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Anti-Photooxidant And Photoprotective Activities Of Ethanol Extract And Solvent Fractions From Corn Cob (*Zea mays*)

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Abstract : Corn cob is one of the food waste-material having the phytochemical component that has healthy benefit. The corn cob was extracted with reflux method using ethanol 80% for 2 hours. After that, the extracts were filtered and the filtrates were combined and concentrated in a rotary evaporator. This crude ethanolic extract was suspended in water and extracted with petroleum ether, ethyl acetate, n-butanol, and water, respectively. The anti-photooxidation effects were evaluated in linoleic acid that containing erythrosine as a sensitizer and exposed under 4000 lux fluorescent light for up to 5 hours. The photoprotective activity was evaluated by sun protection factor (SPF) using spectrophotometry UV-Vis. Ethyl acetate fraction shows the highest total phenolic content followed with butanol fraction, ethanol extract, petroleum ether fraction and water fraction. Ethyl acetate fraction also exhibited the highest anti-photooxidation activity followed by butanol fraction, extract ethanol, petroleum ether, and water fraction. The photoprotective activity of ethyl acetate fraction was the highest, as indicated by higher SPF value as compared with ethanol extract, butanol, water and petroleum ether fractions. There were strong correlations between the total phenolics content and antiradical activity and photoprotective activity with R^2 values are 0.9115, 0.9326 and 0.9975, respectively. These results show that the ethyl acetate fraction of corn cob contains compounds having anti-photooxidation properties and potential as a sunscreen active ingredients.
Keywords : corn cob, solvent fractions, anti-photooxidation, photoprotective, SPF.

Introduction

Sunlight exposure on the skin can lead to photochemical reaction such as photooxidation which produces reactive oxygen species (ROS) such as superoxide anion, singlet oxygen molecule, and hydroxyl radicals. 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, skin aging and cancer[1,2]. Whereas, the oxidation reaction of food components can lead to nutritional losses,

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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, myoglobin and phorphorine present in the systems[4,5,6].

Photooxidation can produce singlet oxygen from oxygen triplet with the presence of components that act as a sensitizer. The sensitizer can transfer its light energy to chemical energy and begin oxidation reaction. The food contains some components that act as a sensitizer, such as colorants (erythrosine). Erythrosine (FD&C Red No. 3) is widely used as a coloring agent for foods, beverages, pharmaceutical preparations, and cosmetics. Synthetic food colorants, which have been used to improve the appearance of food, may act as photosensitizers due to the highly conjugated double bonds. Erythrosine under light can generate reactive oxygen species such as superoxide anion and singlet oxygen[3,7,8]. Skin can also produce singlet oxygen generation when UV light is absorbed by trans-urocanic acid at 345 nm. Exogenous agents and endogenously occurring compounds including porphyrins, flavins, DNA bases, or amino acids and their derivatives like urocanic acid are considered to act as photosensitizing molecules[9,10]. This highly reactive molecule can react with protein or lipid and the reaction products, such as lipid peroxides has lost cellular functionality[11].

This became clear and interesting that the predominant exposure to UV light occurs under everyday circumstances. It is impossible to avoid sun exposure, therefore, everyone needs sun protection. This argues for the application of a daily UV-A protection ingredient. Sunscreens are chemicals that provide protection against the adverse effect of solar and particular UV radiation. Natural substances extracted from plants have been recently considered as potential sunscreens resources because of their ultraviolet radiation absorption in the UV region and their antioxidant activity. Therefore, natural UV-absorbing substances are a suitable alternative for daily skin care. The UV-screening substances of plants, phenolic acids and polyphenols are already in use as the active molecules in a series of cosmetic products against photo-aging.

