Antibacterial Potential of Star Anise (Illicium verum Hook. f.)
Against Food Pathogen Bacteria

EVELINE* AND AGUSTIN NOVITA

Food Technology Department, Faculty of Science and Technology, University of Pelita Harapan, MH. Thamrin Boulevard 1100 Lippo Village, Kelapa Dua, Karawaci, Tangerang, Indonesia.

Star anise (Illicium verum Hook. f.) is spice which is commonly used as spice and flavor enhancer in food. Previous research revealed the presence of active compound which could inhibit bacterial growth. Thus, in order to apply star anise as natural antibacterial agent in food product, a further research concerning antibacterial activity and stability of star anise was conducted. Crude extract of star anise was obtained using ethanol and acetone with maceration method for 3 days, then diluted to 10, 20, 30, 40, and 50% (w/v). Well diffusion was conducted against three food spoilage bacteria (Staphylococcus aureus, Escherichia coli, and Bacillus cereus). Extract from ethanol with 30% concentration was selected as the best extract in which inhibit more than 6 mm inhibition zone with MIC and MBC value: 1.59% and 6.36% (S. aureus), 1.04% and 4.18% (E. coli), and 0.59% and 2.39% (B. cereus). This selected extract was used to test the extract stability against 4 levels of heating temperature (60, 70, 80, and 90°C) for 2 levels of heating time (15 and 30 minutes), and 4 levels of pH (4, 5, 6, and 7). Based on our results, different heating treatment and pH caused extract instability. Star anise extract was more stable at 60°C for 15 minutes heating treatment and pH 4, which resulting the lowest inhibition zone reduction compared to control extract. Star anise extract was categorized as low toxic compound (LC50 = 212.09 ppm). Terpenoids (anethole, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-norpinene, β-caryophyllene, β-bisabolene) was founded as major antibacterial compound in star anise extract; fatty acid (6-octadecenoic acid, hexadecanoic acid, stearic acid) and benzaldehyde (4-anisaldehyde, p-allylanisole) were also founded as minor compound.

Key words: antibacterial, Illicium verum Hook. f., pH, stability, temperature, time

Bunga lawang (Illicium verum Hook. f.) umumnya dimanfaatkan sebagai rempah dan perisa pada makanan. Penelitian terdahulu mengungkap adanya senyawa aktif yang berpotensi menghambat pertumbuhan bakteri. Hal ini mendorong dilakukan penelitian lebih lanjut agar diketahui aktivitas dan stabilitas bunga lawang sebagai senyawa antibakteri alami yang dapat diterapkan dalam produk pangan. Ekstrak kasar bunga lawang diperoleh dari ekstraksi dengan etanol dan aseton selama 3 hari pada konsentasi 10, 20, 30, 40, dan 50% (b/v). Pengujian difusi sumur dilakukan terhadap tiga bakteri perusak pangan (Staphylococcus aureus, Escherichia coli, and Bacillus cereus). Ekstrak 30% dengan pelarut etanol merupakan ekstrak terpilih penghasil zona hambat lebih dari 6 mm dengan MIC dan MBC berurutan sebesar 1,59% dan 6,36% (S. aureus), 1,04% dan 4,18% (E. coli), 0,59% dan 2,39% (B. cereus). Ekstrak terpilih digunakan dalam tahap pengujian stabilitas ekstrak terhadap suhu pemanasan (60, 70, 80, dan 90°C), waktu pemanasan (15 dan 30 menit), dan ph (4, 5, 6, dan 7). Berdasarkan studi ini, konsentrasi suhu 60°C selama 15 menit dan ph 4, kondisi ini menghasilkan ekstrak dengan penurunan zona hambat terkecil terhadap nilai penghambatan ekstrak kontrol. Ekstrak bunga lawang termasuk dalam kategori senyawa antibakteri yang mengandung senyawa antibakteri mayor berupa golongan terpenoid (anethole, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-norpinene, β-caryophyllene, β-bisabolene); dan senyawa antibakteri minor berupa golongan asam lemak (6-octadecenoic acid, hexadecanoic acid, stearic acid) dan benzaldehyde (4-anisaldehyde, p-allylanisole) were also founded as minor compound.

