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Preservation Potential of Galangal Water Extract on Tofu

Alwani Hamad[1], Asmiyenti Djaliasrin Djalil[2], Tina Syah Putri[2], Dwi Hartanti[3]

[1]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Jl. Raya Dukuhwaluh, Banyumas 53182, Indonesia.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Jl. Raya Dukuhwaluh, Banyumas 53182, Indonesia.
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Jl. Raya Dukuhwaluh, Banyumas 53182, Indonesia.

*Corresponding author email: dwihartanti@ump.ac.id

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ABSTRACT

Galangal (*Alpinia galanga* (L.) Willd.) has been long used for medicinal purposes. It showed promising effects on the food-spoilage microorganisms and is commonly used as spices in various cuisines. This study aimed to evaluate the microbial growth inhibitory activity and the preservation potential of galangal water extracts on tofu. The water extracts were prepared by infundation method in the concentrations of 5, 10, and 20%w/v. The phytochemical compounds in the extracts were screened as per standard methods. The tofu was preserved by immersion method for eight days under room temperature with an evaluation time point of two days. The microbial growth inhibitory activity was evaluated by indirect optical density (OD)-based enumeration, and the OD reduction by the extracts was calculated accordingly. The preservation potential was calculated from the changes of the physical characters on tofu evaluated by sensorial analysis. Galangal water extracts contained tannins and terpenoids. It concentration-dependently inhibited microbial growth on tofu, and the extract showed the best OD reduction at a concentration of 20%. It changed the color of tofu but maintained its texture. It also masked the odor of fresh tofu with the aromatic galangal scent. All three tested concentrations showed an equal preservation potential of 2 days. Our data suggested galangal water extract in the optimum concentration of 20% inhibited the microbial growth on tofu and potentially preserved it for two days. This moderate preservation effect might be attributable to its tannins and terpenoids content.

Keywords: Galangal, microbial growth, natural preservative, preservation potential, tofu, water extract.

Introduction

The use of galangal (*Alpinia galanga* (L.) Willd., *lengkuas* in Indonesian, family Zingiberaceae) for medicinal purposes in the traditional systems has been widely described (Chouni and Paul, 2018; Hookheaw and Neamsuvan, 2019; Silalahi et al., 2018).

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The antimicrobial activities of rhizomes of this plant, among others, have been described elsewhere. The essential oil was mainly well studied for the said activity (Hamad et al., 2016; Prakatthagomol et al., 2011; Zhou et al., 2021). However, the antimicrobial activity of galangal water extract was also reported (Pillai et al., 2019; Rini et al., 2018). These microbial activities enabled it to be developed further into a natural food preservative.

Galangal is also commonly used as a spice in Southeast and South Asian cuisines. It is prevalent for protein-based savory meals (Das et al., 2020). Hence, the familiarity with the aroma and taste of galangal might support it as a food preservative with a better acceptance. As the water extract was proven to be more suitable to be applied as a natural preservative for food, this study evaluated the potential use of galangal water extract as the natural food preservative on tofu (Hamad et al., 2019).

Tofu is processed from soya beans and is popular in East and Southeast Asia. It has a relatively short shelf life due to microbial spoilage as it contains water and proteins, around 85 and 8%, respectively (USDA, 2019). In addition, the reports on the contamination of tofu by pathogenic microorganisms were mentioned elsewhere (Ananchaipattana et al., 2012; Lee et al., 2017). Hence, the preservation efforts of tofu for preventing microbial decomposition and pathogenic contaminations were critically needed. This study specifically evaluated the microbial growth inhibitory activity of galangal water extract in tofu and the effects on its physical characters during room temperature storage for eight days.

Research Method

Chemical and Reagents

The galangal rhizomes (collected from Bawang, Indonesia), white firm tofu (bought from a market in East Purwokerto, Indonesia), sterilized water, nutrient broth (Oxoid, Thermo Fisher Scientific, USA), and nutrient agar (Oxoid). Ethanol, chloroform, hydrochloric acid, acetic acid, sulphuric acid, and ferric chloride were bought from Sigma-Aldrich (Merck KGaA, Germany).