Corn (*Zea mays* L.) is one of the most widely cultivated cereals in the world and most popular due to their nutritional value worldwide and rich sources of beneficial anti-oxidants, minerals, vitamins and fiber[12,13]. Corn is one well known of crops and cultivated in developing countries. The utilization of corn seeds as food material resulting corn cob as waste. However, the by-products can be used as functional food ingredients such as phytochemicals, pharmaceuticals, food products, essential oils, seed oil, pectin and dietary fibers[14]. Therefore, corn seed by-products are not only become a good source of bioactive compounds but also usable as several value-added products[15]. Corn cob is a phenolic phytochemical containing biomass which recommended to be used as active antioxidant compound[16,17]. Hossain et al.[18] identified flavonoids from flavonol groups such as quercetine and its glycoside from corn. Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-γ-pyrone structure, that has been reported to act as antioxidants in various biological systems[19,20,21]. Many phenolic compounds have antioxidant properties. These compounds can be used as ingredients in cosmetics, pharmaceuticals, nutraceuticals and food[14]. The objective of this research was to determine the anti-photooxidation activity of ethanol extract and a solvent fraction on erythrosine sensitized photooxidation of linoleic acid and photoprotective activities by determination of sun protection factor of ethanol extract and solvent fractions.

Experimental

Standards and Chemicals

Corn cob was collected from a local market and dried at room temperature until used. The dried corn cob was ground using a blender to 40 mesh. The Folin-Ciocalteu reagent, ethanol, petroleum ether, ethyl acetate, butanol, sodium carbonate and erythrosine used in this experiment were purchased from Merck (Darmstadt, Germany). The Linoleic acid and 1.1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Lois, MO).

Sample Preparation

The corn cob powder (300 g) was extracted with reflux method using 1.5 L of 80% ethanol (3 times) for 2 hours. The extracts were filtered and the filtrates were combined and concentrated using rotary evaporator. The concentrated filtrate was then oven-dried to yield a solid ethanol extract (EE). The ethanol extract was stored at 5°C until used for anti-photooxidant and photoprotective assays.

Solvent Partitioning of Ethanol Extract

Solid EE extract of corn cob (5 g) was suspended in 100 mL of deionized water and then partitioned sequentially with an equal volume of petroleum ether fraction (PEF), ethyl acetate fraction (EAF), butanol fraction (BF) and water fraction (WF) using a separating funnel. Each fraction was concentrated using rotary evaporation and then oven-dried at 37°C to constant weight. To prepare for SOQ and phytochemical assays, the solid residues of the fractions were dissolved in 80% ethanol.

UV Spectra

Extraction of phenolic compounds by different solvent system was monitored by means of UV absorption at 200-400 nm using spectrophotometer (Shimadzu 1800). UV spectra of the crude extract and solvent fractions in ethanol were also measured.

Determination of Total Phenolic Content

The total phenolic content of the ethanol extract and solvent fractions were determined using modified Folin-Ciocalteu colorimetric method from Li et al.[22]. Each sample solution (0.1 mL, 1 mg/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 30 min, 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 Free Radical Scavenger

Determination of free radical activity (scavenger) from ethanol extract and solvent fraction measured by the method from Li et al.[22] with slight modification. A total of 2 mL solution of 92 μM 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol was added 0.5 mL ethanol extract and a solvent fraction. The level of color reduction of the solution shows the efficiency of radical scavenger. The last five minutes of the 30 minutes, the absorbance was measured using a spectrophotometer at 517 nm. Free radical scavenger activity is calculated as a percentage reduction of DPPH color using the equation:

$$\%\,\text{activity} = 1 - \left(\frac{\text{sampel absorbance at 517 nm}}{\text{control absorbance at 517 nm}}\right) x100$$

The IC_{50} value obtained from sample concentration at 50and activity percentage and calculated from a calibrations curve of inhibition percentage against extract or solvent fraction concentration. Test of the samples was carried out in duplicate

Determination of Anti-photooxidation Activity

The procedure of anti-photooxidation activity determination was based on Huang et al.[5], with minor modification. This procedure was to study the effects of ethanol extract and solvent fractions 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. Two mg of ethanol extract, solvent fractions and α-tocopherol (as a positive control) 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 air-tight 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 x 60 cm) for light sources to the glass chamber was 12 cm. Samples were placed on the wire netting which was 10 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 stripped corn oil 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 hydroperoxides[23]. 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.

Sun Protection Factor (SPF) Measurements

Determination of photoprotective activity was conducted by examining SPF *in vitro* value using spectrophotometer (Spectrophotometer UV-Vis Shimadzu 1800)[24,25]. Ethanol extract and solvent fractions were made 10-50 µg/mL in ethanol mixture. Absorbance curve of extract and solvent fraction solution was made in a 1cm cuvette, wavelength of 290 to 320 nm with 5 nm interval. The absorbance of the solution shows the effect of substance which absorbs or reflect UV light in solution. Mansur *et al.*[24] develop a simple mathematical equation to calculate SPF value. SPF = CF x I (λ) x absorbance Note: CF: Correction factor (10), EE: erythemal efficiency, λ: wave length, I: sun light spectrum simulation and Abs: sunscreen product absorbance.