Kata kunci: antibakteri, Illicium verum Hook. f., pH, stabilitas, suhu, waktu

*Corresponding author: Phone: +62-8128187282; Email: eveline.fiti@uph.edu

Available online at http://jurnal.permi.or.id/index.php/mioline
DOI: 10.5454/mi.14.1.3

2016; Wei et al. 2014). The potency of star anise as antimicrobial agent then encouraged further research to increase the possibility to use star anise as natural antibacterial agent in food industry.

Crude extract of star anise was obtained using maceration method with ethanol (polarity index 5.2) and acetone (polarity index 5.1) as solvent for 3 days. The crude extract then diluted into 5 levels of concentration (10, 20, 30, 40, and 50% (w/v)) and tested with well diffusion method against 3 species of...
bacteria (S. aureus, E. coli, and B. cereus). Previous research regarding solvent used in antibacterial compound extraction to apply in food product by Ahmad and Youssef (2015), Dasgupta and Klein (2014), and Das and Kumar (2013) showed that ethanol and acetone work effectively to extract phytochemical compound (flavonoids, alkaloids, triterpenoids, tannins, steroids, and glycosides) which can be used as antibacterial agent against E. coli, S. aureus, and B. subtilis. Aguda and Chen (2016) added that both ethanol and acetone were categorized as Generally Recognized as Safe (GRAS) by Food and Drug Administration (FDA).

The selected concentration was then used to test extract stability against 4 levels of heating temperature (60, 70, 80, and 90 °C) for 2 levels of heating time (15 and 30 minutes), and 4 levels of pH (4, 5, 6, and 7). The heating temperature and time were determined based on previous research by Peter (2001) regarding the stability of trans-anethole in fennel extract (Foeniculum vulgare) at 70°C for 15 minutes heating treatment. Research by Handayani and Sriherfyna (2016) and Surono et al. (2016) also showed that bioactive compounds were commonly degraded at temperature more than 50°C, also the common temperature used in food processing starts around 63°C for 30 minutes. Level of pH used in this research was determined based on the pH of food product (3.5-7.0) and the ability of pathogenic microbes to grow at pH 4.0-9.5 (Surono et al. 2016). Analysis about Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), toxicity analysis using Brine Shrimp Lethality Test (BSLT), and Gas Chromatography-Mass Spectrometry (GC-MS) were also conducted in this research. The results of this research hopefully can be used as source of information, especially regarding optimum conditions of star anise extract for application in food industry.

MATERIALS AND METHODS

Material. Dry star anise (“SAJP” brand), ethanol, acetone, alcohol, dimethyl sulfoxide (DMSO), hexane, NaCl 0.85%, nutrient agar (NA), nutrient broth (NB), HCl 0.1 N, NaOH 0.1 N, pH 4 and 7 buffer solution, and isolates of Staphylococcus aureus (Gram positive bacteria), Escherichia coli (Gram negative bacteria, and Bacillus cereus (spore bacteria).

Sample Preparation Phase Methods. Sample preparation steps that were performed to obtain star anise powder, included size reduction (35 mesh), conducting proximate analysis, and determining optimum time of tested bacteria growth using bacterial growth curve. Total colony used for well diffusion test in this research were 10^4 CFU mL^-1.

Phase I Methods. Phase I research (Fig 1) started with maceration process of star anise powderer (1:10; 20-25 °C) using ethanol and acetone for 3 days. Extraction was executed by constant shaking at 150 rpm. Filtration of the filtrate (using Whatman No.1 and Buchner vacuum) and evaporation (40 °C; 35 rpm; 1 hour) were carried out in order to produce antibacterial compound extract. Extract was then diluted to 5 levels of concentration (10, 20, 30, 40, and 50% (w/v)). Antimicrobial activity test was conducted using well diffusion method in order to determine selected extract which was the best extract concentration in this research.

