Utilization of jasmine flower extract as antimicrobial in tempeh sausage

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Abstract. Bacterial contamination is a major problem for sausage products. Jasmine extracts that has antimicrobial compounds can extend the shelf life of tempeh sausage naturally. The purpose of this study was to tested the effectiveness of using jasmine extract as an antimicrobial agent in tempeh sausage during cold storage 4°C. This study began with preparing jasmine extracts by maceration method using waters, methanol, ethyl acetate and hexane as solvents. Bacteria used in the test were *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. Diffusion disc assay was used with completely randomized design 2 factors and 4 replications. The results showed that the jasmine extracts with a concentration of 25%, 50%, 75% and 100% had an effect to inhibit of the growth of the bacteria. Jasmine extract with ethyl acetate solvent was the most effective to inhibit the growth of bacteria. Concentration of jasmine extracts that can inhibit the growth of bacteria *Escherichia coli* and Staphylococcus aureus was 0.1% and 0.25%. Tempe sausage with the addition of jasmine extracts was tested microbiologically with total plate count, The test results showed that the addition of low concentrations extracts of jasmine on tempeh sausage could decrease the total microbial at cold storage for 5 days.

Keywords: jasmine, antimicrobials, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*

1. Introduction

Jasmine flower is one of the high economic value commodities, its usefulness is not only as an ornamental plant pot and garden, but also as tea deodorizer, perfume industry raw materials, cosmetics, traditional medicine, and complementary in traditional ceremonies. Species *Jasminum sambac* Ait is a species that is very popular and has been crowned as a nation's flower and is widely used for flower arrangements and tea fragrances. Jasmine flowers are usually also extracted into essential oils and the price is very expensive. The content of chemicals in jasmine has the power to relieve stress, depression, and can help regain confidence. Jasmine flowers are also used in biotherapy and perfume industries. Jasmine flowers contain benzilic acetate, linalcohol, benzilic alcohol, indol and jasmon. All of these substances provide jasmine aphrodisiac properties [1-4].

Some of the useful properties of jasmine flowers include: improving digestion, adjuvants in eliminating toxins and heavy metals. Jasmine flowers also help accelerate metabolism and improve

blood circulation associated with aphrodisiac properties. In addition, jasmine flowers are also known for tea consumption, in Chinese civilization jasmine tea is drunk during spring, summer, autumn and especially in winter. Utilization of jasmine flowers (*Jasminum sambac* Ait) as traditional medicine due to the content of active compounds it has. Active compounds in white jasmine flowers (*Jasminum sambac* Ait) revealed the presence of dotriacontanol compounds, oleanolic acid, daucosterol hesperidin isolated from the roots. The content of active compounds such as alkaloids, flavonoids, saponins and tannins in Jasit sambac Ait can also function as antiseptics and antioxidants so that it is believed that plants that contain these compounds can be used as antibacterials [5-7]. Essential oils and methanol extract of jasmine flowers were reported to have antimicrobial activity against *Enterococcus faecalis, Salmonella enteric*, *Streptococcus pyogenes* and *Bacillus cereus* when tested using disc diffusion method and dilution method. Natural preservation by using plants is an alternative. Some plants contain compounds that are antimicrobial which can be used to inhibit growth and kill microbes that are pathogenic, one of which is jasmine flowers. Various roles of plants, especially plants that have medicinal properties can continue to be improved and utilized, it is necessary to explore more deeply through research and testing of disease-causing microorganisms. Based on this background, it is necessary to do research on white jasmine flower extract (*Jasminum sambac* Ait) as a natural antimicrobial material [8-11]. Research on the extraction of jasmine flowers has not been much done, therefore it is necessary to test the extraction of antimicrobial active compounds in white jasmine flowers and see their effect on inhibiting microbial growth. One test can be done by looking at the effect of the extract in inhibiting microbial growth such as *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*. The types of microbes studied in this study can abnormally be pathogenic to humans such as diarrheal diseases by *Escherichia coli* and skin infections, ulcers and ulcers by *Staphylococcus aureus* and *Bacillus cereus* which can cause chronic diarrhea which is very dangerous for humans. In addition, this study also directly saw the application of natural extracts from jasmine flowers into tempeh-based sausage products [12-15].