Statistical Analysis

All experiments were performed in duplicate. Experimental data presented as mean values ± standard deviations and analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range test using SPSS version 18.

Results and Discussion

Extraction and Total Phenolic Content

The results from extraction with ethanol of corn cob (300 g) showed that yield was 4.80% (ethanol extract), whereas the yields of solvent fractions of petroleum ether, ethyl acetate, butanol, and water were 15.49, 20.54, 14.16 and 49.01%, respectively (Table 1). The yield of water fraction (WF) gave higher yields from solvent fractions than ethyl acetate fraction (EAF), petroleum ether fraction (PEF) and butanol fraction (BF). Higher yields for WF could be explained by the presence of tannin compound and a possible extracted protein, carbohydrate group, and components was that soluble in the water. The EAF and BF were all semi-solid with a brownish yellow color. Whereas, for the PEF were all oily with a yellow color. Presence of yellow color in PEF could be explained that they were responsible for the oil. In addition, it must be noted that in PEF, larger amounts of the fraction were obtained as a possible presence of fatty acids.

Tab. 1: Normalization product function which used to calculate SPF (Sayre et al., 1979)

Wavelength (λ, nm)	**EE x I (ternormalization)**
290	0,015
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0837
320	0,018
Total	1

Tab. 2: Yield and phenolic total content of ethanol extract and solvent fractions

Samples	**Yield (%)**	**Phenolic total content (µg/mL)**
Ethanol Extract (EE)	4.80±0.64[a]	50.81± 2.17[a]
Petroleum Ether Fraction (PEF)	16.71±1.40[b]	24.59±0.58[b]
Ethyl Acetate Fraction (EAF)	19.53±0.04c	162.14±3,46[c]
Butanol Fraction (BF)	15.06±0.74[d]	82.65±5.63[d]
Water Fraction (WF)	47.69±2.70[e]	22.35±3.75[e]

Values are means ± SD. Values with the same superscript letter are not statistically significant at the 5% level

Table 2 showed total phenolic content in the extract ethanol and solvent fractions at the sample concentration of 1 mg/mL. The total phenolic of ethyl acetate fraction (EAF) extract (EA) gave higher content (162.14 µg/mL) than butanol fraction (82.65 µg/mL), ethanol extract (81.53 µg/mL), water fraction (22.35µg/mL) and petroleum ether fraction (24.59 µg/mL).

The phenolic compound was extracted with some solvent partitioning that possessed different polarity to separation phenolic compound in corn cob ethanol. Extraction using petroleum ether could dissolve less polar phenolic compounds, whereas ethyl acetate and butanol could dissolve semi polar compounds and the use of water would recover the more polar compounds. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility[26]. Therefore, the semi polar solvents (ethyl acetate, butanol, and chloroform) are considered to be suitable for the solvent partitioning of all plant phenolic and it is a standard extraction procedure for solvent fractions is usually used.

The higher phenolic compound content is seen in semi-polar fraction and could be caused the present kind of phenolic compounds such as phenolic acid groups (ferulic acid, syringic acid, coumaric acid) and flavonoid groups (kaempferol and quercetine) which have been reported from a type of corn[27]. According to Shahidi[26], the total phenol content can be resulted from the sum of phenolic compounds such as simple phenolics (derivatives of hydroxybenzoic and hydroxycinnamic acid), non tannin flavans (anthocyanins, catechins, and leucoanthocyanins), hydrolysable tannins gallic and ellagic acid) and condensed tannins (polymers and copolymers of catechins and leucoanthocyanins).

UV Spectra

Figure 1 showed the absorption spectra of ethanolic extract and solvent fractions measured in ethanol with the wavelength regions of 200-400 nm for the phenolic component. Characteristic phenolic component in ethanol extracts of corn cob was identified by UV spectrometry. Therefore, the variation of components of ethanol extracts of corn cob was partitioned in four different solvents such as n-hexane, ethyl acetate, n-butanol, and water, to fractionate the polar, semi polar and non-polar in the ethanol extract. As shown in Figure 1, the UV spectra of ethanol extract was greatly dependent on the polarity of the extracting solvent. The ethyl acetate, ethanol and butanol fractions exhibited maximum absorbance were at 315, 313 and 281 nm respectively, whereas hexane and water fractions showed no maximum absorption.