Phase II Methods. In phase II (Fig 1), the stability test was performed by setting the extract into different temperature (60, 70, 80, and 90 °C) with different heating time (15 and 30 minutes), and pH (4, 5, 6, and 7); MIC and MBC test; component analysis using GC-MS; and toxicity test (Sangi 2012) were also performed toward selected extract from phase I research.

Analysis. Proximate analysis in phase I research was used to determine the water content of dry star anise powder (AOAC 2005). MIC and MBC in Phase II research were used to analyze the minimum value needed to inhibit bacterial growth and kill 90% of tested bacteria (Bloomfield 1991). In addition, to test the cytotoxicity of extract, we used BSLT method (Sangi 2012), whilst GC-MS test was used to analyze major antibacterial compound in extract.

Experimental Design. Experimental design used in phase I research was Completely Randomized Design with one factor and five levels (10% [A1], 20% [A2], 30% [A3], 40%, [A4]), and 50% [A5]) with three repetition. Experimental design used in phase II research for stability against heating temperature and time was Completely Randomized Design with two factors and three repetition. Temperature factor used four levels which were: 60 (A1), 70 (A2), 80 (A3), and 90°C (A4); while heating time used two levels which were: 15 minutes (B1), and 30 minutes (B2). Stability test against pH value was performed using Completely Randomized Design with one factor and three repetition. Levels of pH were: 4 (A1), 5 (A2), 6 (A3), and 7 (A4).
RESULTS

Phase I. The yield of 72 hours maceration extraction using ethanol was 15.19 ± 0.35% and 12.45±0.37% using acetone. Ethanol is a polar solvent, so it is easy to attract most of the polar in star anise.

The Bacteria Used. Bacteria used in test were in log phase after 6 hours of incubation period. Total colony used for well diffusion in this research were 10⁻¹ CFU mL⁻¹; total colony of S. aureus, E. coli, and B. cereus used in test were 1.9x10⁵ CFU mL⁻¹, 1.8x10⁶ CFU mL⁻¹, and 2.5x10⁶ CFU mL⁻¹.

Inhibition Diameter Based on Extract Concentration. Table 1 showed that extract concentration significantly affecting inhibition diameter (p<0.05). The bigger extract concentration would produce larger inhibition diameter. Table 1 also showed that ethanol was proven to be effective in inhibiting the growth of tested bacteria. At a concentration of 30%, ethanol was able to inhibit the bacteria with more than 6 mm inhibition diameter, it was minimal inhibition according to Khalaphallah (2015); thus, the extract concentration used for the next phase was 30%.

Phase II. Phase II Research was done in order to determine the stability against heating temperature (60, 70, 80, and 90 °C) and heating time (15 and 30 minutes), and pH (4, 5, 6, and 7). In this phase, MIC and MBC test, toxicity test, and component analysis using GC-MS were also done towards selected extract from phase I.

Extract Stability Based on Heating Temperature and Time. Statistical test results (Table 2) showed that stability of antibacterial activity of extract on S. aureus and E. coli was affected by heating temperature and time (p<0.05), but there was no interaction between these two factors; when tested on B. cereus (Table 3), stability of antibacterial activity of extract was affected by both factors (heating temperature and time) interactively (p<0.05). Higher
heating temperature and time tend to produce smaller inhibition diameter. When heated for more than 60°C for 15 minutes heating period, the extract was not showing active antibacterial activity (inhibition diameter < 6 mm).

**Extract Stability Based on pH.** Changes in pH affected the inhibition diameter (p<0.05). The addition of HCl 0.1 M as the acid regulator and NaOH 0.1 M as base regulator on extract gave significant difference compared to control extract with pH ~ 5.66 (the extract without any treatment). Table 4 showed that pH escalation in extract towards neutral produced smaller inhibition diameter, and antibacterial compound in star anise extract was more stable at pH 4-5.  

