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Singlet Oxygen Quenching activity of Silver Nanoparticles Synthesized using Goroho Banana Peel (*Musa acuminata*)

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Abstract : Goroho banana is one of plantain type, locally grown crops in North Sulawesi and potential as excellent source bioactivities including antioxidant phytochemical content and macronutrients.The silver nanoparticles have made by mixing goroho banana peel extract with silver nitrate solution (10^{-3} M) at roomtemperature. The silver nanoparticles were characterized using spectrophotometer UV-Vis and infrared (IR), while the morphology and particle size have determined by SEM (Scanning Electron Microscope). The anti-photooxidative activity of silver nanoparticles with the concentration 40; 60100and200mg/mL were evaluated in linoeat acid emulsion system that containing 5 µg/mL erythrosine as sensitizer and illuminated with fluorecent 4000 lux for 5 hours. TheUV-Vis spectra shows that surface plasmon resonance (SPR) at wavelength 423-442 nm. It proves that there was a reduction of silver ion (Ag^{+}) to silver (Ag^{0}) and indicates the formation of silver particles. The spectra of IR at 4302 cm^{-1} shows that the presence of hidroxyl groups (OH) from polyphenol compounds in goroho banana peel extracts while the IR spectras of silver nanoparticles at the ribbon 3448 cm^{-1}. The characterization with SEM shows that silver nanoparticles have sized by the range at 254-768 nm; 41-98 nm and 53-90 nm. On assessment with the silver nanoparticles having effect on singlet oxygen quenching at all of concentration levels compared to banana goroho extract. The results of this research proves that antioxidant of banana goroho peels extract can act as an agent to synthesized silver nanoparticles possess singlet oxygen quenching acitivity.
Keywords : banana goroho peels, silver nanopartikel, characterization, singlet oxygen quenching.

Introduction

Singlet oxygen induces a unique oxidation process by attacking directly the electron-rich compounds without the free radical involvement. The oxidations of biological components (proteins, lipids, vitamins and DNA) induced by singlet oxygen are associated with various pathological events such as pigmentation, cataract,

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skin aging and cancer[1,2]. Whereas, the oxidation reaction of food components can leads to nutritional losses, production of possible toxicants that make food less acceptable or unacceptable to consumers[3]. Both the oxidation system in various types of endogenous and foods are very susceptible to photooxidation during storage under light, especially when photosensitizers such as chlorophylls, riboflavin, erythrosine, myoglobin and phorphorine present in the systems[4,5,6]. The sensitizer can transfer its light energy to chemical energy and begin oxidation reaction. Rawls and Van Santen[7]reported that singlet oxygen participated in the initiation step of oil oxidation and the reaction rate of singlet oxygen with linoleic acid is about 1450 times greater than that of triplet oxygen.

The undesirable photosensitized lipid oxidation can be reduced by quenching singlet oxygen. Tocopherols, carotenoids, and ascorbic acid can be used for the practical reduction of singlet oxygen oxidation of oils and other oil-soluble components[8]. Tocopherols can be also act antioxidant in autocatalytic condition but, their singlet oxygen-quenching abilities are not effective as the carotenoid. β-carotene is an active $^1O_2$ quencher in soybean oil[9] (Lee and Min, 1990). The bleached and unbleached rosemary oleoresins had quenching effect in the soybean oil on light-sensitised oxidation[10].

In recent years, the use of nanotechnology to healthy benefits is a promising alternative in food and medical field, such as faster diagnosis, bioavailability, drug delivery and tissue regeneration. The use of green synthesis of nanoparticles isevolving and essential approach in nanotechnology. Among plant biological system is much preferable to the biosynthesis of nanoparticles because of the richness of plant divert that provider phytochemicals and antioxidant properties. It is well known that the plant has been used by humans long ago to treat many diseases[11]. The process of biosynthesis of metal nanoparticles by biological agents, is influenced by several factor, including organism types, and concentration of reducers of precursors. Biological agent is thought to be reducing, stabilizing, or both in the process of farming nanoparticle biosynthesis are thought to involve secondary metabolic compounds from plants, such as flavonoids and triterpenoids[12].Besides, this is also due to the abundances of hydroxyl and amino groups that present in these plant resources as reducing and capping agent.Many study had been reported and proved that various spices extracts of plant was good reducing agents that potential for preparing silver nanoparticels such as *Ocimum sanctum,Camellia sinensis,Morinda pubescens,Cymbopogon citrates, and Rhododendron dauricum* [13,14,15,16,17]. The silver nanoparticles are also reported to be nontoxic to human[18].