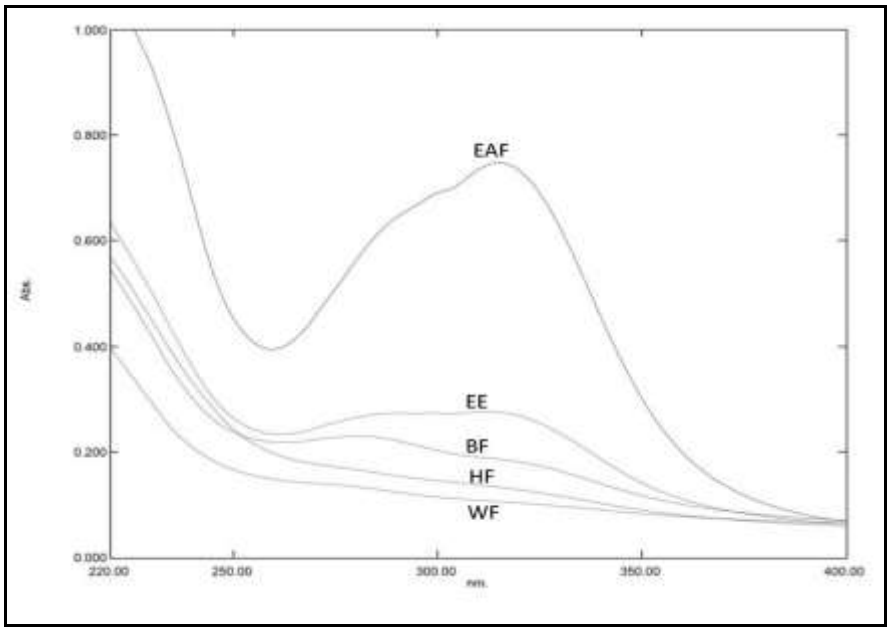


Fig. 1: Absorbance spectral of 20 µg/mL hexane fraction (HF), ethyl acetate fraction (EAF), buthanol fraction (BF), ethanol axtract (EE) and water fraction.

These result proved that UV spectra of the ethanol extract, butanol, and ethyl acetate fractions showed ability for extracting phenolics components. In addition to the UV spectra of ethyl acetate fractions was a more effective solvent for obtaining of phenolic component of corn cob sample. This could be due to the presence of large amounts phenolic compound, especially flavonoid group that is more soluble in ethyl acetate than ethanol and butanol. As shown in Figure 1, it can be seen that ethyl acetate fraction possessed two major absorption peaks in the region 315 dan 285 nm, and gave an absorbance value reading in the region 0.41 to 0.75. These two peaks are commonly referred to as band I (315 nm) and band II (285 nm). According to Markham[29], flavonoid compound having the wavelength regions at 300-330 nm and 275-295 nm are flavanone and dihydroflavonol. The flavanon group having two peaks are commonly referred to as band I (300-350 nm) and band II (270-295 nm). Dihydroflavonol group that have two peaks are commonly referred to as band I (300-320 nm) and band II (270-295 nm)[30].

From Figure 1 also found that UV spectra of EAF having characteristic absorption bands in regions UVA and UVB and capable play role as the photo-protective effect. Sayre et al.[31] stated that the radiation ability of active component in extract to absorb UV associates with sunscreen activity. These results indicate that ethyl acetate fraction which to contain flavonoid compounds and potential as sunscreen active component.

DPPH Radical Scavenging Activity of The Ethanol Extract and Solvent Fractions

The result tested radical scavenging activity (RSA) in DPPH from the ethanol extract and solvent fraction showed ethyl acetate fraction (EAF), butanol fraction (BF) and ethanol extract (EE) having the ability as scavenger free more than 50% at concentration of 1000 µg/mL (Figure 2). In other hand, solvent fractions prepared from ethanol extract of corn cob have radical scavenging activity. The result showed that there was a positive relationship between the amounts of total phenolic compounds in corn cob with radical scavenging activity. For example, ethyl acetate fraction (EAF) showed most phenolic compounds and possessed higher scavenger activity followed a butanol fraction (BF), ethanol extract (EE), petroleum ether fraction (PEF) and water fraction (WF). Many researches showed that extract of a plant as fruits, leaves, and vegetables have a positive correlation among total phenol content and antioxidant activity[32,33].

