Antibacterial and antioxidant activities of pyrogallol and synthetic pyrogallol dimer

Cynthia[1], Ignatia Florence[2], Sutanto Hery[1]* and Darmawan Akhmad[3]

1. Department of Chemical Engineering, Faculty of Life Sciences and Technology, Swiss German University, Tangerang, Banten, 15143, INDONESIA
2. Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, Tangerang, Banten, 15143, INDONESIA
3. Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK Serpong, Tangerang, Banten, 15314, INDONESIA

*hery.sutanto@sgu.ac.id

Abstract

Pyrogallol (1,2,3-trihydroxybenzene) has the ability to perform antibacterial and antioxidant activities. Dimerization of pyrogallol may enhance these activities, however it has not been evaluated by previous studies. The success of pyrogallol dimerization using 2,2-diphenyl–1–picrylhydrazyl (DPPH) method via oxidative coupling reaction was confirmed using Thin Layer Chromatography (TLC), Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (LC-MS-MS) and Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) and it was found that the molecule conversion rate can be increased by the addition of phosphate buffer. Pyrogallol dimer or [1,1'-biphenyl]-2,2',3,3',4,4'-hexaol, had lower Minimum Inhibitory Concentration (MIC) value for both Staphylococcus aureus and Escherichia coli (8 µg/ml) compared to pyrogallol (512 µg/ml and 256 µg/ml respectively).

In agar well diffusion, pyrogallol and its dimer showed the same antibacterial activity against S. aureus but the dimer performed higher activity against E. coli. The antioxidant activity of pyrogallol dimer (at 500 µM 40.37%), measured using DPPH scavenging assay, was lower than pyrogallol (at 500 µM 92.77%). Due to high antioxidant activity of pyrogallol, the study also evaluated its effect to reduce chilling injury symptoms of mangosteen (Garcinia mangostana), which is one of high demanded Indonesian export commodities. Pyrogallol dipped mangosteens tend to have lower chilling injury symptoms such as pericarp hardening, inner pericarp browning, off odor and off flavor of edible arils.

Keywords: Pyrogallol, Dimerization, DPPH, Antibacterial, Antioxidant.

Introduction

Pyrogallol as a phenolic compound has been reported to have antibacterial[1] and antioxidant[2] activities. Dimerization is a process of creating molecular entity A2 from molecular entity A[3]. Since dimerization doubles the phenolic groups of pyrogallol, the antioxidant[4] and antibacterial[5] activities of pyrogallol dimer are expected to be increased, due to more location for electron to be delocalized. However, there were limited studies of pyrogallol dimerization and this dimerized product is not commercially available yet. This research will study the synthesis of pyrogallol dimer using DPPH through oxidative coupling reaction, as proposed by Zhu et al[6] in 2001 and also reproducing the method with a better conversion efficiency of dimer production using common antioxidant activity assay[7].

Pyrogallol is dimerized because number of hydroxyls and degree of polymerization are pivotal for a compound's antibacterial activity[5]. Both harmless and pathogenic microorganisms are found in the body of organisms. Indiscriminate use of antibiotics rises antimicrobial resistance so that the medical treatment would be ineffective. That makes it worth to find a novel antimicrobial agent. Hence, this study aims to provide a new potent of antibiotic by dimerization of pyrogallol.

In addition to antibacterial activity, antioxidant activity is also predicted to be increased by dimerization. However, the antioxidant activities of pyrogallol and its dimer have not been compared yet. A study has researched about pyrogallol dip to the pericarp of lychee to reduce the enzymatic browning process during storage at 4ºC[8]. The study resulted in decrement of peroxidase (POD) and phenylalanine ammonia lyase (PAL) activities in the pericarp of lychee. POD and PAL also play a significant role in chilling injury symptoms of mangosteen.

Mangosteen (*Garcinia mangostana*) is one of Indonesian exotic fruits which is highly demanded by other countries due to its high amount of vitamin C and refreshing taste[9,10]. The crucial stages in exporting agricultural commodities are the storage before, during and after distribution, since the probability of spoilage to occur at these stages is relatively high. Storage at low temperature allows chilling injury to happen in the form of pericarp hardening due to lignin synthesis, browning on the outer and inner part of pericarp, browning and off flavor of edible aril[11]. All chilling injury symptoms were believed to be caused by reactive oxygen species which will allow enzymatic reactions to happen[8,12].

As mentioned before, PAL and POD in lychee, which can be reduced by pyrogallol, contribute in chilling injury of mangosteen. They play specific role as lignin compound biosynthesis, which are trans-cinnamic acid and polymer of monolignol[13]. In addition, POD also contributes in the browning of mangosteen pericarp, as a part of chilling injury

symptoms. Therefore, it is possible to apply pyrogallol or pyrogallol dimer to mangosteen pericarp in order to prevent chilling injury of mangosteen.

Material and Methods

Reagents: Pyrogallol, potassium phosphate buffer pH 7, methanol, ethyl acetate, acetonitrile, n-hexane, barium chloride, sulfuric acid, dimethylsulfoxide (DMSO), nutrient broth, Triton X-100, phenol, polyvinyl polypyrolidone, tris(hydroxymethyl)aminomethane, hydrochloric acid, ethylenediaminetetraacetic acid, magnesium chloride hexahydrate, L-phenylalanine and hydrogen peroxide 30% were acquired from Merck, Germany. DPPH, streptomycin sulfate, dithiothreitol and 4-aminoantipyrine were obtained from Sigma-Aldrich, Germany. Mueller-Hinton broth was obtained from HiMedia, India. Agar bacteriological was obtained from Oxoid, England.