Instrument

The optical density of the samples was read with the UV-Vis spectrophotometer (UV-1240, Shimadzu, Japan).

Methods

The plant materials were authenticated in the Laboratory of Plant Taxonomy, Jenderal Soedirman University, Purwokerto, Indonesia (Ref. 139/FB.Unsoed/TaksTumbVI/2015). The rhizomes were dried by direct sun-drying method and pulverized to the fine degree accordingly. According to the previous study, the water extract was prepared by infundation method in sterilized water at the concentrations of 5, 10, and 20%w/v (Hamad et al., 2017).

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Phytochemical screening was subjected to the obtained water extracts. The presence of alkaloids, flavonoids, saponins, sterols, tannins, and terpenoids in the extract was detected as per standard reagents for the respective compounds (Table 1) (Patel et al., 2016; Zohra et al., 2012).

The tofu was cubed in 2x2x2 cm and immediately immersed in the boiling water to reduce the microorganism load on the surface. The cubes were aseptically placed in 100 ml of water extracts or sterilized water (as the negative control) and tightly closed afterward. They were stored at room temperature and protected from direct light and heat for eight days. On days 2, 4, 6, and 8, tofu from each sample were evaluated for microbial growth and physical characteristics as detailed in our previous works (Hamad et al., 2019; D Hartanti et al., 2019).

Data Analysis

The experiment was conducted in triplicate, and data were presented in mean value ± standard deviation (SD). The effect of extract concentration and storage time on cultured tofu's optical density (OD) was evaluated by two-way ANOVA. The OD reduction of each extract was calculated according to Formula 1.

$$OD\ reduction = \frac{ODc - ODe}{ODc} \times 100\% \quad (1)$$

ODc was the OD of the negative control, while *ODe* was the OD of the tested extract. The comparison between OD reductions among the extracts was calculated by one-way ANOVA. Duncan's test was used as the post-hoc for both analyses. The analysis was conducted on the SPSS ver. 19 (IBM – SPSS, USA), and the difference was considered significant at p-value < 0.05.

Results and Discussion

The galangal water extract contained tannins and terpenoids (Table 1). Tannins are polyphenol compounds with good solubility in water, particularly in the extraction with the higher temperature as in this study (Smeriglio et al., 2017). The presence of tannins in galangal was previously reported in rhizomes collected in Vijayawada (India), Sidoarjo (Indonesia), El-Fayoum (Egypt) (Johnley et al., 2020; Rashad et al., 2016; Rini et al., 2018). On the other hand, terpenoids are slightly soluble in water. However, those h lower molecular weight can be better extracted with water under the higher extraction temperature (Martins et al., 2017). The compounds were detected in the galangal extracts originated from Vijayawada as well as from Qingdao (China) and Telangana (India) (Babu et al., 2017; Johnley et al., 2020; Zhou et al., 2021). Other studies reported detecting flavonoids, alkaloids, steroids, and saponins in the galangal rhizomes extracts, which were not detected in this study (Babu et al., 2017; Rashad et al., 2016). The variation of phytochemical compounds in plant extracts is commonly reported, with the extraction method, solvent, and intraspecific variation as the defining factors (Ciccarelli et al., 2016; L Hartanti et al., 2019; Joshi et al., 2011).

Both concentration of the extract (p-value = 0.000) and storage time (p-value = 0.000) significantly affected the optical density of the media of the cultured tofu. The negative control showed the highest OD, which was comparable to the galangal water extract at a concentration of 5%. The concentration-dependent relationship was observed with the lowest OD was shown by the extract at a concentration of 20%. On the other hand, the overall OD of the media was statistically constant from day 2 to 6 and started to rise on day 8 (**Figure 1**) significantly. OD represented the indirect microorganism enumeration on the tofu. The higher OD indicated the presence of more microorganisms and vice versa. Hence, the galangal water extracts did not exert microbial growth inhibitory activity, while two higher concentrations dependently inhibited the growth of the microorganisms.