2. Research Method

The tools used in this study are: glassware, laminar air flow cabinet, autoclave, blender, flow pipette, caliper, oven, visible spectrophotometer, ose needle, spatula, desiccator, tweezers, bunsen, gas stove, bottle vials, refrigerators, porcelain crusts, analytic scales, incubators, rotary evaporators, desiccators, spatulas, bunsen lamps, petri dishes. Equipment used for calculating colony counts. The ingredients used: jasmine flowers with the criteria of semi-blooming florets, *Staphylococcus aureus* bacteria (ATCC 6538), *Escherichia coli* (ATCC 8938) *Bacillus cereus* (KCCM 40152), chemicals and media namely NA (Nutrient Agar), MHA (Mueller Hinton agar), Nutrient Broth (NB), dimethyl sulfoxide (DMSO), physiological Na Cl 0.9%, 70% aquadest, disc paper (Oxoid), methanol, ethyl acetate, and hexane. The material for bacterial testing is plate count agar (PCA) media. The study was conducted by using a completely randomized design with two factorials. This research consisted of 3 stages, namely 1. Phytochemical test of jasmine flower extract on various types of solvents, 2. Antimicrobial test, 3. Activity tests of inhibition of bacteria Escherichia coli, Staphylococcus aureus, and Bacillus cereus on jasmine flower extracts with various variations. Flow chart of the research method as shown in Figure 1.

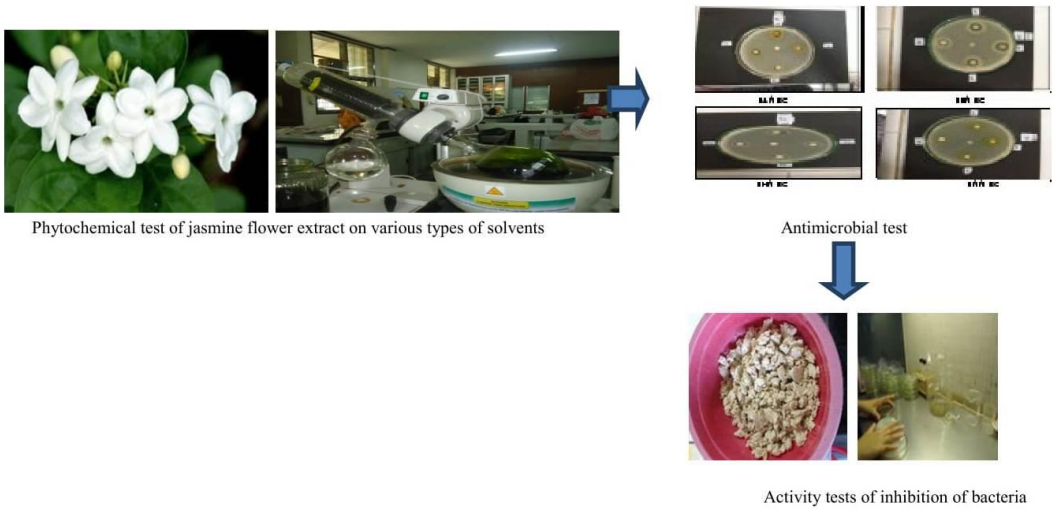

Figure 1. Flowchart of the research method

3. Results and Discussions

3.1. Results of Jasmine flower water powder analysis

The initial stage in analyzing the water content of jasmine flower simplicia is to make jasmine flower powder first by way of sorted and cleaned jasmine flowers then dried in an oven at 40°C for 48 hours, then milled and sifted with a 20 mesh sieve, then obtained flower powder dried jasmine and analyzed its water content. The results of the analysis of water content in jasmine flower powder were obtained at 8.4%. The results of the analysis obtained are smaller than 10%, which is in accordance with the requirements set by Materia Medika Indonesia (MMI). Water content that exceeds 10% can be a good source of media for fungal growth at the time of storage, so it can reduce the quality of simplicia [16-20].

