.

Haryani, D., 2011. Efektifitas lama waktu perendaman daging kerang hijau perna viridis dengan larutan jeruk nipis 25% dalam menurunkan logam berat timbal. Undergraduate Thesis, Diponegoro University, Indonesia.

Jafari, S., S. Esfahani, M.R. Fazeli, H. Jamalifar and M. Samadi *et al*., 2011. Antimicrobial activity of lime essential oil against food-borne pathogens isolated from cream-filled cakes and pastries. Int. J. Biol. Chem., 5: 258-265.

Jayana, B.L., T. Prasai, A. Singh and K.D. Yami, 2010. Study of antimicrobial activity of lime juice against *Vibrio cholerae*. Sci. World, 8: 44-46.

Kataoka, S., 2005. Functional effects of japanese style fermented soy sauce (shoyu) and its components. J. Biosci. Bioeng., 100: 227-234.

Khuwijitjaru, P., K. Chalooddong and S. Adachi, 2008. Phenolic content and radical scavenging capacity of kaffir lime fruit peel extracts obtained by pressurized hot water extraction. Food Sci. Technol. Res., 14: 1-4.

Maddocks, S.E., M.S. Lopez, R.S. Rowlands and R.A. Cooper, 2012. Manuka honey inhibits the development of *Streptococcus pyogenes* biofilms and causes reduced expression of two fibronectin binding proteins. Microbiology, 158: 781-790.

Matan, N., 2012. Antimicrobial activity of edible film incorporated with essential oils to preserve dried fish (*Decapterus maruadsi*). Int. Food Res. J., 19: 1733-1738.

Mboto, C.I., M.E. Eja, A.A. Adejoke, G.D. Iwatt and B.E. Asikong *et al*., 2009. Phytochemical properties and antimicrobial activities of combined effect of extracts of the leaves of *Garcinia kola*, *Vernonia amygdalina* and Honey on some medically important microorganisms. Afr. J. Microbiol. Res., 3: 557-559.

Meliono-Budianto, V.I., 2004. Dimensi etis terhadap budaya makan dan dampaknya pada masyarakat. Makara Sosial Humaniora, 8: 65-70.

Nattadiputra, S., 2009. Kumpulan Kuliah Farmakologi. 2nd Edn., EGC Penerbit Buku Kedokteran, Jakarta, Indonesia.

Nogueira, M.C.L., O.A. Oyarzabal and D.E. Gombas, 2003. Inactivation of *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Salmonella* in cranberry, lemon and lime juice concentrates. J. Food Prot., 66: 1637-1641.

Nuraini, A.D., 2007. Ekstraksi komponen antibakteri dan antioksidan dari biji teratai (*Nymphaea pubescens* Willd). Skripsi Institut Pertanian Bogor, Bogor. http://repository.ipb.ac.id/bitstream/handle/123456789/2532/F07adn.pdf?sequence=4.

Owhe-Ureghe, U.B., D.A. Ehwarieme and D.O. Eboh, 2010. Antibacterial activity of garlic and lime on isolates of extracted carious teeth. Afr. J. Biotechnol., 9: 3163-3166.

Parhusip, A.J.N., B.S.L. Jenie, W.P. Rahayu and S. Yasni, 2006. Pengaruh ekstrak andaliman (*Zanthoxylum acanthopodium* DC) terhadap permeabilitas dan hidrofobisitas *Bacillus cereus*. Institut Pertanian Bogor, Bogor. http://repository.ipb.ac.id/handle/123456789/51027.

Penniston, K.L., S.Y. Nakada, R.P. Holmes and D.G. Assimos, 2008. Quantitative assessment of citric acid in lemon juice, lime juice and commercially-available fruit juice products. J. Endourol., 22: 567-570.

Poemorno, D., S.H. Suseno and A. Wijatmoko, 2004. Pemanfaatan asam cuka, jeruk nipis (*Citrus aurantifolia*) dan belimbing wuluh (*Averrhoa bilimbi*) untuk mengurangi bau amis petis ikan layang (*Decaptems* spp). Buletin Teknologi Hasil Perikanan, IPB, Bogor.

Razak, A., A. Djama and G. Revilla, 2013. Uji daya hambat air perasan buah jeruk nipis (*Citrus aurantifolia* s.) terhadap pertumbuhan bakteri *Staphylococcus aureus* Secara *in vitro*. Jurnal Kesehatan Andalas, 2: 1-8.

Razzaghi-Abyaneh, M., M. Shams-Ghahfarokhi, M.B. Rezaee, K. Jaimand, S. Alinezhad, R. Saberi and T. Yoshinari, 2009. Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. Food Control, 20: 1018-1024.

Seotan, K.O., M.A. Oyekunle, O.O. Aiyelaagbe and M.A. Fafunso, 2006. Evaluation of antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench. Afr. J. Biotechnol., 5: 2405-2407.

Sohn, H.Y., K.H. Son, C.S. Kwon, G.S. Kwon and S.S. Kang, 2004. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai Phytomedicine, 11: 666-672.

Suryawiria, U., 1978. Mikroba Lingkungan. 2nd Edn., Institut Teknologi Bandung, Bandung, Indonesia.

Taiwo, S.S., B.A. Oyekanmi, Y.O. Adesiji, O.O. Opaleye and O.A. Adeyeba, 2007. *In vitro* antimicrobial activity of crude extracts of *Citrus aurantifolia* linn and *Tithonia diversifolia* poaceae on clinical bacterial isolates. Int. J. Trop. Med., 2: 113-117.

Thanaboripat, D., S. Chareonsettasilp, K. Pandee and K. Udomwongsup, 2006. Inhibitory effect of kaffir lime, bitter cucumber and tobacco extract on the growth of *Aspergillus flavus*. KMITL Sci. Technol. J., 6: 18-24.

Tomotake, H., T. Koga, M. Yamato, A. Kassu and F. Ota, 2006. Antibacterial activity of citrus fruit juices against Vibrio species. J. Nutr. Sci. Vitaminol., 52: 157-160.