Table 1. Phytochemical compounds in galangal water extract

Compounds	Test/ reagent	Remarks	Results
Tannins	Ferric chloride	The black solution was observed	Positive
Terpenoids	Salkowski's	Brown ring between solvents was formed	Positive
Alkaloids	Mayer's and Dragendorff's	No reactions	Negative
Flavonoids	Willstatter's	No reactions	Negative
Saponins	Froth test	No reactions	Negative
Sterols	Liebermann-Burchard's	No reactions	Negative

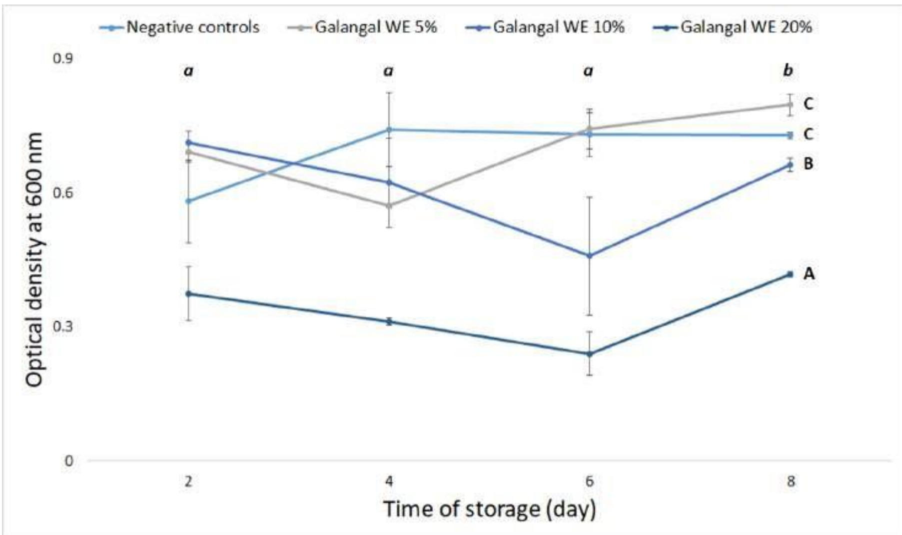

Figure 1. The profile of optical density of the media of the cultured tofu during 8-day storage. WE = water extract. The different upper-case alphabets and those in the lower case indicated a significant difference in optical density among samples and time of storage, respectively, evaluated by two-way ANOVA and Duncan's test at $p < 0.05$

The hindered microbial growth on tofu until day six might indicate that the extract's actions were mediated by prolonging the lag phase of the microbial growth (Rialita, 2014). The direct tofu culture on the nutrient agar supported the OD-based microbial growth inhibitory activity of galangal water extracts. The microbial growth was clearly shown in tofu treated with negative control and galangal water extracts at two lower tested concentrations. In contrast, a much sparser profile was observed in those in the concentration of 20% group (**Figure 2**).

Our result was similar to previous studies on the antimicrobial activity of galangal rhizome extracts. Galangal n-hexane, ethanol, water extracts and essential oils showed various degrees of activity against vast arrays of microorganisms (Hamad et al., 2016; Oonmetta-aree et al., 2006; Prakatthagomol et al., 2011; Rini et al., 2018). Tannins and terpenoids might contribute to the antimicrobial activity of galangal water extract. Tannins of rain tree leaves and terpenoids of lemongrass were previously mentioned to exert the said activity (Halabi and Sheikh, 2014; Sari et al., 2015; Zarin et al., 2016). The OD reduction represented the efficacy of the inhibitory activity of the galangal water extract. The highest tested concentration of galangal water extract consistently exerted the best OD reduction in each evaluation time. It gradually increased from day 2 to day six and started to decrease on day 8. On the other hand, the extracts at 5 and 10% concentrations showed an equal OD reduction on days 2 and 4.