Microorganisms: The bacteria used in the *in vitro* test were *Staphylococcus aureus* and *Escherichia coli* and were kindly obtained from Microbiology Laboratory of Swiss German University, Indonesia.

Mangosteen: 350 mangosteens with maturity index of 5 (red-purple color) were obtained from local supermarket and harvested from a mangosteen farm in Bogor, Indonesia.

Pyrogallol Dimer Synthesis: The pyrogallol dimer was synthesized using three methods. First method was proposed by Zhu et al[6]: Pyrogallol 150 mg and DPPH 350 mg are dissolved in 10 mL of acetonitrile. The mixture is kept in dark for 2 days. Second and third methods were adapted from Lewis[7]: 100 µL of 10 mg/mL pyrogallol solution is mixed with 4,5 mL of potassium buffer solution (pH 7) and 400 µL methanol. The third method was made without buffer. 10 mL of 0.1057 mM DPPH solution is added and the mixture is incubated in a dark place for 30 minutes. The conversion efficiency of all methods was compared using TLC (n-hexane:ethyl acetate = 2:1).

Pyrogallol Dimer Separation: Method of Meloan[14] was used for this assay. The mixture from the chosen method that has the highest conversion rate of pyrogallol is added to separatory funnel along with 20 mL ethyl acetate and 20 mL aquadest. After two layers appeared, the desired upper layer is retained while the bottom layer is discarded. The dimer will then be characterized using LC/MS/MS (Waters Corporation UPLC Acquity 1, USA) with gradient of acetonitrile, formic acid 0.1% and water as mobile phase and ^{1}H-NMR (Jeol JNM ECA-500, Japan) at 500 MHz using methanol-d3 as solvent.

Agar Well Diffusion Assay: Solutions of 0.2% streptomycin, 1% pyrogallol and 1% synthesis product expected to be pyrogallol dimer were made. Streptomycin was dissolved in distilled water while pyrogallol and the dimer were dissolved in DMSO. Afterwards, a colony from an overnight bacterial inoculum from both *S. aureus* and *E. coli* was put into sterile saline solution and the turbidity was adjusted visually to the turbidity of 0.5 McFarland standard.

Subsequently, 1000 µl of bacterial suspension was pipetted into the sterile Petri dish. Then, the solution of Mueller Hinton agar was poured into the petri dish. Four wells were created on the agar and filled with 30 µl of DMSO as negative control, streptomycin as positive control, pyrogallol and pyrogallol dimer. The Petri dishes were incubated overnight at 37°C. After incubation, the bacterial growth around each well was observed. The diameter of the zone of inhibition around the well was measured and recorded[15]. The experiment was run with two replications. The result was analyzed statistically using f-test and continued with t-test.

Minimum Inhibitory Concentration (MIC) Determination: The MIC of pyrogallol and pyrogallol dimer was determined in broth microdilution assay. A final inoculum size of $5x10^5$ CFU/ml was prepared in the Mueller-Hinton Broth for each bacterium. A series of two-fold dilution concentration ranging from 1024 µg/ml to 2 µg/ml was prepared for pyrogallol and pyrogallol dimer. In 96-well microtitration plate, the bacterial inoculum was placed in each well and mixed with pyrogallol and pyrogallol dimer solution as the antibacterial agent. The plate was incubated overnight in the incubator at 37°C. The data were taken as duplication. The result was recorded as the lowest concentration of pyrogallol and pyrogallol dimer that completely inhibit growth of bacteria in microdilution wells detected by unaided eye[16].

Antioxidant Activity Comparison: Method of Xie and Schaich[17] with modification was used for this assay. Pyrogallol and pyrogallol dimer solution were made with concentrations of 100, 200, 300, 400 and 500 µM using DMSO as solvent. 0.2 mM DPPH solution was added with 1:1 volume ratio and the mixtures were incubated for 30 minutes. The absorbances of each mixture were observed using UV-Vis Spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA) at 515 nm, using DMSO as blank and DPPH: DMSO (1: 1) as control. The antioxidant activity is measured as DPPH inhibiting activity using the formula below:

$$\text{DPPH Inhibiting Activity (\%)} = \frac{A\ control - A\ sample}{A\ control} x\ 100\%$$

Dipping, Air Drying and Cold Storage of Mangosteens: Method of Jing et al. (2013)[8] was used for this assay. Mangosteens are immersed into 0 and 1 mM pyrogallol for 3 minutes and then air dried for 30 minutes at 25°C. Five mangosteens were packed in polyethylene bags (0.03 mm thick and 250 × 200 mm) and stored at 4°C for 15 days. Samples of five mangosteens were analyzed in every 5 days for firmness tests, PAL and POD activities. Samples of thirty mangosteens are used for sensory tests to observe browning

on inner pericarp, off flavor of edible aril and overall acceptance on the firmness of mangosteen.

