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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目： Screening of bioactive phytochemicals from mangrove fruits
Title of Dissertation （マングローブ果実からの生理活性植物成分の選別）

学位論文要約：
Dissertation Summary

Mangroves are a highly productive forest with various important economic and environmental functions. The rich resources of mangroves have been widely used by coastal people of the tropics for a thousand years. Many human communities have traditional dependence on mangroves for their survival by utilizing a wide range of natural products and their surrounding waters. The uses of mangroves fall in two major categories. Firstly, from ecological function, mangroves as a buffer area to protect the coast from erosion, stabilize the sedimentation, and purify the coastal polluted water. Secondly, mangroves provide various natural products such as timber, fuel, food, and medicine and also as a source of chemical constituents. The chemical constituents derived from mangroves are used not only for the medicinal purpose by indigenous people for centuries but also for functional food consumption because of their bioactivities.

In Indonesia, the resources from mangroves, mainly for wood or timber products, charcoal, tannin, dyes, beverages, traditional medicine, and food have been utilized traditionally for centuries by the local communities who live in the coastal area. For instance, in particular area of Pamekasan Regency, East Java, the fruit part of dominant species of mangrove, *Rhizophora stylosa*, has been processed as food products. *Rhizophora stylosa* is part of family *Rhizophoraceae* and bears viviparous seed. Their germination and the seedling development actually occur on parent plant. When the seedling matured and ready for regeneration, the fruit part (including cotyledon) is left useless on the tree. The fruit part is, therefore, used as the main ingredient of food products within the local society in this area, which is known as coffee and tea mangrove.

Coffee and tea mangrove have been manufactured by the household industry since 2012 and 2015, respectively. The coffee mangrove is composed of the powders of mangrove fruits, gingers (*Zingiber officinale*), and Javanese long peppers (*Piper retrofractum*); all ingredients are prepared through sun drying, roasting, and grinding processes. The supplemental materials, ginger, and Javanese long pepper are added as the flavor to enhance the coffee taste.

Meanwhile, tea mangrove is a plain coarse powder from mangrove fruits without roasting process and addition of supplemental materials. Local folks who daily take both products recognize physiological activities, such as rejuvenation and fitness improvement. It means that some bioactive compounds in these products may exhibit such effects; however, no scientific evidence has been elucidated and further study is desirable to clarify their phenomena; therefore, investigation of the bioactive compounds of mangrove food products (coffee and tea mangrove) and their medicinal potentials: antioxidant activity, α-glucosidase inhibitory activity, and anti-bacterial activity have been conducted in this research.

In the first step of our study, the bioactive compounds and the antioxidant activity were screened from coffee and tea mangrove. Furthermore, three raw materials of coffee mangrove were also investigated to clarify their potencies. After being subjected to DPPH assay, from five crude extracts, tea mangrove showed strongest antioxidant activity with IC_{50} 4.13 μg/mL. Then, tea mangrove was successfully fractionated using different polarity of solvents such as n-hexane, n-hexane and ethyl acetate, acetone, and methanol and the fractions were subjected to the antioxidant assay using DPPH. As a result, acetone and methanol fractions exhibited high activity compared to the others with IC_{50} 4.38 μg/mL and IC_{50} 2.66 μg/mL, respectively.

The acetone fraction was purified by HPLC was performed using ODS column (Mightysil, RP-18 GP 250–20, Kanto Chemicals Co.), eluted with 100% of methanol at flow rate 3 mL/ min, and gave fractions A1, A2, A3, A4, A5, and A6, but only A2 and A3 indicated antioxidant activity and, therefore, they were subjected to further purification using HPLC were performed by silica-gel semi preparative column (Wakosil 5SIL, Wako Chemicals Co.), eluted with n-hexane and ethyl acetate (50%:50%). From fraction A2, four compounds were obtained (AS1-AS4) based on the peaks which amounted to only 14.1 mg. Most of A2 that could not be separated well seemed to be oligomer or polymer-like as methanol fraction; A3 was rather single compound. The fractions were identified as caffeine (A3) and from A2: AS1 (N,N-dimethyl-L-alanine), AS2 (quercetin-3-O-galactopyranoside), AS3 (dodecanoic acid), and AS4 that had the ^{1}H-NMR spectrometric results similar to that of AS2.