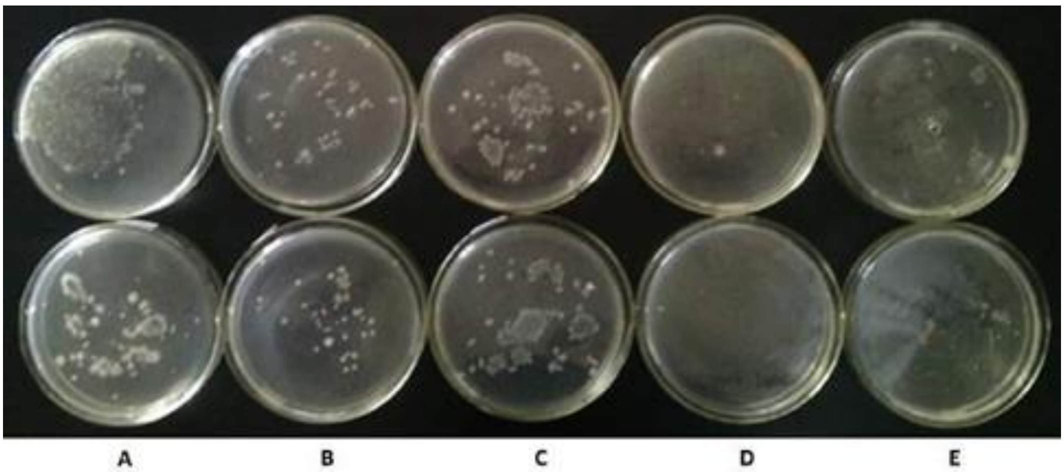

Figure 2. The profile of microbial growth on tofu after treated with galangal water extracts for 8 days. A = the negative control, B = 5%, C = 10%, D = 20%, E = the media control

From day six onward, the higher concentration of the extracts generated, the better OD reduction (**Figure 3**). The activity with concentration-dependent manner, as seen on days 6 and 8, was previously reported in the water extracts of galangal (Rini et al., 2018). The galangal water extracts changed the color of tofu from white to light brown (**Figure 4**). Tannins might cause the changed tofu color as they were interacted with the proteins on the surface (Wang and Heinonen, 2017).

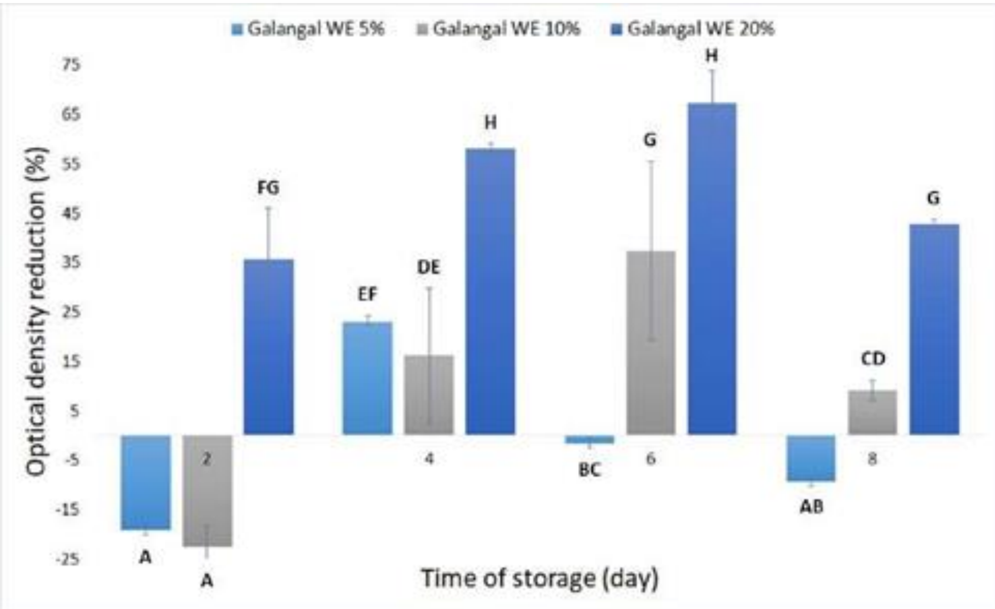

Figure 3. The profile of optical density reduction by the galangal water extract during 8-day storage of tofu. WE = water extract. The different alphabets in each bar represented the significant difference in optical density reduction, evaluated by one-way ANOVA and Duncan's test at $p < 0.05$