The banana plant is classified as a great perennial treelike, tropical herb of the genus *Musa* and the family Musaceae. Banana is one of fruit that much comsumption in the worldwide for better fresh (common banana)orin process (plantain). The plantain is a type of banana, usually cooked before eating, known as *Musa paradisiaca.* Goroho banana is one of a type of plantain, locally grown crops in North Sulawesi and that are not familiar to people outside North Sulawesi, compared to other varieties of banana. The utilization of banana fruits as food material resulting banana peels which is often ignore or as waste product. Gorohobanana and sap peel are a phenolic, flavonoid, saponin, and tannin phytochemical containing biomass which recommended to be used as active antioxidant compound[19].The other research reported that peel extracts of nine varieties banana in India exhibited antioxidant and phytochemical content which are rich in compounds with free radical scavenging activity[20]. Besides, banana feel of *Musa sapientum* indicated the presence of glucoce, fructose, sucrose and maltose[21].Sundaram et al.[22]suggest that the unripe banana peel sample had higher antioxidant potency than ripe and leaky ripe.

In the present study, phytochemicals antioxidant extracted from goroho banana peel has been used to synthesize silver nanoparticles. The abundances of phenolic groups present in the antioxidants extract is expected to reduce silver ions to silver metal so that antioxidant constituents in present goroho banana peel can be interaction with metallic surface by capping agent through phenolic extracts. The objective of this research was to synthesize silver nanoparticles using goroho banana, characterization by UV-visible spectrophotometry, infrared (IR), scanning electron microscope (SEM) and determine singlet oxygen quenching activity.

Materials and Methods

Goroho banana was collected from a local market in Manado, North Sulawesi, Indonesia. The Folin-Ciocalteu reagent, ethanol, Tween 20, silver nitrate, sodium carbonate, and erythrosine used in this experiment were purchased from Merck(Darmstadt, Germany). The linoleic acid and gallic acid were purchased from Sigma Chemical Co. (St. Lois, MO).Deionized water was used throughout the reaction.

Preparation of plant extract

Goroho banana was washed to remove impurities, dried at room temperature then sliced into small pieces using stainless steel knife. About 1 g of fresh banana peel was boiled in 50 mL of deionized water for 15min and cooling was filtered using filter paper (Whatman No. 1), furthermore this treatment is called sample A. After that the extract was centrifuged at 3000 g for 10 min and then stored in screw tube at 5^{o}C until examined. The same procedure was done for extracting3g (sample B) and 5g (sample C) fresh banana peel.

Synthesis of silver nanoparticles

Silver nanoparticles were synthesized from the aqueous peel extract of goroho banana by reducing silver nitrate. Banana peel extract (20, 60, 100 and 200 mg/ml) was used to reduce 10 mL of 1 mM silver nitrate solution. The appearance of reddish brown color after 60 minutes indicates the formation of silver nanoparticles and the solution was centrifuged at 12000 rpm for 15 min. The recovered nanoparticle sample was used for characterization and singlet oxygen quenching studies.

Characterization of silver nanoparticles

Silver nanoparticles that have been synthesized are than characterized to determine the characteristics of silver nanoparticles. Analysis performed for characterization using UV-Vis (Shimadzu Seri 1800),FTIR Spectroscopy was measured using spectrum FT-IR in the scanning range 450-4000cm^{-1}(Shimadzu FTIR), scanning electron microscope (SEM) and the SEM. This instrument helped to check the presence of various elements in the powdered nanoparticles.