The methanol fraction did not exhibit clear peaks on the chromatogram by HPLC. Therefore, the precipitation method was conducted to purify this fraction, and the precipitate was analyzed by NMR spectra. The results from ^{1}H and ^{13}C NMR indicate that this fraction is a typical polymer of condensed tannins, containing procyanidin and prodelphinidin units. The precipitate compound was also methylated at room temperature with KOH and dimethyl sulfate in order to be analyzed using GPC column (TSK gel G2000HS, Toso Co.) with chloroform to determine the degree of polymerization. Based on the appearance of peaks on chromatogram, the average DP (degree of

polymerization) is estimated to be around 4 and 5.

As the final stage, the six compounds isolated were subjected to DPPH test to clarify their antioxidant activity, and condensed tannin, which mainly constitutes the acetone and methanol fractions of tea mangrove extract, had high antioxidant activity (IC_{50} 2.69 μg/mL) compared with the other compounds. The condensed tannins in mangrove food products or mangrove fruit extracts must be the potential antioxidant agent.

In the second step of our study, the inhibitory activity of coffee and tea mangrove on α-glucosidase and anti-bacterial activity against *Streptococcus mutans* have been evaluated. The potential of coffee and tea mangrove were tested to inhibit the activity of α-glucosidase enzyme from yeast; and *p*-NPG as a substrate. Mangrove powder, ginger and Javanese long pepper powder, green tea, black tea, regular coffee, and the two positive controls (acarbose and quercetin) were also tested with the same manner. The inhibitory mechanism of sample with high inhibitory activity was further analyzed using Lineweaver-Burk plots.

Coffee and tea mangrove including mangrove powder strongly suppressed α-glucosidase activity compared to the other samples and the positive controls. At the lower concentration 0.05 μg/mL, mangrove extracts indicated the % inhibition for α-glucosidase activities were ranged between 25.51 and 34.68, and at the highest concentration of 0.15 μg/mL, the extracts inhibited α-glucosidase were ranged from 74.17% to 83.36%; while their IC_{50} values were ranged from 0.07 to 0.08 μg/mL, without any statistical differences. Then, the extracts (coffee and tea mangrove including mangrove powder) were analyzed for their inhibitory mechanism using Lineweaver-Burk plots.

Mangrove food products (coffee and tea mangrove including mangrove powder) showed mixed competitive inhibition. The Lineweaver-Burk plot regression reveals the inhibitors affect the reduction in Vmax and the increase in Km. The Inhibition constants value of mangrove extracts binding with free enzyme (Ki) were lower than the value of enzyme substrate complex (Ki'). The lower value of Ki than of Ki' and the increasing value of Km were indicating the extracts had higher affinity for free enzyme than for the enzyme-substrate complex (Ki < Ki'). A possible reason for the extracts showing mixed competitive inhibition because of condensed tannins with emerged more diversity in the structures (e.g., the coexistence of A-type and B-type condensed tannins; propelargonidin, prodelphinidins, and procyanidin), they tend to form a mixed competitive inhibition. This might arise from mutual interference of condensed tannins with a different structure.

Meanwhile, the anti-bacterial activity of coffee and tea mangrove including mangrove powder, ginger and

Javanese long pepper powder were evaluated by disk diffusion method in comparison with 0.2% aqueous solution of chlorhexidine as a positive control. The results showed these mangrove products also had anti-bacterial activity in disk diffusion method through measuring inhibition zone. The anti-bacterial activities of products were increased with the increasing of concentration of the extracts. At lower concentration (1 mg/mL) the diameters of inhibition zones were ranged from 2.33 to 8.67 mm; and at highest concentration (25 mg/mL) the diameters of inhibition zones were ranged from 9.17 to 13.33 mm. Our previous study showed that the mangrove food products, in particular, the acetone and methanol fractions of tea mangrove were found to be mostly composed of the condensed tannins. Tea and coffee mangrove, and mangrove powder were all originated from *R. stylosa* fruit, and it means that the potent activity of those products to inhibit α-glucosidase and as an anti-bacterial agent might be of the condensed tannins.

In conclusion, the mangrove food products or mangrove fruit extract contains 6 bioactive compounds with condensed tannins as the active main compound, exhibited the strongest antioxidant activity compared with the other compounds. On the evaluation of anti α-glucosidase and anti-bacterial activity, mangrove food products (coffee and tea mangrove) showed the inhibitory activity for α-glucosidase and the anti-bacterial activity against *S. mutans*. The both activities of the mangrove extracts might be induced by the presence of condensed tannins. Coffee and tea mangrove can be the potential of antioxidant, anti α-glucosidase and anti-bacterial agent due to the presence of condensed tannins as the active main compound.

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