**MIC and MBC.** MIC and MBC value were determined based on selected extract's (30%
concentration) inhibition zone. MIC value was the minimum concentration needed to inhibit 90% growth of tested bacteria, while MBC value was the minimum concentration needed to kill 90% of tested bacteria (Zadrazilova et al. 2015). MIC and MBC test results were 1.59% and 6.36% for S. aureus, 1.04% and 4.18% for E. coli, and 0.59% and 2.39% for B. cereus (Table 1).

**Toxicity.** Selected star anise extract was categorized as low toxic compound (LC$_{50}$ = 212.09 ppm) in its function as antibacterial compound that contains as follows (in the order of the best antibacterial potential).

**GC-MS.** Star anise extract contained major antibacterial compounds in the form of terpenoids (anethole, β-caryophyllene, β-bisabolene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-norpinene, β-Linalool, p-allylanisole, Trans-γ-bisabolene); and minor antibacterial compounds in the form of fatty acids (6-octadecenoic acid, hexadecanoic acid, stearic acid) and benzaldehyde (methoxy acetophenone, 4-anisaldehyde, 3-propenylphenol).

**DISCUSSION**

Star anise extract made using ethanol for 3 days extraction time at 30% concentration effectively exerted best inhibition toward three food spoiling bacteria (Staphylococcus aureus, Escherichia coli, and Bacillus cereus). According to Baldosano et al. (2015), most of the compounds in star anise tend to be polar, thus the amount extracted using ethanol solvent was greater than acetone solvent. More active compounds found in an extract would produce larger inhibition diameter (Khasanah 2014). According to Khalaphallah (2015), antimicrobial compound was categorized as active if the inhibition diameter is more than 6 mm. Antibacterial activity test result of star anise extract using ethanol was proven to be effective in inhibiting the growth of tested bacteria. Tannin and phenolic compounds were antimicrobial agent that tend to be polar, thus both were extracted more in ethanol extract and resulting greater inhibition zone than acetone extract (Iloki-Assanga et al. 2015; Wijayanti, 2016; Medini et al. 2014; Chouksey et al. 2013).

MIC and MBC value against the three tested bacteria respectively were: 1.59% and 6.36% (S. aureus), 1.04% and 4.18% (E. coli), and 0.59% and 2.39% (B. cereus). The more susceptible the bacteria to anethole (Mohammed 2009). Different structure and outer cell membrane component caused different MIC and MBC value in S. aureus (Gram positive) and E. coli (Gram negative), this was also affected by membrane sensitivity against specific antimicrobial compounds. Gram negative bacteria have more complex cell wall which contain thin peptidoglycan (10-50%). Antimicrobial compounds destroy outer cell membrane through porins (hydrophilic pathway) then separate phospholipids and lipopolysaccharides. Gram positive bacteria don't have outer cell membrane, but it's composed with thicker peptidoglycan (90%), thus generally harder to be penetrated by antimicrobial compounds.

Star anise extract was unstable toward heating treatment and changes in pH value; but heating treatment at 60 °C for 15 minutes and pH 4-5 was able to exert closest result in inhibiting bacterial growth when compared to control extract. Generally as the heating temperature and time escalated, the antibacterial compounds lost its stability (thermolabile) because chemical structure of the compounds was degraded during heating and the antimicrobial activity decreased (Turek and Stintzing 2013; Sant’Anna et al. 2012; and Zhang et al. 2014). Ardiansyah (2002) reported that in certain specific condition, antibacterial activity could also increase as the temperature increases. Degradation of extract compounds formed new compounds which also have the potential to inhibit microorganism growth. Oxidized anethole compound would form 4-methoxy benzaldehyde (4-anisaldehyde) and anis ketone which act as antimicrobial compound (Okamoto et al. 2002; Fahlbusch et al. 2003; Kosalec et al. 2005).