Determination of total phenolic content

To detect the involvement of phenolic content in reducing and capping agents for silver nanoparticles, 20; 60, 100 and 200 mg/mLsolution containing silver nanoparticles was prepared. Procedure was determined using modified Folin-Ciocalteu colorimetric method from Li et al.[23]. Each sample solution (0.1 mL) was added to Folin-Ciocalteu reagent (0.1 mL, 50%) in a test tube and then this mixture was vortexed for 3 minutes. After intervals of 3 minutes, 2 mL of Na_2CO_3 2% solution was added. After incubation at room temperature for 30min, the mixture was kept in the dark for 30 minutes. The supernatant was measured using a spectrophotometer at 760 nm. The standard curve was prepared using different concentrations of gallic acid and the results were expressed as gallic acid equivalents in milligrams per milligram extract.

Determination of singlet oxygen quenching activity

The procedure of singlet oxygen quenching activity determination was based on Suryanto et al.[24], with minor modification. This procedure was to study the effects of banana peel extract and silver nanoparticles on photosensitized oxidation of linoleic acid emulsion. Linoleic acid (1.5 g) was added with 0.2 mL of Tween 20 and 1.8 mL of distilled water. The emulsion was then stirred for 3 minutes at room temperature. After that, samples were added with 16 mL distilled water and stirred for 30 minutes. Banana peel extract and silver nanoparticles (20, 60, 100, and 200 mg/ml) were added 10 mL of the emulsion that contained 5 µg/mL of erythrosine as a photosensitizer. Emulsion samples (10 mL) were transferred into a 30 mL serum bottle. The bottles were airtight sealed with Teflon septa, wrapped with aluminum and then placed in the light box. The light intensity of the sample level was 4,000 lux, at room temperature. The light storage box consisted of two rectangular chambers: a glass chamber (60 cm x 30 cm x 50 cm) for sample storage and the wooden box (70 cm x 50 cm x60 cm) for light sources to the glass chamber was 12 cm. Samples were placed on the wire netting which was10 cm above the bottom of glass chamber. The light source, 65-watt cool white fluorescence lamps (Philips)was placed on the 4,000 lux. The temperature of the light storage box was kept constant at room temperature. Photooxidation stability of linoleic acid was evaluated by analyzing samples periodically for conjugated diene hydroperoxides and the conjugated diene absorbance was measured at 234 nm. Results were calculated as hydroperoxide in millimoles per kilogram of oil using an absorptivity of 26000 for linoleate hydroperoxides23.Samples of oil-in-water emulsion (0.30 µL) were into the tube and dissolved with 5 mL of ethanol absolute, the absorbance was measured at 234 nm. Hydroperoxide value will be measured every time interval of an hour. The experiment was carried out in triplicate.

Result and Discussion

Synthesis of silver nanoparticles

The formation of silver nanoparticles can be detected with observing the color changes of prepared solutions. Figure 1 shows that the color changes from pale yellow to yellowish-brown during the reaction, indicating the synthesis of silver nanoparticles, whereas the silver nitrate solution without addition of banana peel extract and banana peel extract using as control. In this study is color change occurred a few minutes. This color formation was due to the presence of the surface plasmon resonance, an optical characteristic of silver nanoparticles[25].Many food and medicinal plants containing phytochemical compounds such as phenolic, tannin, flavonoid, alkaloids, glycocides, and amino acid have been reported to possess strong ability to act as reducing and capping agents for silver nanoparticles[26,27,28, 29,30,31].

Figure 1. Change of color synthesis of silver nanoparticles. Note: Extract (1 g goroho banana peel), 1 g (sample A), 3 g (sample B) and 5 g sample C

Alhabsyi et al.[32] and Kurniawan et al.[33]reported that peel and gum of banana goroho contain of phenolic, flavonoid and tannin having antioxidant properties. The availability of this compound with redox properties makes banana peel extract possesses ability for green synthesis of nanoparticles colloids so that plays role to reduce Ag^{+} to Ag^{0}. According to Ahmad et al.[34]that mechanism for the reduction of silver in plant extracts is releasing of an electron when formation of phenolic radicals from phenolic reduces the silver ions or electron released during glycolysis for conversion of NAD to NADH led to transformation of $AgNO_3$to form nanoparticles. Therefore, the color change shows the process of the reduction of silver ions for initiation of forming silver nanoparticles.