Pericarp Firmness: Method of Dangcham et al[12] was used for this assay. Whole mangosteen fruits were tested using a GY-4 fruit penetrometer (Total, NK/HP Series, China), completed with a cylindrical plunger 0.5 cm in diameter and pressed 0.5 cm into the pericarp at two points at opposite sides on the fruit. The force was recorded in newtons (N). The rest of pericarp was used for chemical analysis.

Enzyme Extraction: Method of Dangcham et al[12] was used for this assay. 2 grams of mangosteen pericarp was mixed together with 20 mL of extraction buffer and 0.2 gr of polyvinyl polypyrolidone and then was centrifuged at 18,000 G and 4°C for 30 minutes. The extraction buffer consists of 100 mM Tris HCl (pH 7.5), 1 mM EDTA, 5 mM magnesium chloride, 2.5 mM dithiothreitol and 0.05% Triton X-100. The enzyme extract can only be used for less than 12 hours and it is suggested to keep it at 5-10°C to prevent degradation.

PAL Activity: Method of Camm and Towers[18] with modification was used for this assay. 0.1 mL enzyme extract was reacted with 2.4 mL L-phenylalanine solution in 10 gr/L Tris Buffer (pH 8.5) and then the mixture was incubated for 1 hour at 37°C. The reaction was stopped using 0.5 mL of 5 M hydrochloric acid. Spectrometry analysis at 290 nm was used to measure the absorbance of transcinnamic acid.

POD Activity: Method of Morita et al[19] with modification was used for this assay. 100 µL enzyme extract was mixed with 600 µL extract buffer, 800 µL of 100mM 4-aminoantipyrine, 800 µL of 1 mM phenol and 20 µL of 100mM hydrogen peroxide. The absorbance was then measured using UV-Vis Spectrophotometer at 512 nm after 90 seconds of enzyme extract addition. The mixture lasts 4.5 minutes only before it is degraded.

Sensory Test: Method of Category Scoring Test by Meilgaard et al[20] was used for this assay. Two mangosteens (controlled and treated) were served to 30 untrained panelists. Panelists were asked to score each mangosteen in term of firmness acceptability, level of inner pericarp browning, aroma of edible aril and flavor of edible aril. Coffee powder was provided to neutralize olfactory senses and plain crackers with mineral water were provided to neutralize taste buds.

Results and Discussion

Pyrogallol Dimer Synthesis and Characterization: Mixtures from the first, second and third method were analyzed in TLC, with n-hexane and ethyl acetate as the mobile phase for the determination of method that has the highest conversion rate of the product. The results showed that the second method or antioxidant assay method with addition of potassium phosphate buffer effectively yielded the product (Rf = 0.90).

The second mixture then underwent liquid-liquid extraction with ethyl acetate and water as the immiscible solvents in order to separate the reaction product. Two layers were formed where water was located in lower layer or as aqueous phase along with methanol and buffer while ethyl acetate was in the upper layer as organic phase together with the dimeric compound of pyrogallol. The upper layer was taken as the extract containing expected product. Before other further analysis step, the extract was subjected to rotary evaporator to remove the solvent.

In order to determine the molecular weight of the product, it was then further analyzed using Liquid Chromatography and tandem Mass Spectroscopy (LC-MS/MS). The molecular weight of pyrogallol dimer should be 250 g/mol while the result of LC-MS/MS was detected at retention time of 4.2 mins with m/z 249 $[M-H]^+$.

As for Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR), the signals in the spectral data of the product were present in the chemical shift of 7 ppm (2H, *d* 5-H) and 6.85 ppm (2H. *d* 6-H). The mixture with addition of potassium phosphate buffer was more able to efficiently yield dimer rather than the other two methods due to the impact of potassium and phosphate ions in increasing dimerization process[21]. Therefore, the reaction product was confirmed to be pyrogallol dimer. The mechanism of the dimerization process through oxidative coupling reaction was proposed as shown in figure 1.

Antibacterial Activity Analysis: The analysis of antibacterial activity using agar well diffusion method and broth microdilution method was done to determine the higher antibacterial activity between pyrogallol and the dimer of pyrogallol over *S. aureus* as the representative of Gram-positive bacteria and *E. coli* to represent Gram-negative bacteria. In agar well diffusion, the antibacterial activity of streptomycin, pyrogallol and pyrogallol dimer was detected by the appearance of Inhibition Zone (IZ) around the agar well, as presented by table 1. The experiment was run with two replications. The data of inhibition zone diameters of the sample were statistically analyzed using f-test: two samples for variances and continued with t-test: two samples with unequal variances.

According to statistical analysis using f-test: two samples for variances and t-test: two samples with unequal variances, there was significant difference in the antimicrobial activity of pyrogallol against *S. aureus* and *E. coli*. However, pyrogallol dimer showed no significant difference in the activity towards *S. aureus* and *E. coli*. The statistical analysis also showed that against *S. aureus*, pyrogallol and dimer pyrogallol had no significantly different activity. While against *E. coli*, it can clearly be seen from table 1 that pyrogallol dimer had higher antibacterial activity compared to pyrogallol which showed no inhibition activity.

The different ability of pyrogallol as the antibacterial agent against both bacteria might be related to the complexity of Gram-negative bacteria's wall compared to Gram-positive bacteria. The wall of Gram-negative bacteria has a peptidoglycan layer and outer membrane that is composed of double layer phospholipids that are linked to inner membrane by lipopolysaccharides providing barrier that allows Gram-negative bacteria to be more resistant[22].