Escalation in pH also affected extract stability. The presence of fatty acids, such as hexadecanoic acid, 6-octadecenoic acid, and stearic acid in extract made the extract more stable at pH 4-5. pH can affect the absorption of fatty acids in bacteria. As the pH increase, effectiveness of fatty acid absorption would decrease, thus the antibacterial activity also decreased (McGaw et al. 2002). Purbowati et al. (2016) and Pan et al. (2014) added that phenolic compounds in the extract are damaged by increased pH and causing the antibacterial activity became less effective. In reverse, a decrease in pH (an increase in H$^+$ ions) causes bacterial cytoplasm became less stable and it needs more energy to restore the cell's internal pH to normal state. Cell metabolism will be disturbed as more energy required to keep the normal state of cell's internal pH, then the bacteria cells will die over time (Naufalin et al. 2006). The decrease in pH also increases the stability and effectiveness of phenolic compounds which are more hydrophobic in acidic conditions thereby facilitating the dissolution of
bacterial membrane fat (Purbowati et al. 2016; Pan et al. 2014).

Toxicity value (LC₅₀) indicates the safety level of extract to be applied in food products. This test was done as the first step in other complex toxicity test. Based on the principle of Brine Shrimp Lethality Test (BSLT), the more amount of compound needed to kill 50% of shrimp larvae, then the compound will be categorized as more non-toxic. Juntiarti et al. (2009) divides the toxicity level of LC₅₀ into three categories which are: LC₅₀ > 1000 ppm as non-toxic, 30 < LC₅₀ < 1000 ppm as low toxic, and LC₅₀ < 1000 ppm as toxic. Test result showed that star anise extract had LC₅₀ of 212.09 ppm (low toxic). Nakamura (1996) reported that veranisatin A, B, and C were toxic compound which contributed in star anise toxicity test, but the concentration of veranisatin A, B, and C in dry star anise were low, respectively, 0.00016%, 0.00010%, and 0.000015% and it's categorized as safe to use in medicine and food products. FDA already classified Illicium verum Hook. f. as generally recognized as safe (IOFI 2017). Dewi et al. (2012), Soctan and Oywole (2009), and Yunilla (2016) added that the natural antinutrient bioactive components formed by plant metabolism can also be toxic, such as glycosides, tannins, lignin, triterpenoids, oxalates, and amino acids. Low toxicity is considered safe and harmless if consumed as herbal intake at the right dose (Yunilla 2016). Further in vivo research is needed as dose reference for the application in food products that are safe for humans (BPOM 2014).

The major antibacterial compound in star anise extract, including terpenoids (anethole, β-caryophyllene, β-bisabolone, 2,6-dimethylyl-6-(4-methyl-3-pentenyl)-2-norpinene, β-Linalool, p-allylanisole, Trans-γ-bisabolene); and minor antibacterial compound, including fatty acids (6-octadecenoic acid, hexadecanoic acid, stearic acid) and benzaldehyde (methoxy acetophenone, 4-anisaldehyde, 3-propenylphenol). All component were categorized as essential oil that could act as antibacterial agent (Attokaran, 2017; Aly et al. 2016; Taniguchi et al. 2014; Herman et al. 2016; Andrade et al. 2015; Okatomo et al. 2002; Fahlfusich et al. 2003; Chong et al. 2015; Carneiro et al. 2017; Dahham et al. 2015; McWilliams, 2006; Courtois et al. 2012; Rai et al. 2011; Marteau et al. 2016; Karimi et al. 2015; Uitterhaegen et al. 2016). Okamoto et al. (2002), Tisserand and Young (2014), and Kosalec et al. (2005) added that oxidized anethole will form anis ketone which also acts as antimicrobial agent. In the recent study of Alhajj et al. (2019) showed that star anise alcoholic extract have antibacterial and antifungal activities and could be used as natural antimicrobial agent. The stability of star anise volatile essential oil and its antibacterial activity can be significantly enhanced by using HPCD encapsulation (Zhang et al. 2018).

REFERENCES

Aguda R, Chen CC. 2016. Solubility of nutraceutical compounds in generally recognized as safe solvents at 298K. Int J Chem Eng Appl. 7(5):289-294. doi: 10.18178/ijcea.2016.7.5.591.

Ahmad AF, Youssef SH. 2015. Chemical composition and bioactive properties of Illicium verum (star-anise) extracts prepared by different methods. JCBPSC. 5(2):1160-1170.

