ANTI-PHOTOOXIDATIVE EFFECT OF PHENOLIC EXTRACT FROM CLOVE PARASITE (*Flower*) IN SYSTEM MODEL

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ABSTRACT

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Clove parasite is a herbal plant commonly used in north sulawesi. Its traditionally used to cure many kind of disease, especially cancer. The objectives of this study was to determine antiphotooxidation activities of clove parasite flower on linoleic acid 0,03 M under illuminate with 4000 lux fluoresence lamp for 5 hours. Analyses of antiphotooxidation activities based on Lee *et al*. (1997) method and analyses of rancidity of linoleic acid was determine with AOCS 1990 methods. The addition of clove parasite flower extracts in the reaction mixture showed antiphotooxidation activities on linoleic acid 0,03 M. The mixture with extract show lower peroxide value compare with control without extract after 5 hours light exposure at room temperature. Activities as antiphotooxidation of clove parasite extract may cause by phenolic compound on this plant.

Keywords: clove parasite, linoleic acid, antiphotooxdation, peroxide value

INTRODUCTION

Parasite is a plant which live in other plant and get nutrition by absorb from the plant where it live. The Parasite which live in a plant, named according to the plant where it live, for example parasite which live in a clove plant named clove parasite. Parasite traditionally used by people to cure many diseases, especially cancer. Based on that uses, clove parasite have a great potential to develop as an antioxidant component.

Free radical included reactive oxygen species (ROS) is a relative unstable molecule, have one or more unpaired electron in outer orbit. Some of ROS are hydroxyl radical ($^{\bullet}OH$), superoxyde anion radical ($O_2^{\bullet-}$), hidroperoxide (H_2O_2) and singlet oxygen ($^1O_2$). Between ROS above, singlet oxygen act as biologycal oxidant, show unique oxidant character and so reactive on biological component such as protein, lipid, vitamins and DNA (Foote, 1970; Jung *et al*., 1998; King and Min, 2002; Choe and Min, 2005).

Not like another ROS, singlet oxygen is a ROS species non radical electrophilic (Min and Boff, 2002; Choe and Min, 2005). However, singlet oxygen may influence oxidation process by attacking the component directly which rich with electrons without free radical presence. Oxidation on biologycal component which induced by singlet oxygen correlated with many kind of patologycal process such as pigmentation, cataract, skin ageing and cancer (Davies and Goldberg, 1987; Shahidi, 1997: Haliwell and Guttridge, 2001).

Although there's no data that study about anti-photooxidative activity or *singlet oxygen quencher* (SOQ) from clove parasite flower.

MATERIALS AND METHODS

Materials and Tools

Clove parasite was obtained from a local farm at Minahasa district, North Sulawesi when summer 2008. Some materials used in this research were qualify for professional analyses: amylum, acetic acid, ethanol, potassium iodide, sodium thiosulphat, filter paper whatman no. 42 and erythrosine purchased from MERCK (Darmstadt, Germany). Linoleic acid purchased from SIGMA Chemical Co. (St. Lois, MO). The tools used in this research are laboratory glass, microburrete, micropippete, rotary evaporator vacuum Eyela N-1000 and Spectrophotometer UV-Vis Milton Roy Spectronic 501.

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Sample preparation

Clove parasite were cleaned from dust and other external material. After that, flower part of clove parasite was separated from other parts. Sustainable sample was freeze dry until water component <5%. Furthermore, clove parasite flower sample (CPF) was blended to 40 mesh.

Extraction of clove parasite flower

Ten gram CPF powder was transferred to Erlenmeyer glass than extracted with 100 mL ethanol 95% for 24 hours. Sample filtered with filter paper Whattman no. 42. The filtrate that had been obtained was evaporated using rotary evaporator to obtain phenolic extract of clove parasite flower. Extract was measured and refrigerated in -20 ^{o}C before analyses.

Total phenolic content

The content of total phenolic was measured using Jeong et al., 2002 method. Briefly, the clove parasite extract (1 mL) was mixed with 0,1 mL of Folin-Ciocalteu reagen 50% and 2 mL of Na_2CO_3 2% and sentrifuged at 13400 rpm for 5 min. The absorbance of extract was read at 750 nm with spectrophotometer Milton Roy 501 after 30 min incubation at room temperature. The resut was expressed as galli acid equivalen.

Effect of phenolic extracts on photostability of erithrosine

To study about the presence of singlet oxygen in photodegradation, five ppm erythrosine contain 200 ppm phenolic extract was prepared. Sample from the mixture was taken 10 mL and transferred to serum bottle 30 mL equipped with a plastic cover and aluminium foil. The bottle was illuminated in a light box (4000 lux) for 5 hours. Sample without extract was used as a control. An erythrosine changing was determine using spectrophotometer method (λ = 524 nm). Another experiment was done in condition without light.