As for determination of MIC value, broth microdilution is one of the most appropriate methods. After pyrogallol and pyrogallol dimer were mixed with the bacteria in the microtitre plate, the plate was then incubated overnight. The result was recorded as the lowest concentration of pyrogallol and pyrogallol dimer that completely inhibit growth of bacteria in microdilution wells detected by unaided eye[16] and displayed in table 2.

Generally, it can be seen from table 2 that pyrogallol dimer has the lower MIC value to inhibit the visible growth of both *S. aureus* and *E. coli* in the microtitration plate which is 8 µg/ml. On the other hand, pyrogallol needed higher concentrations to inhibit *S. aureus* and *E. coli*, which are 512 µg/ml and 256 µg/ml respectively. This result supported that the presence of hydroxyl group in a phenolic compound is responsible for its antibacterial property. As the number of hydroxyl group increases, the antibacterial activity of a compound increases as well[23]. In this case, pyrogallol dimer has more hydroxyl groups (six groups of –OH) rather than its monomer (three groups of –OH).

The mechanism of action of phenolic compounds which is also believed to be the mechanism of pyrogallol, as antibacterial agents has been previously reviewed and some researches have proposed that antimicrobial effect of phenolic compound is possibly due to their ability to alter microbial cell permeability resulting in the loss of macromolecules from the interior side of cell wall of bacteria, for example ribosome and Na glutamate[24].

Antioxidant Activity of Pyrogallol Dimer: Pyrogallol has higher antioxidant activity (92.37% at 500 µM) than its dimer (40.77% at 500 µM) as shown in figure 2. This can be caused by lower bond dissociation enthalpy of O-H in the monomer than the dimer[27].

Effect of Pyrogallol on Chilling Injury of Mangosteen: Pyrogallol dipped mangosteen had less firm pericarp after 15 days of storage at 4°C and both less activities of PAL and POD enzymes (figure 4 and 5). The ability of pyrogallol as radical and oxygen scavenger may contribute to these results[2,8].

PAL contributes to the conversion of L-phenylalanine (one of alkaloids in the pericarp of mangosteen) into trans-cinnamic acids (one of lignin compounds). In order to perform the conversion, PAL requires oxygen[26]. Pyrogallol (Electrode potential = 0.612 V) is a strong antioxidant which means it is very easy to be oxidized[27]. The amount of oxygen required for the activity of PAL is therefore reduced by the presence of pyrogallol and resulted in lower lignification process and lower firmness of mangosteen pericarp. However, if the results of firmness (figure 3) and PAL activity (figure 4) are compared, it can be seen that the increasing firmness is not parallel with increasing PAL activity. Therefore, it can be concluded that there are other factors contributing in the firmness of mangosteen pericarp other than lignin production. A study has found that the hardening of mangosteen stored at 8°C for 6 days was caused by the leakage of electrolytes from pericarp cells[28,29].

Many damages in fruit pericarp happen due to membrane deterioration which allows enzyme and substrate to encounter and react. The membrane deterioration can be caused by peroxidation of lipid membrane by reactive oxygen species (ROS). Jing et al[8] found that pyrogallol prevented increasing membrane permeability by scavenging the ROS, therefore enzyme and substrate cannot encounter and therefore they do not react. These explain the lower PAL activity of treated mangosteen than its control on day 5. A study found that PAL activity and the expression of PAL mRNA are very susceptible to oxygen level and also temperature change not concerned in this experiment. Duration and temperature change when the mangosteen was moved from chiller to room temperature and it may increase the PAL activity after 5 days of storage[12].

POD is crucial in the polymerization of lignin precursor monomers such as p-coumaril, coniferyl and sinapyl alcohols[12]. A study found that POD assists the polymerization because it converts cynamyl alcohols to their radical forms which will further bind into polymer of lignin[13]. Antioxidant such as pyrogallol has the ability to scavenge free radicals via hydrogen atom transfer (HAT), electron transfer (ET), or sequential proton loss-electron transfer (SPLET)[27]. The presence of pyrogallol is able to neutralize the free radical forms of cynamyl alcohols and therefore the polymerization into lignin compounds does not occur. This condition resulted in lower firmness of pericarp of the pyrogallol dipped mangosteen.

POD is known to assist the polymerization of lignin precursors by converting them into free radical forms. Pyrogallol is theoretically able to scavenge these free radicals and therefore avoid polymerized lignin to be synthesized. The theory seems to state that pyrogallol has no direct effect to the activity of POD, but it reduces the probability of polymerization to happen. Much damage in fruit pericarp happens due to membrane deterioration, which allows enzyme and substrate to encounter and react. The membrane deterioration can be caused by peroxidation of lipid membrane by reactive oxygen species (ROS). Jing et al[8] in 2013 found that pyrogallol prevented increasing membrane permeability by scavenging the ROS, therefore enzyme and substrate cannot encounter and therefore they do not react.

However, the sensory acceptances of treated and non-treated mangosteen had no clear trends (figure 6) which can be caused by human and environment factors disturbing the sensory assay[20]. Panelists overall pericarp firmness acceptance of controlled and treated mangosteen is decreased from 6.3 to 5.9 and 5.6 respectively. This condition explains that the increment of firmness which makes the mangosteens harder to open will reduce the acceptability of mangosteen firmness.