Allhaj MS, Qasem MAA, Nabi ARJE, Al-Murarraj SI. 2019. In vitro antibacterial and antifungal effects of high levels of chinese star anise. Braz J Poult Sci. 21(1):1-8. doi: 10.1590/1806-9061-2016-0427.

Andrade TCB, De Lima SG, Freitas RM, Rocha MS, Islam T, Da Silva TG, Militao GCG. 2015. Isolation, characterization and evaluation of antimicrobial and cytotoxic activity of estragole, obtained from the essential oil of Croton zeintheri (Euphorbiaceae). An Acad Bras Cienc. 87(1):173-182. doi: 10.1590/0001-3765201520140111.

Aly SE, Sabry BA, Shaheen MS, Hathout AS. 2016. Assessment of antimycotoxigenic and antioxidant activity of star anise (Illicium verum) in vitro. J Saudi Soc Agric Sci. 15:20-27. doi: 10.1016/j.jssas.2014.05.003.

AOAC. 2005. Official methods of analysis of the association of official analytical chemists. Washington: AOAC Inc.

Arđiansyah. 2002. Kajian aktivitas antimikroba ekstrak daun beluntas (Pluca indica L.). Bogor: Institut Pertanian Bogor.

Attokaran M. 2017. Natural food flavors and colorants 2nd ed. Chicago: John Wiley & Sons.

Badal S, Delgoda R. 2017. Pharmacognosy: fundamentals, applications and strategy. Cambridge: Elsevier Inc.

Baldosano HY, Castillo BMG, Elloran CDH, Bacani FT. 2015. Effect of particle size, solvent and extraction time on tanning extraction from Spondias purpurea bark through soxhlet extraction. Manila: Presented at the DLSU Research Congress, March 2-4.

Bloomfield SF. 1991. Assessing antimicrobial activity. Oxford: Blackwell Scientific Publication.

Carneiro NS, Alves CCF, Egea MB, Martins CHG, Silva TS, Bretanha LC, Beleste MP, Micke GA, Silberira EV, Miranda MLD. 2017. Chemical composition, antioxidant, and antibacterial activities of essential oils from leaves and flowers of Eugenia klotzschiana Berg
Chong SP, Osman MF, Bahari N, Nuri EA, Zakaria R, Abdul-Rahim K. 2015. Agarwood inducement technology: a method for producing oil grade agarwood in cultivated *Aquilaria malaccensis* Lamk. J Agrobiotech. 6:1-16.

Chouksey D, Upmanyu N, Pawar R. 2013. Central Nervous System Activity of *Illicium verum* Fruit Extracts. Asian Pac J Trop Med. 6(11):869-875. doi: 10.1016/S1995-7645(13)60155-8.

Courtois EA, Baraloto C, Paine CET, Petronelli P, Blandinieres PA, Stien D, Houel E, Bessiere J, Chave J. 2012. Differences in volatile terpenes composition between the bark and leaves of tropical tree species. *Phytochemistry*. 82:81-88. doi: 10.1016/j.phytochem.2012.07.003.

Das MP and Kumar SS. 2013. Preliminary phytochemical analysis of *Illicium verum* and *Wedelia chinensis*. Int J PharmTech Res. 5(2):324-329.

Dasgupta A, Klein K. 2014. Antioxidants in food, vitamins and supplements: prevention and treatment of disease. San Diego: Elsevier.

Dewi CSU, Soedharna D, Kawaroe M. 2012. Komponen fitokimia dan toxsisitas senyawa bioaktif dari lamun *Enhalus acoroides* dan *Thalassia hemprichii* dari pulau Pramuka, DKI Jakarta. Jurnal Teknologi Perikanan dan Kelautan. 3(2):23-27.

Fahlbusch KG, Franz-Josef H, Panten J, Pickenhagen W, Schatkowski D, Kurt B, Garbe D, Surburg H. 2003. Flavors and fragrances in Ullmann’s encyclopedia of industrial chemistry. Weinheim: Wiley-VCH.