Characterization of silver nanoparticles

Spectra UV-Vis is used to determine the characterization of the nanoparticles formed by peak absorption spectrum. Characterization of colloidal silver nanoparticles is using a spectrophotometer UV-visible at wavelength (200-800 nm). Goroho banana peel extract (GBPE) has a peak absorbance spectrum at 276 nm wavelength of 0.89 presented in Figure 2.The UV-visible spectrum obtained with change after the $AgNO_3$ solution was mixed with BPE and obtained the absorbance peak at 426.50-452.00 nm (Figure 2) which is the silver nanoparticles absorption area in observation for 60 minute. The results are significantly different than the maximum absorption wavelength for GBPE. It means that there has been a reduction process (Ag^{+}) to (Ag^{0}).

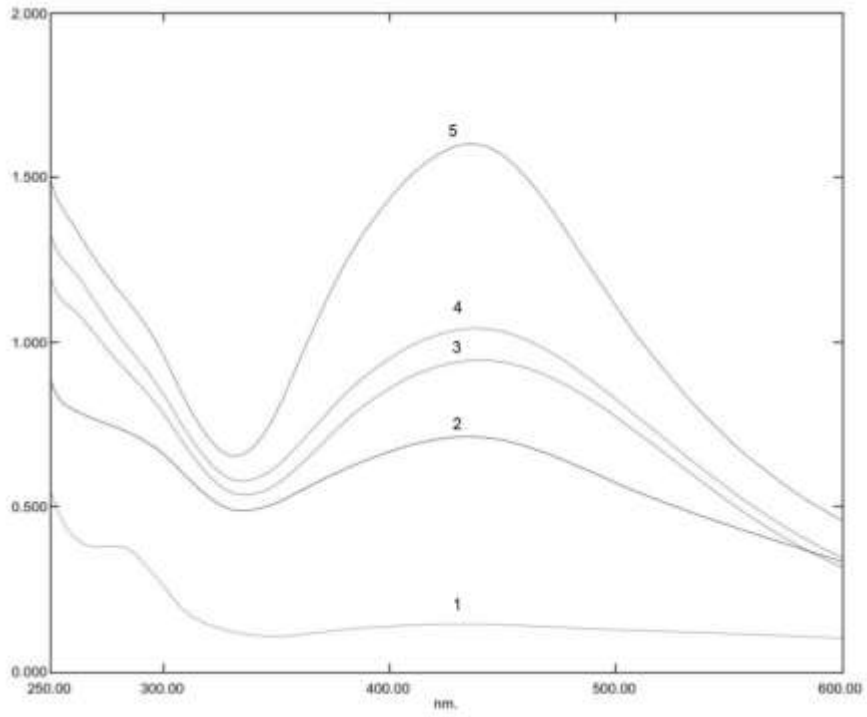

Figure 2. UV-visible spectra of silver nanoparticles synthesized using goroho banana peel extracts. Note: 1. Goroho banana peel extract (GBPE), silver nanoparticles (40 mg/mL), 2.silver nanoparticles (60 mg/mL), 3. silver nanoparticles (100 mg/mL), 4. silver nanoparticles (200 mg/mL).

Figure 2 shows the wavelength values resulting from the synthesis values resulting from the synthesis of silver nanoparticles undergo significant changes over time so that it can be concluded that the resulting silver nanoparticles are relatively stable. While for extract value of GBPE have time order that is the amount of silver nanoparticles that have been formed increase along with the increase of time. A large absorbance corresponds to number of nanoparticles formed. The process of silver nanoparticles is suspected because of the ability of phenolic compounds to reduce silver ions (Ag^+) into silver nanoparticles. When it is in its ion form, Ag will repel each other because of the charge of a similar charge, but after it is reduced to Ag^0, the aromatic charge of Ag became neutral, allowing the Ag intermediate Ag approach each other and interact with each other through the bond between the metals to form a cluster of nanosize.