Browning in the inner pericarp of mangosteen can be caused by some enzymes including polyphenol oxidase (PPO) and peroxidase (POD). These enzymes will react with their substrates when the membrane deteriorates due to lipid peroxidation. Pyrogallol, as an antioxidant, is able to scavenge the reactive oxygen species (ROS) by performing radical delocalization[8]. Therefore, the browning of inner pericarp can be reduced.

The acceptability of odor has a strong relation to the acceptability of flavor, since flavor is the combination of taste, odor and chemical feeling of a food product[20]. Odor or aroma is carried by volatile substances which are transmitted by gas. Aroma and flavour profiles of mangosteen have been studied and it revealed that there are around 51 volatile compounds responsible for aroma and flavor of mangosteen. Off odor may be caused by lipid peroxidation and metabolism of microorganism presented in the aril[20,30]. Mangosteen aril consists of 0.6% fat[31]. The presence of pyrogallol may avoid the peroxidation of lipid, therefore the side product of lipid peroxidation responsible for off odor (e.g. acetaldehyde) will not be synthesized[32].

The presence of microorganisms such as fungi may also produce metabolites responsible for off odor, usually fermentative metabolite such as ethanol[32]. This theory was strengthened with the fact that fungi growth was detected in the controlled mangosteens at day 10 and 15 of storage.

The potential pathogens[33] which can grow on mangosteen are *Lasiodiplodia theobromae, Pistalotiopsis sp., Penicillium sp.* and *Phomopsis sp., Diplodia gossypina, Pestalotia sp., Gloesporium sp.* and *Rhyzopus nigricans*. Since pyrogallol also has antimicrobial effects, the higher flavor acceptability of treated mangosteen may be caused by the microbial inhibiting activity of pyrogallol.