Handayani H, Sriherfyna FH. 2016. Ekstraksi antosidak dari sirsak metode ultrasonik bath (kajian rasio bahan: pelarut dan lama ekstraksi). Jurnal Pangan dan Agroindustri. 4(1):262-272.

Herman A, Tambor K, Herman A. 2016. Linalool affects the antimicrobial efficacy of essential oils. Curr Microbiol. 72(2):65-72. doi: 10.1007/s00284-015-0933-4.

Iloki-Assanga S, Lewin-Lujan LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL, Haines DD. 2015. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. BMC Res Notes. 8:396-410. doi: 10.1186/s13104-015-1388-1.

International Organization of the Flavor Industry (IOFI). 2017. Global reference list of natural complex substances/natural flavouring complexes available from https://jr.chemwatch.net/galleria/LEGSSREGS/40-1-11-547-0-0-AA-20170217.pdf.

Juniarti, Osmeli D, Yuhermita. 2009. Kandungan senyawa kimia, uji toksisitas (brine shrimp lethality test) dan antiosidan (1,1-diphenyl-2-picrylhidrazyl) dari ekstrak daun saga (*Abus precatorius* L.). Makara Sains. 13(1):50-54. doi: 10.7454/mss.v13i1.378.

Karimi E, Jaafar HZ, Ghaseemzadeh A, Ebrahimi M. 2015. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of *Labisia pumila* Benth. Biol Res. 48(1):9-14. doi: 10.1186/0717-6287-48-9.

Khalaphallah R. 2015. Antimicrobial activity of some heterocyclic compounds and herbal extracts on plant pathogens. Chem Sci Rev Lett. 4(13):171-178.

Khasanah I. 2014. Ekstrak etanol daun kersen (*Montingia calabura* L.) sebagai antibakteri terhadap *Staphylococcus agalactiae* penyebab mastitis subklinis pada sapi perah. Malang: Universitas Brawijaya.

Kosalec I, Pepeljnjak S, Kustrak D. 2005. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., *Apiaceae*). Acta Pharm. 55:377-385.

Marteau C, Guittard R, Penverne C, Favier D, Rataj VN, Aubry JM. 2016. Boosting effect of *ortho*-propenyl substituent on the antioxidant activity of natural phenol. Food Chem. 196:418-427. doi: 10.1016/j.foodchem.2015.09.007.

McGaw LJ, Jager AK, Staden JV. 2002. Antibacterial effects of fatty acids and related compounds from plants. S Afr J Bot. 68:417-423. doi: 10.1016/S0255-6299(15)30677-7.

McWilliams M. 2006. Nutrition and dietetics 8th ed. Jurong: Pearson Education Inc.

Medini F, Fellah I, Ksouri R, Abdelly C. 2014. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. J Taibah Uni Sci. 8(3):216-224. doi: 10.1016/j.jtusci.2014.01.003.

Mohammed MJ. 2009. Isolation and identification of anethole from *Pimpinella anisum* L. Fruit oil: an antimicrobial study. J Pharm Res. 2(5):915-919.

Nakamura T, Okuyama E, Yamazaki M. 1996. Neurotropic substances/natural flavouring complexes available from https://jr.chemwatch.net/galleria/LEGSSREGS/40-1-11-547-0-0-AA-20170217.pdf.

Nakamura T, Okuyama E, Yamazaki M. 1996. Neurotropic substances/natural flavouring complexes available from https://jr.chemwatch.net/galleria/LEGSSREGS/40-1-11-547-0-0-AA-20170217.pdf.

Nakamura T, Okuyama E, Yamazaki M. 1996. Neurotropic substances/natural flavouring complexes available from https://jr.chemwatch.net/galleria/LEGSSREGS/40-1-11-547-0-0-AA-20170217.pdf.
Okamoto K, Narayama S, Katsoo A, Shigematsu I, Yanase H. 2002. Biosynthesis of p-anisaldehyde by the white-rot basidiomycete *Pleurotus ostreatus*. J Biosci Bioeng. 93(2):207-210. doi: 10.1263/jbb.93.207.