3.2. Jasmine flower extraction

In the process of extraction of jasmine flowers begins with the preparation and supply of samples with a wet weight of 7 kg, then the jasmine flower is dried using an oven at 40 ^{0}C for 48 hours, after drying the weight becomes 2.4 kg. The dried jasmine flower is then ground and sieved with a 20 mesh sieve to obtain dried jasmine flower powder. Jasmine flower powder then weighed 480 grams each and then macerated with four types of solvents (water, methanol, acetylate and hexane) for 3 days to obtain the first maceration results of greenish brown colored solution, followed by second and third heating. The results of maceration were then on the rotary evaporator to obtain thick jasmine extract. The extract was then weighed and obtained the results of jasmine flower extract from each solvent, namely: jasmine flower extract with water solvent 19 g, jasmine flower extract with methanol 49.1g, jasmine flower extract with ethyl acetate 11.6 g and jasmine flower extract with hexane solvent 6.9g.

3.3. Phytochemical Test Results of Jasmine Flower Extract

Phytochemical testing aims to determine and identify the components of bioactive compounds contained in jasmine flower extract. The components of the active compounds identified in this study include: alkaloids, steroids/triterpenes, saponins, tannins, flavonoids, phenolics and glycosides. From the results of the tests showed the presence of several bioactive compounds contained in jasmine flower extract with types of water, methanol, ethyl acetate and hexane solvents which can be seen in Table 1. below

Table 1. Phytochemical test results of jasmine flower extract

Bioactive Compounds	Solvent jasmine flower extract			
	water	methanol	ethyl acetate	hexane
Alkaloid	+	+	-	-
Steroid/Triterpen	+	+	+	+
Saponin	+	-	-	-
Tanin	+	+	-	-
Flavonoid	-	+	+	-
Fenolik	+	+	+	-
Glikosida	-	+	-	+

Description: (+) = Contains a class of compounds
(-) = Does not contain compounds

The results of testing jasmine flower extract in each type of solvent, showed that the most optimal solvent to attract bioactive compounds was methanol solvent. From the results of phytochemical screening tests, it was found that hexane solvents found in compounds such as alkaloids, steroids/triterpenes, and glycosides. Ethylacetate solvents obtained for compounds such as steroids/triterpenes, flavonoids, and phenolics, whereas in methanol solvents, almost all compounds can be withdrawn by methanol solvents except for saponin compounds. The ability of methanol to attract almost all bioactive compounds is because methanol is a universal solvent that can attract most of the chemical compounds in plants, where methanol has a polar group (-OH) and nonpolar group (CH) so that it can attract the polar and nonpolar [21]. Likewise with water solvents that are able to attract many compounds such as alkaloids, steroids/triterpenes, saponins, tannins, and phenolics. Saponin has the potential as an antimicrobial compound because of its ability to reduce the permeability of the bacterial cell wall so that it can enter the bacterial cytosol and inhibit its growth.

Saponins are generally in the form of glycosides so they tend to be polar. Saponin compounds are substances that when interacting with the bacterial cell wall the cell wall will break or. Saponins will interfere with the surface tension of the cell wall, so when the surface tension is disrupted the antibacterial substances will easily enter the cell and will disrupt the metabolism until bacterial death finally occurs [22]. The next compound is tannins found in extracts in methanol and water solvents, tannins in plants can inhibit microbial activity. The mechanism of action of tannins as antimicrobials is related to the ability of tannins to inactivate the molecules in the host cell found on the cell surface. Tannins are phenol compounds that cause damage to the cell wall polypetides. The mechanism of tannin inhibition occurs by means of bacterial walls which have been lysed by saponin and flavonoid compounds, thus causing tannin compounds to easily enter bacterial cells and coagulase the protoplasm of *S. aureus* and *E. coli* bacterial cells [23]. From the preliminary results of antimicrobial activity, ethylacetate extract is the most potential extract in inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* bacteria. In the results of phytochemical screening it was found that jasmine extract with ethyl acetate solvents contained bioactive compounds, one of which was flavonoids. Flavonoids are one of the secondary metabolites produced by a plant that can be found in the leaves, tubers, roots, wood, bark, pollen, flowers and seeds. Flavonoids in plants function to regulate growth, regulate photosynthesis, regulate the work of antimicrobials and antiviral agents, and regulate the work of antiseptors.

3.4. Antimicrobial activity of jasmine flower extracts against Escherichia coli

The types of solvents used in jasmine flower extract had a highly significant effect ($p < 0.01$) on the inhibition zone of Escherichia coli growth. The relationship between the effect of the type of solvent on the zone diameter inhibition of the growth of *Escherichia coli* bacteria can be seen in Figure 2.