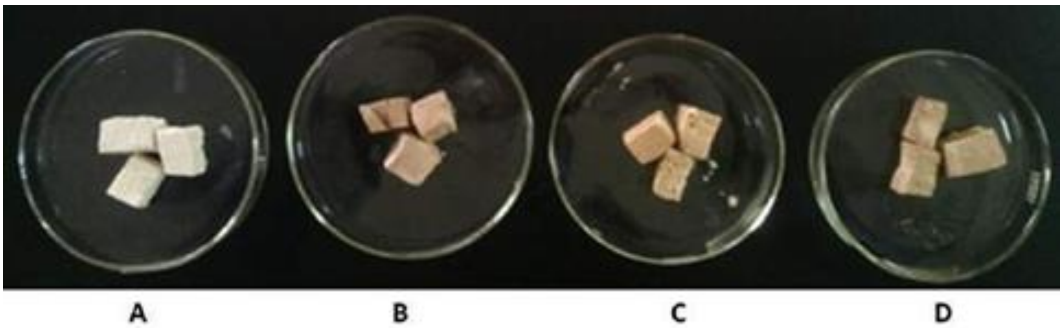

Figure 4. The physical characters of tofu after treated with galangal water extract for eight days. A = the negative control, B = 5%, C = 10%, D = 20%

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The microbial metabolism caused the deterioration of tofu. The decomposition of the proteins caused off-odor, while the off-texture was originated from the breakdown of macromolecules (Wang et al., 2019). The extracts masked the fresh tofu odor with the hint of aromatic galangal one that delayed the start of noticeable off-odor in tofu. In the negative control, tofu started to be off- odor on day 6, while those treated with the extract in all tested concentrations were off-odor on day.

Similarly, the extracts also delayed the start of the off-texture in tofu. Those in the negative control group started to be friable in the 6, while those in galangal water extract groups remained firm until the final day of storage. A further preference testing is needed to assess if the consumer likes the changes on tofu caused by the applications of galangal water extract (Lawless and Heymann, 2010).

The preservation potential of galangal water extracts was calculated by comparing the start of any signs of tofu deterioration in the extract groups to that of the negative control. The galangal water extracts delayed the deterioration of tofu for two days. All three tested concentrations showed the same preservation potential on tofu for two days. Hence, the concentrations of extract did not affect the preservation potential. The positive effects of galangal on the preservation of milkfish have been reported elsewhere. The water extracts at the concentrations of 10% diminished the bacterial count on milkfish and generated a preferred smell, color, and texture profile. The juice also prolonged the shelf life of milkfish up to 46 hours (Florensia et al., 2012; Inayah and Bestari, 2018).

The overall results of all evaluated parameters in this study suggested that the optimum concentration of galangal water extract for the preservation of tofu was 20%. It generated the best antimicrobial inhibitory activity while maintaining the physical characters of tofu as in other tested concentrations. The best activity shown by the highest tested concentrations might relate to the higher tannins and steroids content. In the infundation process, the degree of compounds extractions was increased with the number of plant materials available up to the saturation condition is achieved (Sahne et al., 2016).

Conclusion

Galangal water extracts containing tannins and terpenoids showed a significant microbial growth inhibitory activity and a moderate preservation potential on tofu during 8-day storage at room temperature. The inhibitory activity was in concentration-dependent manner with the optimum concentration of 20%.

Reference

Ananchaipattana C, Hosotani Y, Kawasaki S, et al. (2012) Bacterial contamination of soybean curd (tofu) sold in Thailand. *Food*

Science and Technology Research 18(6): 843–848.