Effect of phenolic extract on photostability linoleic acid

To study about anti-photooxydative activities of phenolic extract using Lee *et al*. (1997) with less modification. The effect of phenolic extract on singlet oxygen oxydation of 0,03 M linoleic acid using 1000 ppm phenolic extract in ethanol and contain 15 ppm erythrosine as sensitizer. Sample from the mixture was taken 10 mL and transferred to serum bottle 30 mL equipped with a plastic cover and aluminium foil. Sample without extract was used as a control. The bottle was illuminated in a light box (4000 lux) for 5 hours. Peroxide value was evaluated every hour using AOCS (1990) method. Another experiment was done in condition without light.

Statistical Analyses

Experimental data were analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range test (DMRT) using SPSS version 15 for windows and $p < 0,05$ considered to be statistically significant.

RESULT AND DISCUSSION

Total Phenolic Content

Detemination of total phenolic was done to study the potential of extract to be antioxidant. In this study, total phenolic in clove parasite extract was determined using galic acid standart (mg/kg). Total phenolic in extract was determine based on activity of phenolic compound in extract that react with fosfomolibdat–fosfotungstat acid in Folin–Ciocalteu (kuning) reagen become blue solution. Total phenolic content in root, stem, leaves and flower extract were 221,45; 229,45; 239,64 and 242,55 mg/kg, respectively showed in Figure 1.

Extract obtained from root contain the highest total phenolic content compared with another parts of clove parasite. High total phenolic content in root were predicted caused by phenolic compound in root was polar then that phenolic component in extract was more solute in ethanol.

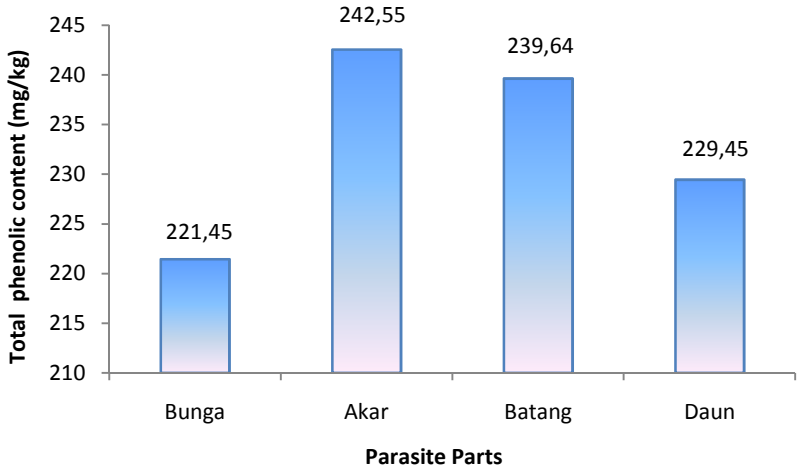

Figure 1. Total phenolic content from clove parasite parts

Total phenolic content in clove parasite extract related with its antioxidative activity. Antioxidative ability of clove parasite extract cause the present of chemical compound that can act as antioxidant.

Effect of phenolic extracts on photostability of erithrosine

After done a photooxidation process to study the effect of phenolic extract on erythrosine photodegradation, a graphic was obtained that interpreted the changing of 5 ppm erythrosine with 200 ppm phenolic extract for 5 hours under fluorescent light exposure (4000 lux). Erythrosine as photosensitizer could absorb the light and transformed to its exitated state. Moreover, erythrosine become unstable sensitizer on triple state (^{3}Sen*). Sensitizer could transfer its energy to triplet oxygen becoming singlet. To study the presence of singlet oxygen in erythrosine photodegradation, phenolic extract of clove parasite flower was added in this mixture. If the addition of phenolic extract could increase the stability of erythrosine, indicated that singlet oxygen involve in erythrosine photodegradation.