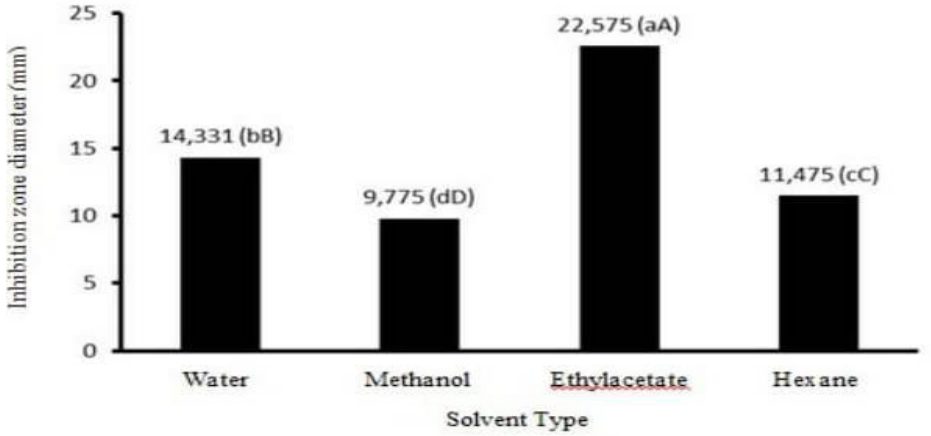

Figure 2. Effect of extract types with inhibiting zones of *Escherichia coli* bacteria growth

Based on Figure 2. each solvent has a significantly different effect. The highest inhibitory zone was obtained at ethylacetate solvent, which was 22.575 mm, while the lowest inhibition zone diameter was obtained in methanol solvent which was 9.775 mm. Based on the test results obtained it can be said that jasmine flowers have the ability to inhibit the growth of *Escherichia coli* bacteria. This is because in jasmine flowers contain several active compounds that have the potential as natural antimicrobials such as flavonoids which have antibacterial activity, phenol compounds such as tannins, triterpenoid/steroid compounds that have antifungal activity. Flavonoids are part of phenol compounds that are widely found in nature, whose mechanism of action is based on the denaturation of bacterial cell proteins that can cause cell death. Ethyl acetate extract of jasmine flower is the most effective extract in inhibiting the growth of *Escherichia coli* bacteria, because the ethylacetate extract has a hydrophilic and lipophilic balance so that it is more optimal in damaging the bacterial cell wall component [24,25]. Based on the results of the study, it was found that jasmine flower extract in each concentration could inhibit the growth of *Escherichia coli* bacteria at the lowest concentration of 25% to the highest concentration of 100%.The relationship between the effect of jasmine flower extract concentration on *Escherichia coli* growth zone diameter can be seen in Figure 3.

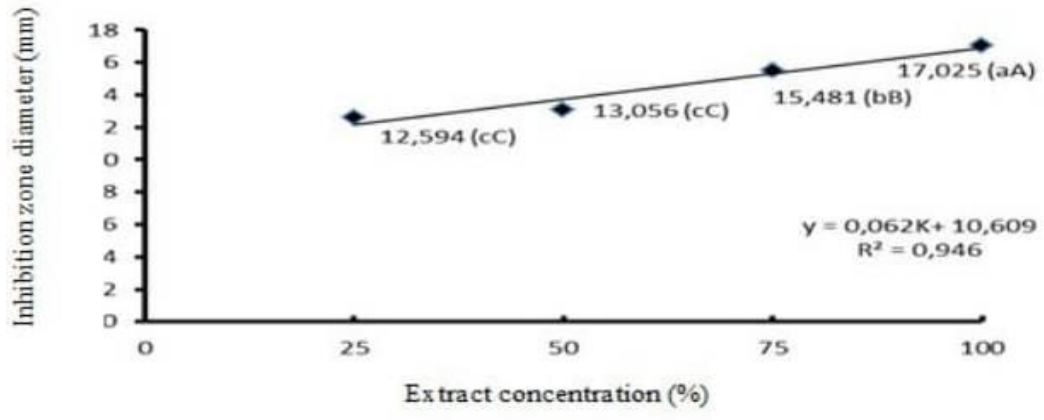

Figure 3. Effect of jasmine flower extract concentration on Escherichia coli growth zones

Giving different concentrations shows different effects on the resulting inhibition zones. The diameter of the inhibitory zone also depends on the absorption of antibacterial substances into the agar plate and the sensitivity of the bacteria to these antibacterial substances. According to, the factors that influence the occurrence of inhibitory zones are the ability to diffuse antimicrobial materials into the media and their interactions with the microbes tested, the number of microbes tested, microbial growth rates, and microbial sensitivity to antimicrobial materials. In the results of the study showed that jasmine flower extract can be made into a satisfying antibacterial category. antimicrobial activity of jasmine flower extract against *Staphylococcus aureus*. The relationship between the effect of solvent types on the diameter of the growth inhibition zone of *Staphylococcus aureus* can be seen in Figure 4.