Silver nanoparticles with infrared (IR)

The spectra of FTIR has used to determine the interaction of functional groups found in goroho banana peel extracts by silver nanoparticles before and after the reduction process of Ag^+ to Ag^0 ions. The result of FTIR spectra could used to identify the probably of functional groups which a part to reduce silver ions. Based on figure 3 shows that the banana peel extracts (BPE) seen a shift on wavelength from banana goroho extracts after and before reduced.The spectra of FTIR shows that the band has wided and strong on wave numbers 1620,21 cm^{-1} and 1404,14 cm^{-1} made by the presence of aromatic groups (C=C) in goroho banana peels extract. The band has wided and strong on wave numbers 3402,43 cm^{-1} shows that typical absorption of hydroxyl groups (-OH) from polyphenolic groups in goroho banana peels extract. The peek of wave numbers 1743,65 cm^{-1} shows that the absorb of carbonyl group (C=O). The spectrum on wave numbers 1080,14 and 1103,28 cm^{-1} be found the absorb of ether group. While on the spectrum of silver nanopartikel (SNP) the result of reduce using by banana peels extract shows a shift in numbers on -OH, C=O and C-O groups with wave numbers have continually 3448,72 cm^{-1}, 1604,77 cm^{-1}, 1072,42 cm^{-1}, and 1026,13 cm^{-1}.

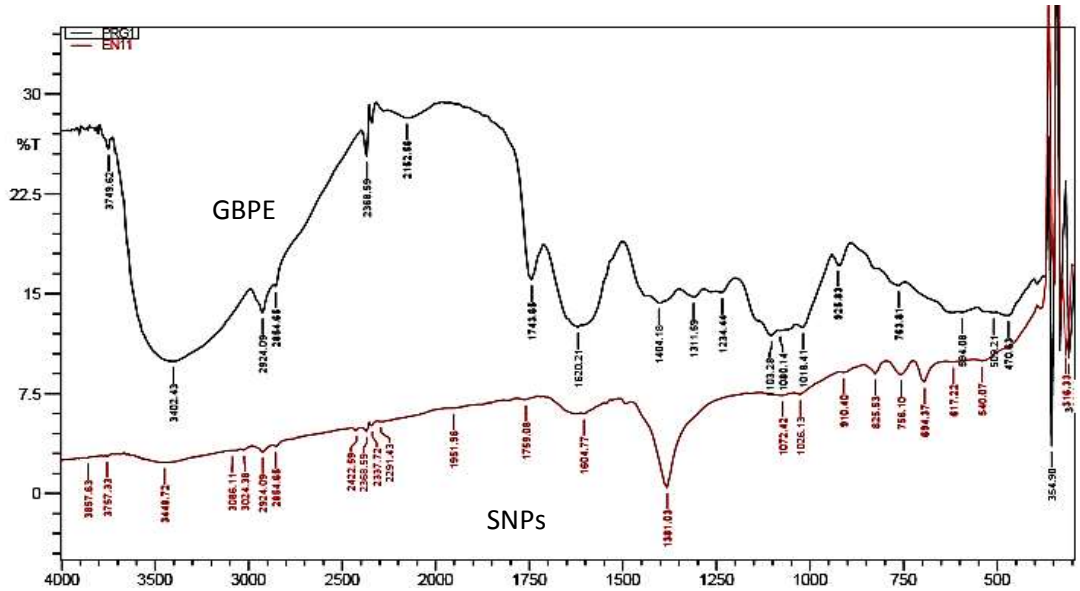

Figure 3. The spectra of FTIR by goroho banana peels extract (GBPE) and silver nanoparticles (SNPs)