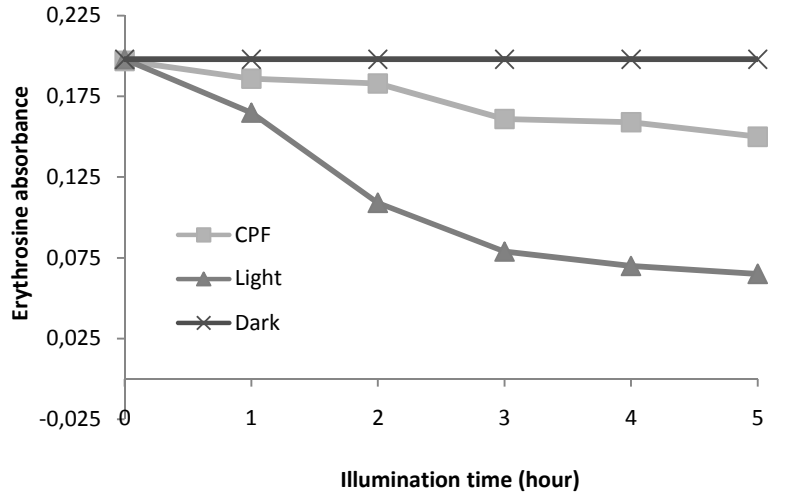

Figure 2. The effect of phenolic extract from clove parasite flower on erythrosine photodegradation (CPF, clove parasite flower; Light, condition with light; Dark, condition without light)

Figure 2 showed that phenolic extract of clove parasite flower can defense the stability of erythrosine compared with erythrosine mixture without extract under fluorescent light exposure. Light effect on erythrosine step by step decrease followed with photodegradation time. Picture 1

show that phenolic extract of clove parasite flower more inhibited on erythrosine photodegradation. This fact proved that the changing of erythrosine was less than control without extract ($p<0,05$). The data that has been obtained show phenolic extract of clove parasite flower has activity to prevent erythrosine bleaching with light intensity 4000 lux ($p<0,05$), an erythrosine bleaching is related with the presence of singlet oxygen on erythrosine photodegradation.

This result indicated that the activities of phenolic extract of clove parasite flower related with singlet oxygen quenching activity. Activity to defense erythrosine bleaching from phenolic extract related with the presence of phenolic, flavonoid and tannins component in extract that react with erythrosine as long as light exposure or that phytochemical component could act as photostability in mixture.

Effect of phenolic extract on photostability linoleic acid

This research was done to study the effect of secondary metabolite on photostability fatty acid. Unsaturated fatty acid such as linoleic acid 0,03 M was used as a substract on antiphotooxidative system with fluorescent light (4000 lux). In photooxidation oil and lipid, initial process to generate peroxide was over than its decomposition process. Therefore, oxidative stability of linoleic acid in this research can be study by the changing of hidroperoxide that expressed as peroxide value. The effect of phenolic extract of clove parasite flower on photooxidation linoleic acid illuminated with fluorescent light (4000 lux) can be see on picture 3 below:

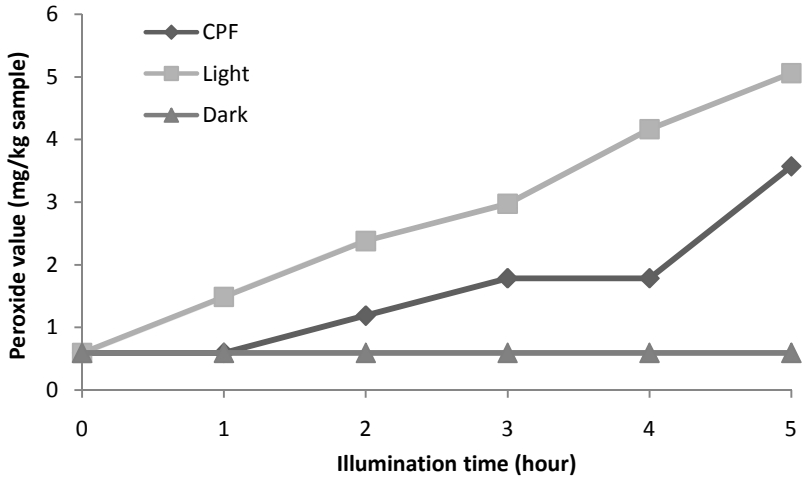

Figure 3. The effect of phenolic extract of clove parasite flower on photostability linoleic acid illuminated with fluoresence lamp (4000 lux) for 5 hours (CPF, clove parasite flower; Light, condition with light; Dark, condition without light)

Linoleic acid contain 5 ppm erythrosine as sensitizer illuminated with fluoresence lamp (4000 lux) on picture 2 above show the changing of peroxide value significantly ($p>0,05$). The presence of erythrosine significantly influenced peroxide value of linoleic acid under fluorescent light exposure for 5 hours. Erythrosine that used in this study could increase the production of singlet oxygen cause photooxidation with unsaturated fatty acid in this case linoleic acid. In addition of 200 ppm phenolic extract in 0.03 M linoleic acid mixture with 5 ppm erythrosine as sensitizer and control in dark condition under illumination for 5 hour does not give any changing of peroxide value significantly ($p<0,05$).