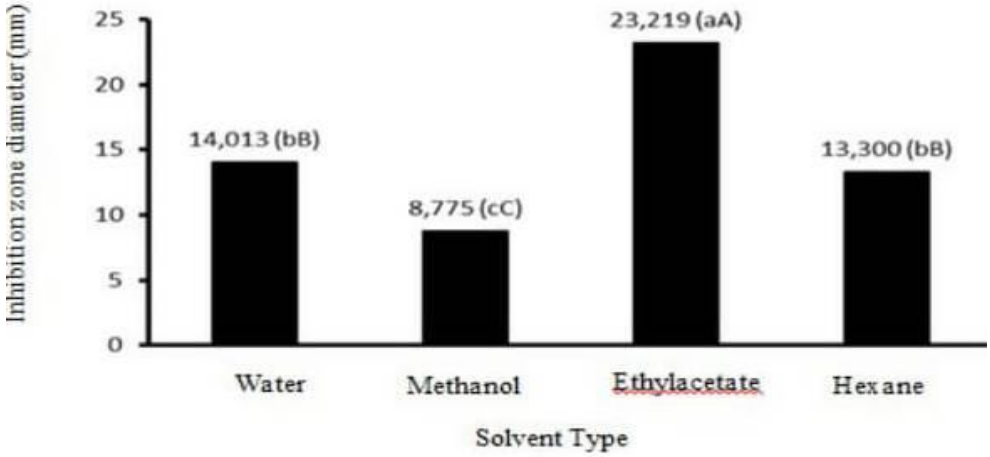

Figure 4. Effect of extract types on *Staphylococcus aureus* bacterial growth zone

The content of steroid compounds in ethyl acetate diffuses more easily and is able to inhibit bacterial growth. Steroids have non polar to semi polar properties. The results of the study explained that the height and diameter of the zones of growth inhibition of *Staphylococcus aureus* jasmine flower extract produced by water, methanol, ethyl acetate and hexane were caused by the properties of each solvent in attracting antibacterial compounds. The use of solvents in extracting foodstuffs will affect the results of testing the bioactive compounds of these foods. Polar compounds are more soluble in polar solvents and non-polar compounds are more soluble in non-polar solvents. Jasmine flower extract has a very significant different effect on the zone diameter of the growth inhibition of *Staphylococcus aureus* bacteria. *Staphylococcus aureus* which is a gram positive bacterium. Generally gram-positive bacteria are more sensitive to antibacterial compounds compared to gram-negative bacteria because the cell walls of gram-positive bacteria have no lipopolysaccharide layer so that antimicrobial compounds that are hydrophilic or hydrophobic can pass through the cell wall of gram-positive bacteria through a mechanism of passive diffusion then interact directly with peptidoglycan in cells bacteria that are growing and causing cell death. Factors that influence the occurrence of inhibitory zones are the ability of diffusion of antimicrobial materials into the medium and their interaction with the tested microbes, the number of microbes tested, microbial growth speed of the test and the level of microbial sensitivity to antimicrobial material, affecting the diameter of the hamlet growth zone.

4. Conclusion

In this research there are almost all bioactive compounds such as: alkaloids, steroids/triterpenes, saponins, tannins, flavonoids, phenolics and glycosides contained in jasmine flower extract with various solvents with different polarity levels. Methanol extract is the most effective extract in attracting all bioactive components. The types of jasmine flower extract with various kinds of solvents gave a very significant effect ($p < 0.01$) on the growth inhibition of Escherichia coli, Staphylococcus aureus, and Bacillus cereus. Jasmine flower extract with ethylacetate solvent is the most effective extract in

inhibiting microbial growth. Analysis of Total Plate Count (TPC) showed that the content of jasmine flower extract had a highly significant effect ($p < 0.01$) on the number of microbes found in tempe sausages. Where the TPC results show the addition of 0.1% and 0.25% jasmine flower extract can reduce the number of microbes for 5 days of storage. The concentration of jasmine flower extract had a significantly different effect on the taste of tempe sausage($p < 0.05$), where the higher concentration of jasmine flower extract added to the sausage tempe would decrease panelists' preference for taste. This is because the two basic ingredients of sausage namely tempeh and jasmine flowers already have a specific basic taste at first.