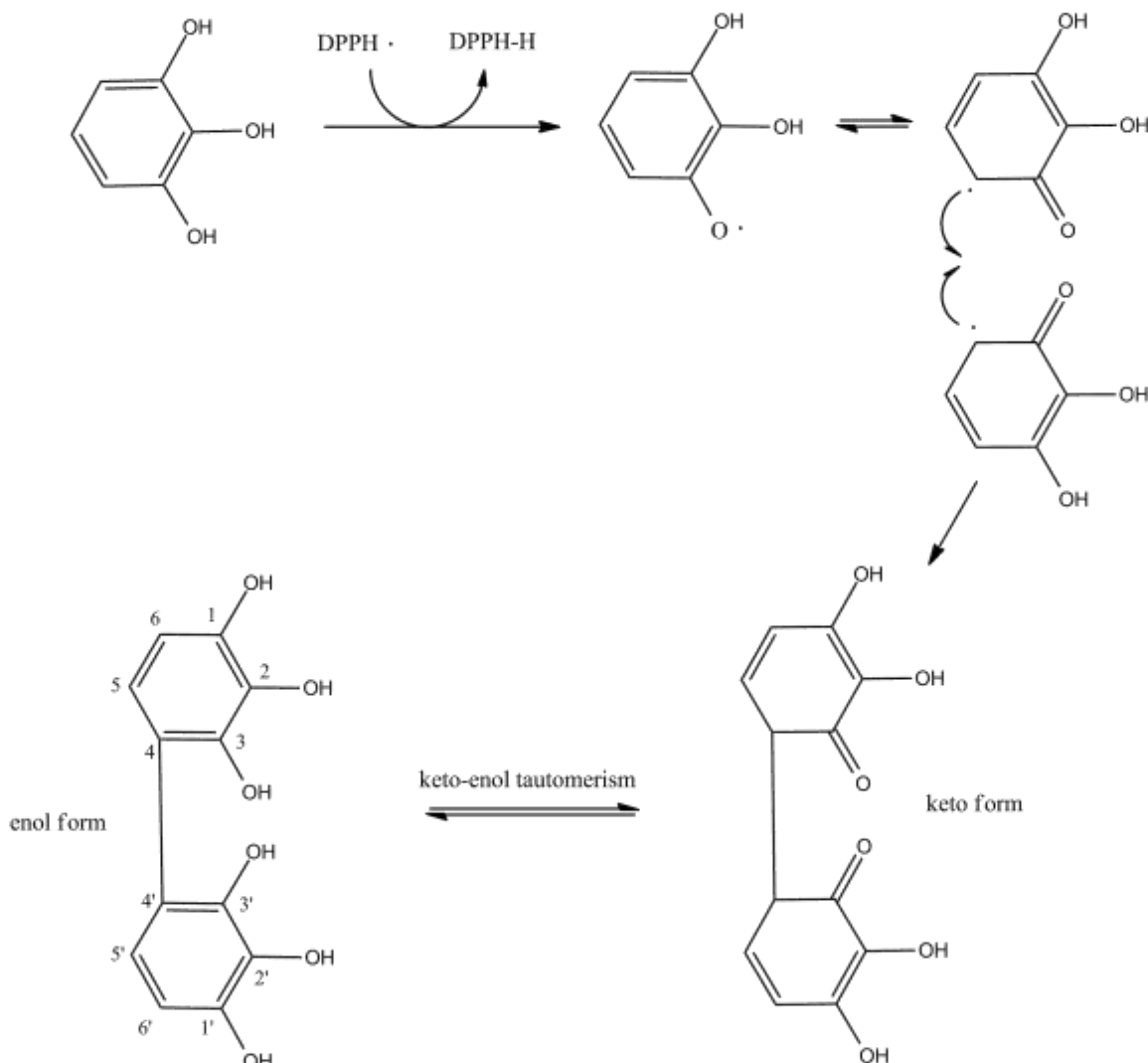

Figure 1: Proposed mechanism of dimeric product formation

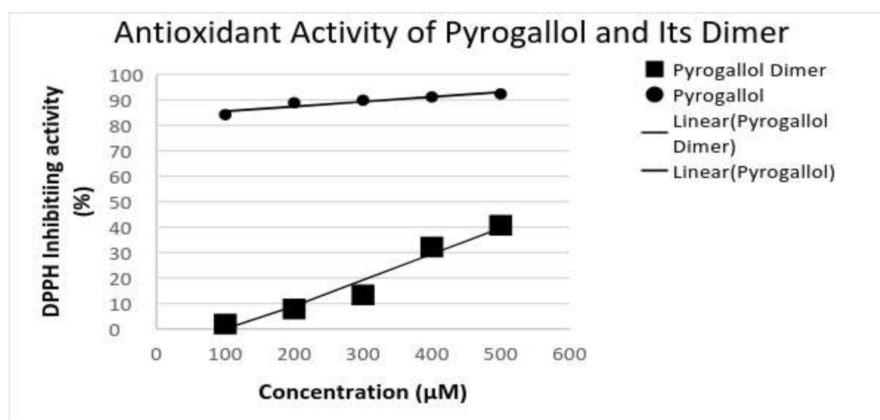

Figure 2: Antioxidant activity of pyrogallol and its dimer

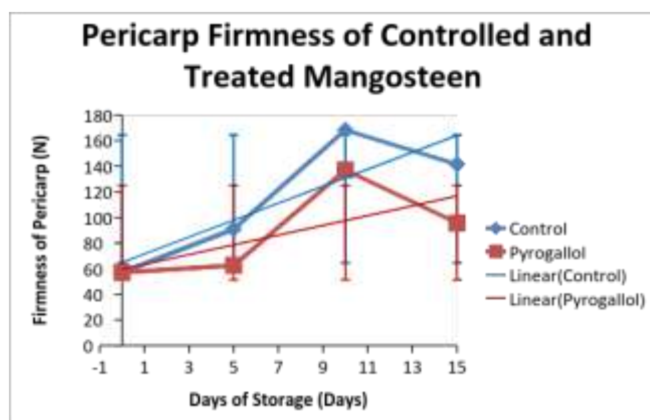

Figure 3: Graph of Pericarp Firmness of Controlled and Treated Mangosteen Stored at 4°C for 15 days

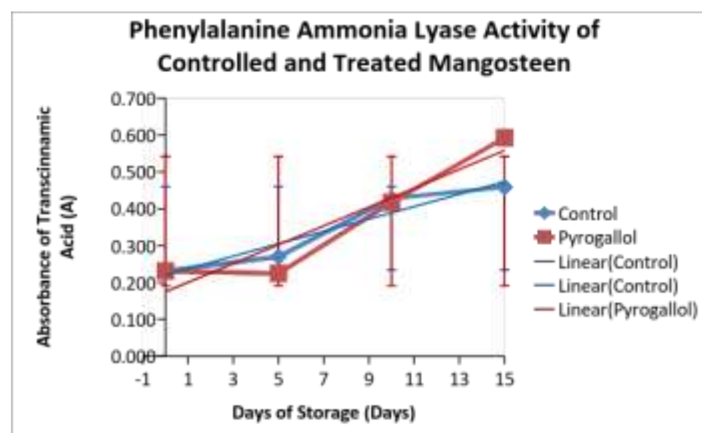

Figure 4: Graph of PAL Activity of Controlled and Treated Mangosteen Stored at 4°C for 15 days

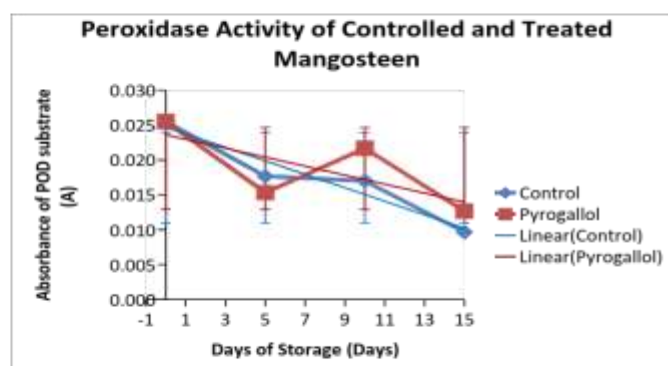

Figure 5: Graph of POD Activity of Controlled and Treated Mangosteen Stored at 4°C for 15 days