Babu ND, Moulika V, Krishna BM, et al. (2017) Phytochemical and anthelmintic activity of *Alpinia galanga* Linn. *Journal of Pharmacognosy and Phytochemistry* 6(4): 2049–2051.

Chouni A and Paul S (2018) A review on phytochemical pharmacological potential of *Alpinia galanga*. *Pharmacognosy Journal* 10(1): 9–15.

Ciccarelli D, Giovanelli S and Pistelli L (2016) Essential oils from *Anthemis maritima* flowers: Infraspecific variability along the Adriatic Coast (Italy). *Chemistry & Biodiversity* 13(5): 561–570.

Das G, Patra JK, Gonçalves S, et al. (2020) Galangal, the multipotent super spices: A comprehensive review. *Trends in Food Science & Technology* 101: 50–62.

Florensia S, Dewi P and Utami NR (2012) Pengaruh ekstrak lengkuas pada perendaman ikan bandeng terhadap jumlah bakteri pengaruh ekstrak lengkuas pada perendaman ikan bandeng terhadap jumlah bakteri. *Life Science* 1(2): 78–86.

Halabi MF and Sheikh BY (2014) Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. *BioMed Research International* 2014: Article ID 906239.

Hamad A, Alifah A, Permadi A, et al. (2016) Chemical constituents and antibacterial activities of crude extract and essential oils of *Alpinia galanga* and *Zingiber officinale*. *International Food Research Journal* 23(2): 837–841.

Hamad A, Anggraeni W and Hartanti D (2017) Potensi infusa jahe (*Zingiber officinale* R.) sebagai bahan pengawet alami pada tahu dan daging ayam segar. *Jurnal Aplikasi Teknologi Pangan* 6(4): 177–183.

Hamad A, Nurlaeli E, Pradani DY, et al. (2019) Application of lemongrass as natural preservatives for tofu. *Jurnal Teknologi dan Industri Pangan* 30(2): 100–109.

Hartanti D, Djalil AD, Yulianingsih N, et al. (2019) The effect of infusion of *Syzygium polyanthum* (Wight) Walp. leaves as natural preservative chicken meats. *Jurnal Kefarmasian Indonesia* 9(1): 19–27.

Hartanti L, Yonas SMK, Mustamu JJ, et al. (2019) Influence of extraction methods of bay leaves (*Syzygium polyanthum*) on antioxidant and HMG-CoA Reductase inhibitory activity. *Heliyon* 5(4): article ID e01485.

Hookheaw S and Neamsuvan O (2019) Ethnopharmacology in treatment of hypertension from Yala Provinces, Thailand. *Srinakharinwirot Science Journal* 36(1): 13–31.

Inayah and Bestari AC (2018) Efektivitas lengkuas (*Alpinia galanga*) dalam mengawetkan ikan bandeng. *Jurnal Sulolipu Media Komunikasi*

Sivitas Akademika dan Masyarakat 18(1): 96–101.

Johnley IIR, Somasundaram G, Salwe KJ, et al. (2020) Anti ulcerogenic and anti-oxidant activity of *Alpinia galanga* rhizomes aqueous extract in indomethacin induced gastric mucosal damage in wistar albino rats. *Biomedical and Pharmacology Journal* 13(1): 55–60.

Joshi B, Sah GP, Basnet BB, et al. (2011) Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *J Microbiol Antimicrob* 3(1): 1–7.

Lawless HT and Heymann H (2010) Preference Testing. In: *Sensory Evaluation of Food*. 2nd ed. New York: Springer, pp. 303–324.

Lee DY, Kwon KH, Chai C, et al. (2017) Microbial contamination of tofu in Korea and growth characteristics of *Bacillus cereus* isolates in tofu. *LWT* 78: 63–69.

Martins MAR, Silva LP, Ferreira O, et al. (2017) Terpenes solubility in water and their environmental distribution. *Journal of Molecular Liquids* 241: 996–1002.