The shift of wave numbers clearly visible between the spectra of BPE and SNPs, what vibrations OH groups of SNPs have missed the absorb compared at BPE. The shift of wave numbers shows that there is interaction between functional groups with silver nanoparticel cause oxidation due to the reduction process of silver nanoparticles. The absorb of number wave 1026,13 cm^{-1} shows there is absorb Ag^{+} ions that formed metalopolymer. Modrzejewska et al.[35]explaining there is change in the fingerprint area on lower wave numbers (650-400cm^{-1}) allows a reduction reaction on Ag^{+} to Ag^{0} ions. It's indicated by a weak absorption peak in wave numbers 540,07 cm^{-1}.Carillo-Lopez et al.[25](2004), explain that -OH groups of terpenoids and flavonoids in leaf extracts had a part in the reduction of silver ions and -COO group participates in stabilization of nanoparticles. The other research also explain that -OH groups participates in reduction-oxidation process, carbonyl and carboxylate groups involved stabilization particles[36,37] (Huang et al., 2007; Cruz et al., 2012). From the result of characterization FTIR could conclude that the interaction between silver nanoparticles of banana peels extract has occured by O atoms on OH groups of phenolic compounds.

Characterization of silver nanoparticles with SEM

Scanning electron microscope (SEM)analysis aims to show morphology particles and sample has analyzed by SEM could seen on Figure 4. The characterization by SEM shows silver nanoparticlesat different concentrationshave a size with the ranged 254-768 nm (A); 41-98 nm (B) and 53-90 nm (C).Based on the results obtained, it was found that the particle size distribution of silver nanoparticles of sample A was lower than sample B and sample C. As samples concentration is increased, the average of particle sizes which analyzed using scanning electron microscope will increased.

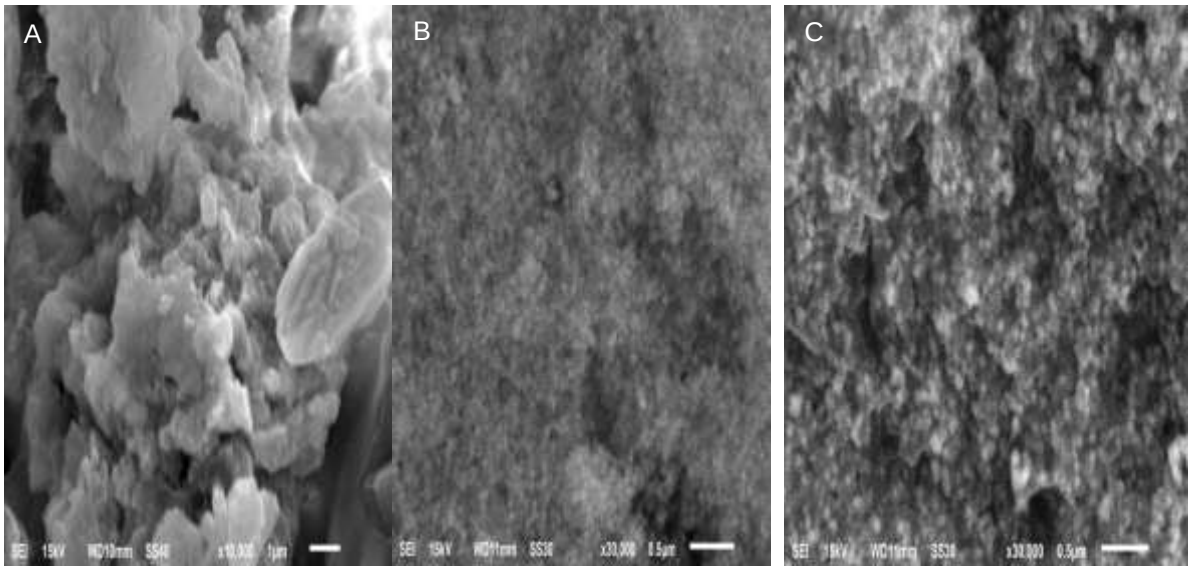

Figure 4. The morphology analysis of silver nanoparticles using by SEM with enlargement 10000 and 30000 times (scale of 0,5-1 µm).Note: 1 g (sample A), 3 g (sample B) and 5 g sample C

The morphology of silver nanoparticles have analyzed by SEM is white between fiber. The goroho banana peels extract containing phenolic content, flavonoid and steroid what have antioxidant activity could reduce Ag^{+} to Ag^{0}. Phenolic is the most dominant compound shown in the phytochemical test of goroho banana peels extract. Phenolic compound in banana peels extract is a group of hydrolyzed tannins with gallic acid as its basic structure.