Pan X, Mak LM, Nakako H. 2014. Efficacy of essential oils on inactivation of *Escherichia coli* O157:H7 in vegetable juice. Food Sci Technol Res. 20(5):1043-1049. doi: 10.3136/fstr.20.1043.

Peter KV. 2001. Handbook of herbs and spices. Boca Raton: CRC Press.

Pan X, Mak LM, Nakako H. 2014. Efficacy of essential oils on inactivation of *Escherichia coli* O157:H7 in vegetable juice. Food Sci Technol Res. 20(5):1043-1049. doi: 10.3136/fstr.20.1043.

Peter KV. 2001. Handbook of herbs and spices. Boca Raton: CRC Press.

Purbowati ISM, Syamsu K, Warski E, Sri H. 2016. Stabilitas senyawa fenolik dalam ekstrak dan nanokapsul kelopak bunga rosella pada berbagai variasi pH, suhu dan waktu. *Agrointek*. 10(1):31-40. doi: 10.21107/agrointek.v10i1.2023.

Rai M, Acharya D, Rios JL. 2011. Ethnomedicinal Plants: Revitalization of traditional knowledge of herbs. Boca Raton: CRC Press.

Sangi M, Momuat L, Kumaunang M. 2012. Uji toksisitas dan skrining fitokimia tepung gabah pelepah aren (*Arenga pinnata*). Jurnal Ilmiah Sains. 12:128-134. doi: 10.35799/jis.12.2.2012.716.

Sant’Anna V, Cladera-Olivera F, Brandelli A. 2012. Kinetic and thermodynamic study of thermal inactivation of the antimicrobial peptide P34 in milk. Food Chem. 130:84-89. doi: 10.1016/j.foodchem.2011.07.001.

Sivakumar P, R NS, B JP, M JT, N, P, S, K H. 2016. Antimicrobial activity of selected spices - a bio preservative approach. *J Chem Pharm Sci*. 9:304-307.

Shete HG, Chitanand MP. 2014. antimicrobial activity of some commonly used Indian spices. *Int J Curr Microbiol App Sci*. 3:765-770.

Soetan KO, Oyewole OE. 2009. The need adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds. *Afr J Food Sci*. 3:223-232.

Surono IS, Subdibyo A,Waspodo P. 2016. Pengantar keamanan pangan untuk industri pangan. Yogyakarta: Deepublish.

Taniguchi S, Shinonaga YH, Tamaoki D, Yamada S, Akimitsu K, Gomi K. 2014. Jasmonate Induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. *Plant Cell Environ*. 37:451-461. doi: 10.1111/pce.12169.

Tisserand R, Young R. 2014. Essential oil safety: a guide for health care professionals 2nd ed. China: Elsevier.

Wei L, Hua R, Li M, Huang Y, Li S, He Y, Shen Z. 2014. Chemical composition and biological activity of star anise *Illicium verum* Extracts against maize weevil, *Sitophilus zeamais* adults. *J Insect Sci*. 14:1-13. doi: 10.1093/jis/14.1.80.

Wijayanti MN. 2016. Uji Aktivitas antioksidan dan penerapan kadar fenolik total ekstrak etanol buah buni (*Antidesma bunius* (L.) Spreng) dengan metode 2,2-diphenyl-1-picrylhydrazyl (DPPH) dan metode folin-ciocalteu. Yogyakarta: Universitas Sanata Dharma.

Yunilla I. 2016. Kajian aktivitas dan stabilitas antibakteri ekstrak daun bintaro (*Cerbera oddolam Gaertn.*). Tangerang: Universitas Pelita Harapan.

Zhang J, Han J, Oyeleye A, Liu M, Liu X, Zhang L. 2014. Extraction methods of natural products from traditional Chinese medicines. *Methods Mol Biol*. 1263:177-185. doi: 10.1007/978-1-4939-2269-7_14.