Oonmetta-aree J, Suzuki T, Gasaluck P, et al. (2006) Antimicrobial properties and action of galangal (*Alpinia galanga* Linn.) on *Staphylococcus aureus*. *LWT - Food Science and Technology* 39(10): 1214–1220.

Patel MR, Panchal HS and Saluja AK (2016) Identification of terpenoids and steroidal compounds in *Caryota urens* leaves by column chromatography and various spectroscopic techniques. *World Journal of Pharmacy and Pharmaceutical Sciences* 5(5): 1610–1622.

Pillai MK, Ismail R, Sasidharan S, et al. (2019) Antioxidant and antimicrobial activities of rhizome extracts from Malaysian species of *Alpinia galanga* and *Alpinia officinarum*. *Pharmacology Online* 1: 366–375.

Prakatthagomol W, Klayraung S and Okonogi S (2011) Bactericidal action of *Alpinia galanga* essential oil on food-borne bacteria. *Drug Discoveries & Therapeutics* 5(2): 84–89.

Rashad NGE-D, Mostafa UE-S and El-Hadidy EM (2016) Effect of galangal (*Alpinia galanga*) as hypoglycemic and hypolipidemic agents in diabetic Albino rats. *Egyptian Journal of Nutrition* 31(4): 337–359.

Rialita T (2014) *Efektivitas antibakteri kombinasi minyak atsiri Zingiber officinale var. Rubrum dan Alpinia purpurata K. Schum dan aplikasinya pada model pangan*. Institut Pertanian Bogor.

Rini CS, Rohmah J and Widyaningrum LY (2018) The antibacterial activity test galanga (Alpinia galangal) on the growth of bacteria *Bacillus subtilis* and *Escherichia coli*. *IOP Conference Series: Materials*

Science and Engineering 420: article ID 012142.

Sahne F, Mohammadi M, Najafpour GD, et al. (2016) Extraction of bioactive compound curcumin from turmeric (*Curcuma longa* L.) via different routes: A comparative study. *Pakistan Journal Biotechnology* 13(3): 173–180.

Sari PP, Rita SW and Puspawati MN (2015) Identifikasi dan uji aktivitas senyawa tanin dari ekstrak daun Trembesi (*Samanea saman* (Jacq.) Merr) sebagai antibakteri Escherichia coli. *Jurnal Kimia* 9(1): 27–34.

Silalahi M, Nisyawati, Walujo EB, et al. (2018) Etnomedisin tumbuhan obat oleh Subetnis Batak Phakpak di Desa Surung Mersada, Kabupaten Phakpak Bharat, Sumatera Utara. *Jurnal Ilmu Dasar* 19(2): 77–92.

Smeriglio A, Barreca D, Bellocco E, et al. (2017) Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology* 174(11): 1244–1262.

USDA (2019) *National Nutrient Database for Standard Reference Legacy Release: Tofu*. Washington Available at: https://ndb.nal.usda.gov/ndb/foods/show? ndbno=16427.

Wang B and Heinonen M (2017) Protein-tannin interactions of tryptic digests of α-lactalbumin and procyanidins. *Journal of Agicultural and Food Science* 65(1): 148–155.

Wang C, Du Q, Yao T, et al. (2019) Spoilage bacteria identification and food safety risk assessment of whole soybean curd. *Indian Journal of Microbiology* 59: 250–253.

Zarin MA, Wan HY, Isha A, et al. (2016) Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang. *Food Science and Human Wellness* 5(2): 65–75.

Zhou C, Li C, Siva S, et al. (2021) Chemical composition, antibacterial activity and study of the interaction mechanisms of the main compounds present in the *Alpinia galanga* rhizomes essential oil. *Industrial Crops and Products* 165: article ID 113441.

Zohra SF, Meriem B, Samira S, et al. (2012) Phytochemical screening and identification of some compounds from mallow. *Journal of Natural Products Plant Resources* 2(4): 512–516.