The total phenolic contents of silver nanoparticles

The total phenolic contents of goroho banana peels extract (GBPE) and silver nanoparticles (SNPs) have evaluated with concentration 40; 60, 100 and 200mg/mL. If the addition of $AgNO_3$ solution in GBPE could decrease phenolic total content of SNP solution, indicating phenolic compounds were involved in the reducing agent. Figure 5 shows total phenolic contentof GBPE and SNPs. The results indicate that GBPE showed a higher phenolic total content than SNP, whereas at the same concentration levels, GBPE showed also higher phenolic total content than SNPs. The phenolic total content of GBPE and SNPs increased with increasing concentration ($p<0.05$). It means that there are involved phenolic compounds to reduce silver ions (Ag^{+}) into synthesis of silver nanoparticles. Besides, it is also possible of nonphenolic compounds are involved in syntheses of silver nanoparticles. Some study reported that plant extract is known to contain flavonoid, phenolic, tripenoid, phloroglucinol dialdehyde diterpene derivatives are responsible in the syntheses and stabilization of silver nanoparticles[17,38,39].

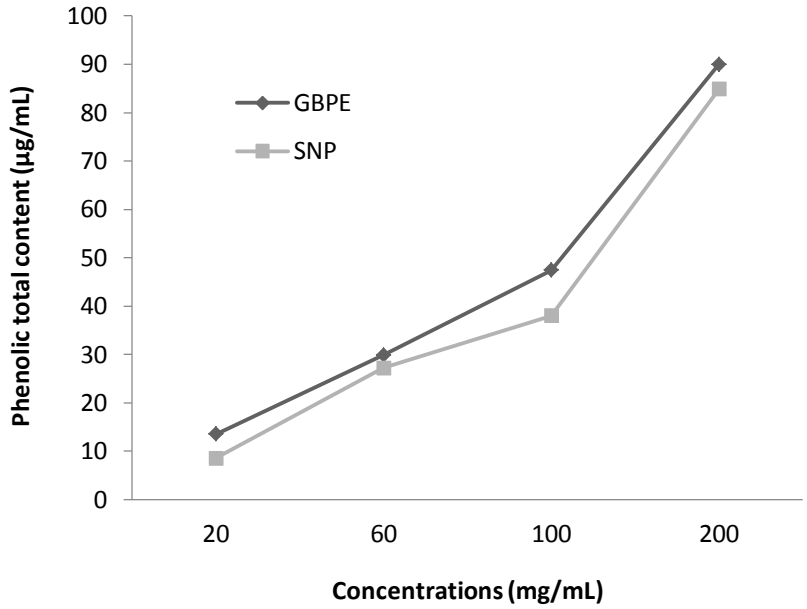

Figure 5. Total phenolic contents of silver nanoparticles (SNP) and banana peels extract (GBPE)

Alhabsyi et al.[32]reported that goroho banana peel was extracted with three solvents (methanol, ethanol and acetone) containing more phenolic components and act as antioxidant. Therefore, these components present in GBPE are good electron donors and could reduce with silver ions to form silver nanoparticles by converting Ag^{+} to Ag^{0}.The mechanism of the plant phenolic may be explained to syntheses of silver nanoparticles in the plant extract[39].

Singlet oxygen quenching activity of silver nanoparticles

The effects of 40, 60, 100, and 200 mg/ml silver nanoparticles and peel extract of goroho banana on peroxide value of linoleic acid emulsion which exposed with 4000lux light are presented in figure 6. Silver nanoparticles (SNPs) of 200 mg/mL showed the highest effect in singlet oxygen quenching followed by concentration of 140, 100, 60, and 20 mg/mL for 5hexposurefluorescent light ($p<0.05$). It means that SNPs act as singlet oxygen quenching in photooxidation linoleic acid with presence of sensitizer erythrosine during 5h light exposure. Min and Boff [40]stated that singlet oxygen can be produced by triplet oxygen with the presence of light and sensitizer. The presence of sensitizer can increase oxidation reaction, make it absorb energy from light to form hydroperoxide by photooxidation reaction. Photooxidation of singlet oxygen in linoleic acid generate hydroperoxide at conjugated double bond 9-OOH and 13-OOH and unconjugated 10-OOH and 12-OOH. Autooxidation of triplet oxygen on linoleic acid produce hydroperoxide at position conjugated 9-OOH and 13-OOH[41].The structure 1,4-pentadiena inside linoleat makes it very easy to oxidize. There is double bond fatty acids weaken C-H bond on carbon atoms close to double bond so that the release of H is easier.