References

[1] Acton J C, Saffle R L 2007 Stability of oil in water emulsion *J Food Sci* 35(6): 852-854
[2] Duggan C, Walker W A and Watkins J B 2003 Nutrition In Pediatrics 3rd Edition. London: BC Decker 3(1): 223-233
[3] AOAC 1995 Official Methods of Analysis of The Association of Official Analytical Chemists. Washington : AOAC
[4] Bergmann W and Feeney R J 2015 The isolation of a new thymine pentoside from sponges. J Am Chem Soc 72: 2809-2810
[5] Arora D S and Bhardwaj 2010. Antibacterial activity of some medicinal plants, *Geo Bios* 24: 127-131
[6] Proksch P, Edrada R A and Ebel R, 2012 Drugs from the seas current status and microbiological implications *Appl Microbiol Biotechnol* 59: 125-134
[7] Taylor M W, Radax R, Steger D and Wagner M 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol* Rev 71: 295-347
[8] Brandly P J, Migaki G and Taylor K E 2006. Meat Hygiene, 3rd Edit. Lea and Febiger Philadelphia
[9] Brody 2010 Food Technology Packaging 59 (2): 65 – 66
[10] Ravi Varshney, Matthew J and Budoff 2016 *garlic and heart disease*. *The Journal of Nutrition*, Volume 146, Issue 2, 1 Pages 416S–421S
[11] Cappucino J G and Sherman, 1996. Microbiology: a laboratory manual 4th Ed Addison-Wesley Publishing Company
[12] Beena Uppal 2015 A Comparative Study of Bacterial and Parasitic Intestinal Infections in India Journal of Clinical and Diagnostic Research ISSN-0973-709x
[13] Depkes RI 1995 Farmakope Indonesia Departemen Kesehatan Republik Indonesia, Jakarta
[14] Depkes RI 2000 Parameter Standar Umum Ekstrak Tumbuhan Obat Cetakan Pertama. Jakarta : Direktorat Jendral Pengawasan Obat dan Makanan
[15] Dewan Standarisasi Nasional 1995 SNI 01-3818 Bakso Daging Dewan Standardisasi Nasional Jakarta
[16] Grace D 2015 Food Safety In Developing Countries: An Overview A learning resource for DFID Livelihoods Advisers
[17] Zheng Z, Bao B, Jian Y and Xiu T 2004 Studies on chemical constitutents in roots of Jasminum sambac. Zhongguo Zhong Yao Za Zhi 29(3): 237-239
[18] Berrue F, McCulloch M W, Boland P, Hart S and Harper M K. 2012 Isolation of steroidal glycosides from the Caribbean sponge Pandaros acanthifolium. *J Nat Prod* 75: 2094-2100
[19] Zarringhalami S, Sahari M A and Hamidi E Z 2009 Partial Replacement of Nitrite by Annatto as a Colour Additive in Sausage. *Meat Sci*. 81:281-284
[20] Kubo I, Muroi H and Kubo A 1995 Antibacterial activity of long-chain alcohols against Streptococcus mutans *J. Agric Food Chem* 40 (6): 999-1003
[21] Hougton P J, and Raman. 2010. Laboratory Handbook for The Fractionation of Natural Extract. Chapman and Hall London
[22] Harborne J B. 2008. Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan. Bandung: Penerbit ITB

[23] Marthanda P. 2015. Food Safety A Global Public Health Concern. *JMAJ* Vol.58, No.4
[24] Kanazawa A and Ikeada 2010 Laboratory Handbook for the Fractionation of Natural Extract. London: Chapan and Hall
[25] Forrest J G, Alberle E D, Hendrick H B, Judge M D and Merkel R A 2013. Principles of Meat Science W H Freeman, San Fansisco

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