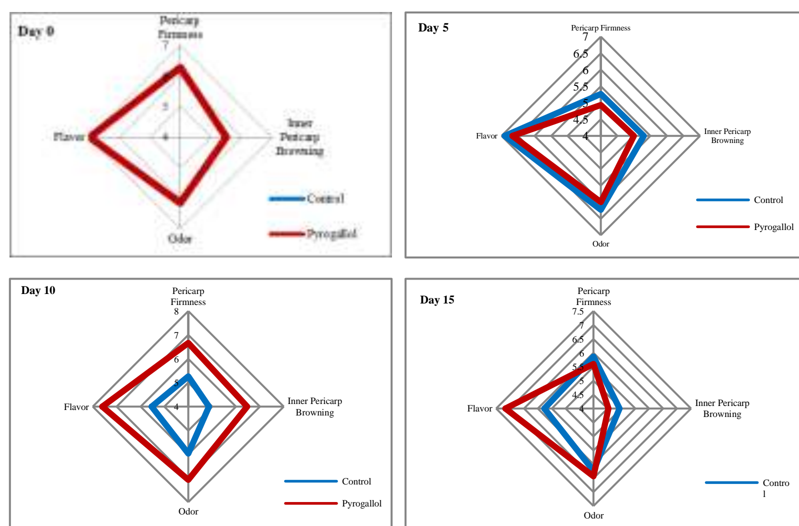

Figure 6: Sensory evaluation of treated and non-treated mangosteens

Table 1
Inhibition zone of pyrogallol and pyrogallol dimer (include 5mm well diameter)

Sample	Diameter of Inhibition Zone (mm)	
	S. aureus	*E. coli*
Pyrogallol	9.5 ± 0.7	-
Pyrogallol Dimer	11.5 ± 0.7	12
Streptomycin	18.75 ± 0.35	20.25 ± 0.35

Table 2
MIC values of pyrogallol and pyrogallol dimer by broth microdilution assay

Sample	MIC value (µg/ml)	
	S. aureus	*E. coli*
Pyrogallol	512	256
Pyrogallol Dimer	8	8

Conclusion

The dimerization of pyrogallol using DPPH method was confirmed by TLC [(Rf =0.90), LC/MS/MS (Rt = 4.20 mins and $^{m}/_{z}$ = 249.04080)] and ^{1}H-NMR (δ 7.00 ppm, d and δ 6.85 ppm, d) as a success, despite having excess unreacted pyrogallol. Dimerization method proposed by Lewis[7] with (pH 7; Method 2) and without (pH 5; Method 3) the addition of potassium phosphate buffer was able to reproduce same product as Zhu et al[6] with a better conversion efficiency of pyrogallol. Dimerization occurred through oxidative coupling reaction consisting of radical polymerization and ketoenol tautomerism.

The antibacterial activity of pyrogallol dimer and pyrogallol was assessed and compared by agar well diffusion and broth microdilution method. In agar well diffusion, pyrogallol and its dimer had the same antibacterial activity to *S. aureus* but the dimer tends to have higher activity against *E. coli*. Pyrogallol dimer also had lower MIC value for both bacteria (8 µg/ml) compared to pyrogallol with MIC value of 512 µg/ml and 256 µg/ml against *S. aureus* and *E. coli* respectively. In conclusion, pyrogallol dimer tends to have a higher antibacterial activity compared to its monomer.

The dimer of pyrogallol synthesized using method 2 has lower antioxidant activity (% Inhibition = 40.77% at 500 µM) than pyrogallol (% Inhibition = 92.37% at 500 µM), therefore pyrogallol was used as dipping agent for mangosteen. Pyrogallol dipping agent has effect on chilling injury symptoms of mangosteen stored at 4°C for 15 days, however its effect to sensory properties of mangosteen was unclear.

Acknowledgement

The authors would like to express their gratitude to Faculty Research Fund of Swiss German University, Research Center for Chemistry of Indonesian Institute of Sciences and all people who helped accomplishing this research.

References

1. Lima V.N., Oliveira-Tintino C.D., Santos E.S., Morais L.P., Tintino S.R., Freitas T.S., Geraldo Y.S., Pereira R.L., Cruz R.P., Menezes I.R. and Coutinho H.D., Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Gallic acid, caffeic acid and pyrogallol, *Microbial Pathogenesis*, **99**, 56-61 **(2016)**

2. Furuno K., Akasako T. and Sugihara N., The contribution of the pyrogallol moiety to the superoxide radical scavenging activity of flavonoids, *Biology Pharmaceutical Bulletin*, **25**, 19–23 **(2002)**

3. PAC 66, 1077, Glossary of terms used in physical organic chemistry, (IUPAC Recommendations 1994), 1106 **(1994)**

4. Contreras-Guzman E. and Strong F., Determination of total tocopherols in grains, grain products and commercial oils, with only slight saponification and by a new reaction with cupric ion, *Journal of Agricultural and Food Chemistry*, **30(6)**, 1109-1112 **(1982)**

5. Xia E., Deng G., Guo Y. and Li H., Biological Activities of Polyphenols from Grapes, *International Journal of Molecular Sciences*, **11(2)**, 622-646 **(2010)**

6. Zhu N., Wang M., Wei G., Lin J., Yang C. and Ho C., Identification of reaction products of (−)-epigallocatechin, (−)-epigallocatechin gallate and pyrogallol with 2,2-diphenyl-1-picrylhydrazyl radical, *Food Chemistry*, **73(3)**, 345-349 **(2001)**