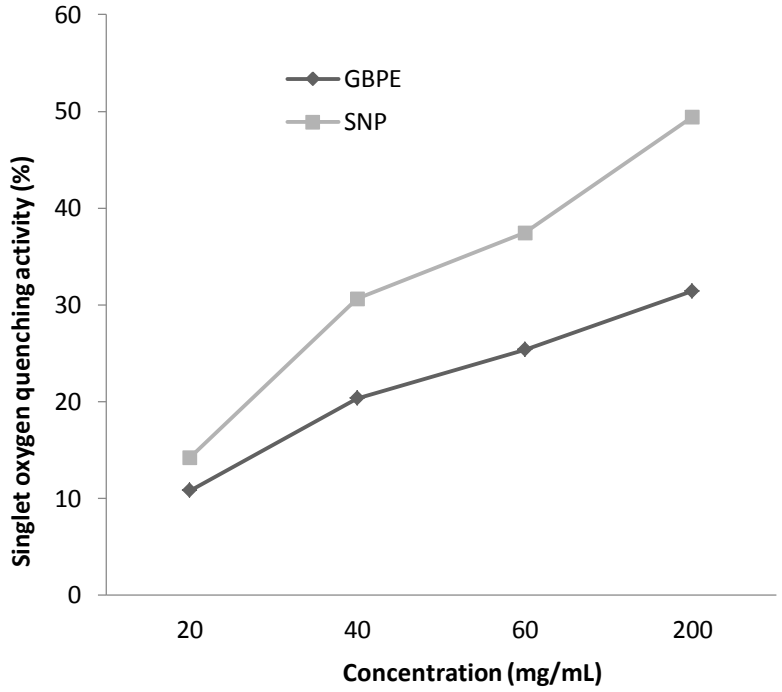

Figure 6. Singlet oxygen quenching activity of silver nanoparticles (SNPs) and banana peels extract (BPE)

The obtained peroxide values were used to calculate the percentage of singlet oxygen quenching (Figure 6). Silver nanoparticles showed the highest singlet oxygen quenching compared by banana peel extract at all level of concentration. Singlet oxygen quenching of SNPs 200, 100, 60, and 40 mg/mL were 49.44; 37.44; 30.63 and 14.24% respectively, while GBPE were 31.44; 25.39; 20.36 and 10.82%. SNPs of concentration at 200 mg/mL are effective nanoparticles to stabilize singlet oxygen generation from erythrosine sensitizer as showed by the increasing of singlet oxygen inhibition. It means that the concentrationaffects the silver nanoparticles to prevent photooxidation in linoleic acid emulsion. Therefore the silver nanoparticles play role in influnceby photooxidationwith better precentage than banana peel extract.

Conclusions

Based on this result, the antioxidant of goroho banana peel extract possess the ability to synthesize silver nanoparticles colloidal solution of 423-442nm at room temperature for 30 minute. The FTIR spectra revealed the presence of different functional groups like hydroxyl (O-H stretch), Alkanes (-C-H- stretch), Alkene (C=C- stretching), Aromatic (C=C stretching), Alkane (-C-H bending), Ether (C-O stretching), Alkene (=C-H bending).The characterization of silver nanoparticles by SEM studies revealed the range at 254-768 nm (A); 41-98 nm (B) and 53-90 nm (C). Silver nanoparticles possess the ability as singlet oxygen quencher. The enhancement of silver nanoparticles concentration showed stronger singlet oxygen quenching compared with peel extract of goroho banana.

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