Anti-photooxidative activity from phenolic extract of clove parasite flower in linoleic acid mixture can hold the production of hidroperoxide probably cause by the presence of components in phenolic extract of clove parasite flower that react with erythrosine under illumination or the component in phenolic extract of clove parasite flower may act as anti-photooxidation component in that mixture. Some of secondary metabolite such as phenolic component may act as anti-photooxidation. Phenolic component is a natural antioxidant that commonly used as exhibit lipid oxidation in food. In the other hand, α-tocopherol reported as a singlet oxygen quencher in soybean oil (Jung *et al*., 1991). In Contras with, peroxide

value on sample without erythrosine did not show any differences after 5 hour illumination with fluorescent lamp. Cause without the presence of erythrosine as photosensitizer will not accelerate the production of singlet oxygen, so peroxide value of sample after illumination did not change (*stable*).

Besides controling the intensity of light and decrease oxygen capasity, utilizing quenching agen was the best way to minimalize oxidation from singlet oxygen. Natural component in food such as tocopherol, carotenoid and ascorbic acid can act as singlet oxygen quencher efectively. Quenching agent was involved in minimalizing the production dan activity of singlet oxygen on some food oxidation stages (Min dan Boff, 2002).

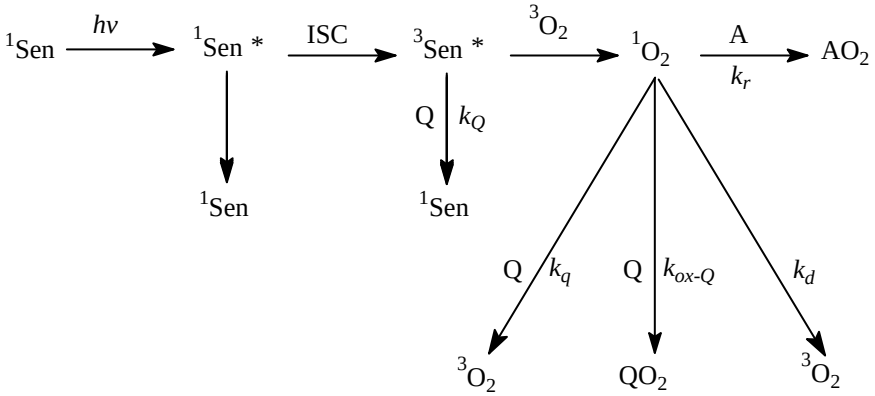

Figure 4 Singlet oxygen production and its reaction with subtract A produced oxidation product AO_2. Production AO_2 can be delayed from reaction between $^3Sen^*$ or $^1O_2$ quencher agent (Min dan Boff, 2002)

Figure above show how quenching agent such as quencetin may be involved to minimize the development of singlet oxygen activity at several stages I the oxidation of food. Figure above shows the development of singlet oxygen and its subsequent reaction with compound A to perform the oxidazed product (AO_2). At every stage in this reaction, there is at least 3 alternate route which if we taken would minimized the oxidation of the compound (A). The first step is represent whe a sensitizer (Sen) such as erythrosine in oil absorb energy and becomes an excited singlet sensitizer ($^1Sen^*$). The return of excited singlet sensitizer ($^1Sen^*$) to ground state without intersystem crossing (isc) to form excite triplet sensitizer ($^3Sen^*$). The second represent the reaction with a quenching agent (Q) at a rate represent as k_q, returning to excited triplet sensitizer ($^3Sen^*$) to ground state (^1Sen) pior to reaction with triplet oxygen. The excited triplet sensitizer ($^3Sen^*$) may react with triplet oxygen ($^3O_2$) to perform singlet oxygen ($^1O_2$). Following its reaction, there are three fates for singlet oxygen in food: (1) it may naturally decay to the round state at a rate represented as k_d ; (2) it may react with siglet-state compound (A) at a rate represent as k_r forming the oxydazed product AO_2 and (3) it may destroyed by quenching agent by either combining with the quencher at a rate represent as k_{ox-Q} to perfor the product QO_2 or by passing its energy to the quenching agent and returning to free triplet oxygen at a rate represent as k_q (Min and Boff, 2002)

CONCLUSION

Phenolic extract of clove parasite flower which added in linoleic acid system may minimalyse the increasing of peroxide value of linoleic acid. This study showed that phenolic extract of clove parasite flower seems to have antiphotooxidative activities on linoleic acid that illuminated by fluoresence lamp (4000 lux) for five hours.

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