7. Lewis M.J., Natural Product Screening: Anti-oxidant Screen for Extracts, Protocol No. 15.3b **(2012)**

8. Jing G., Huang H., Yang B., Li J., Zheng X. and Jiang Y., Effect of pyrogallol on the physiology and biochemistry of litchi fruit during storage, *Chemistry Central Journal*, **7(1)**, 19 **(2013)**

9. Kondo S., Ponrod W., Kanlayanarat S. and Hirai N., Relationship between ABA and chilling injury in mangosteen fruit treated with spermine, *Plant Growth Regulation*, **39**, 119–124 **(2003)**

10. Indriani N.L.P., Lukitariati S., Nurhadi and Anwarudinsyah M.J., Studi kerusakan buah manggis akibat getah kuning, *Jurnal Hortikultura*, **12(4)**, 276-283 **(2002)**

11. Choehom R., Ketsa S. and van Doorn W.G., Chilling injury in mangosteen fruit, *Journal of Horticulture Science Biotechnology*, **78**, 559 – 562 **(2003)**

12. Dangcham S., Bowen J., Ferguson I. and Ketsa S., Effect of temperature and low oxygen on pericarp hardening of mangosteen fruit stored at low temperature, *Postharvest Biology and Technology*, **50(1)**, 37-44 **(2008)**

13. Higuchi T., Biosynthesis of lignin, In Higuchi T., Biosynthesis and Biodegradation of Wood Components, Academic Press, USA, New York, 141-160 **(1985)**

14. Meloan C., Chemical separations, 1st ed., Wiley, New York **(1999)**

15. Bonev B., Hooper J. and Parisot J., Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method, *Journal of Antimicrobial Chemotherapy*, **61(6)**, 1295-1301 **(2008)**

16. Chakraborty S., Mahapatra S. and Roy S., Biochemical characters and antibiotic susceptibility of *Staphylococcus aureus* isolates, *Asian Pacific Journal of Tropical Biomedicine*, **1(3)**, 212-216 **(2011)**

17. Xie J. and Schaich K., Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity, *Journal of Agricultural and Food Chemistry*, **62(19)**, 4251-4260 **(2014)**

18. Camm E. and Towers G., Phenylalanine ammonia lyase, *Phytochemistry*, **12(5)**, 961-973 **(1973)**

19. Morita Y., Yamashita H., Mikami B., Iwamoto H., Aibara S., Terada M. and Minami J., Purification, crystallization and characterization of peroxidase from *Coprinus cinereus*, *Journal of Biochemistry*, **103**, 693-699 **(1988)**

20. Meilgaard M., Civille G. and Carr B., Sensory evaluation techniques, CRC Press Inc. 2000 Corporate, Boca Raton **(1991)**

21. Takaya Y., Terashima K., Ito J., He Y., Tateoka M., Yamaguchi N. and Niwa M., Biomimic transformation of resveratrol, *Tetrahedron*, **61(43)**, 10285-10290 **(2005)**

22. Nazzaro F., Fratianni F., De Martino L., Coppola R. and De Feo V., Effect of essential oils on pathogenic bacteria, *Pharmaceuticals*, **6(12)**, 1451-1474 **(2013)**

23. Ultee A., Bennik M. and Moezelaar R., The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*, *Applied and Environmental Microbiology*, **68(4)**, 1561-1568 **(2002)**

24. Lambert P., Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria, *Journal of Applied Microbiology*, **92(s1)**, 46S-54S **(2002)**

25. Evans C., Choma C., Peterson W. and Misawa M., Bioconversion of trans-cinnamic acid toL-phenylalanine in an immobilized whole cell reactor, *Biotechnology and Bioengineering*, **30(9)**, 1067-1072 **(1987)**

26. Riahi S., Moghaddam A.B., Ganjal M.R. and Norouzi P., Determination of the oxidation potentials of pyrogallol and some of its derivatives: theory and experiment, *Journal of Theoretical and Computational Chemistry*, **6(2)**, 331-340 **(2007)**

27. Noichinda S., Effect of modified atmosphere condition on quality and storage life of mangosteen (*Garcinia mangostana L.*) fruit, 74 f, Doctorate Thesis, Kasetsart University, Bangkok, Thailand **(1992)**

28. Florkowski W., Postharvest handling, Academic Press, San Diego **(2014)**

29. Saqib M., Mahmood A., Akram R., Khalid B., Afzal S. and Kamal G.M., Density functional theory for exploring the structural characteristics and their effects on the antioxidant properties, *Journal of Pharmaceutical and Applied Chemistry*, **1(2)**, 65-71 **(2015)**

30. MacLeod A. and Pieris N., Volatile flavour components of mangosteen, *Garcinia mangostana*, *Phytochemistry*, **21(1)**, 117-119 **(1982)**

31. Nio O.K., Daftar analisis bahan makanan, Fakultas Kedokteran Universitas Indonesia, Jakarta **(1992)**

32. Manurakchinakorn S., Intavong P., Yuennan P., Tonwattana S. and Pankong A., Effect of storage conditions on quality attributes of fresh-cut mangosteen, *Acta Horticulturae*, **857**, 251-258 **(2010)**

33. Castro M., Anjos V., Rezende A., Benato E. and Valentini S., Postharvest technologies for mangosteen (*Garcinia mangostana L.*) conservation, *Food Science and Technology (Campinas)*, **32(4)**, 668-672 **(2012)**.