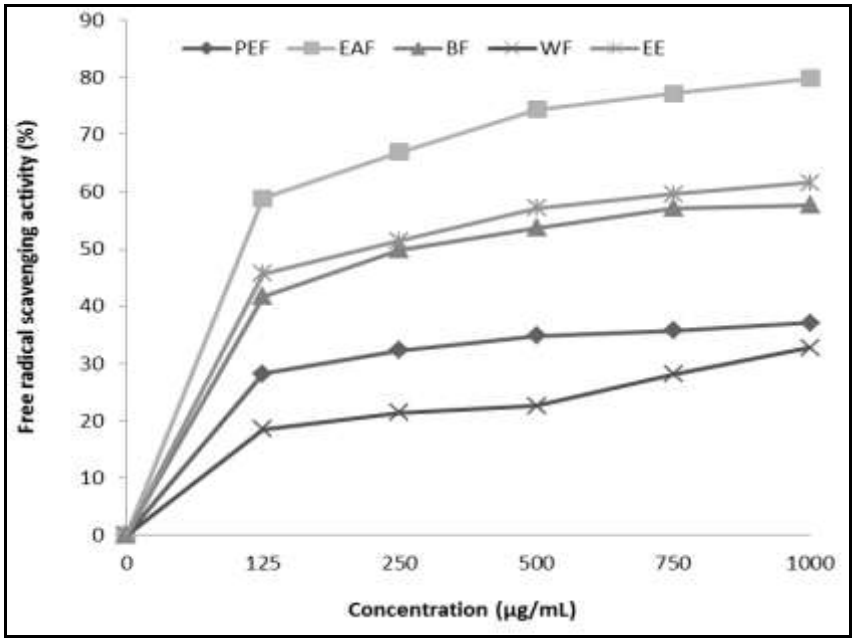


Fig. 2: Effects of ethanol extract and solvent fractions at different concentrations on scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. (PEF: petroleum ether fraction, EAF: ethyl acetate fraction, BF: butanol fraction, WF: water fraction and EE: ethanol extract).

Figure 2 showed that all fractions and ethanol extract examined were found to possess DPPH-scavenging activity, indicating that these two fractions (EAF and BF) of phenolic compounds were used the most potent DPPH scavengers. The effects of ethanol extract and solvent fractions on scavenging DPPH free radical at the concentration ranging from 250 to 1000 µg/mL. For solvent fractions and ethanol extract, as the concentrations increased from 250 to 1000 µg/mL, scavenging activity of both fractions and extracts were

increased significantly ($p<0.05$). However, radical scavenging activity between EAF or BF and EE obtained from the same material were statistically significant. Ethyl acetate fraction (EAF) was assumed that most of the phenolic compounds consisting mainly of flavonoid components were present in the fraction. Ethyl acetate fraction at concentration 250 to 1000 µg/mL exhibited excellent antiradical activities from 75.25 to 87.19and. Effect of EAF at 1000 µg/mL on radical scavenging activity was compared to that α-tocopherol as a positive control. EAF exhibited the lower antiradical activity than α-tocopherol at 1000 µg/mL (95.16%).

Tab. 3: The free radical scavenging activity of extract ethanol and solvent fractions as expressed by IC_{50}

Sample	**IC_{50} (mg/mL)**
Petroleum ether fraction (PEF)	22.52 ± 0.07^{a}
Ethyl acetate fraction (EAF)	0.05 ± 0.01^{b}
Butanol fraction (BF)	0.31 ± 0.01^{c}
Water fraction (WF)	24.29 ± 0.06^{a}
Ethanol extract (EE)	0.21 ± 0.02^{d}
α-Tocopherol	0.02 ± 0.01^{e}

Values are means ± SD. Values with the same superscript letter are not statistically significant at the 5% level

Table 3 showed IC_{50} values of ethanol extract and solvent fractions to scavenge 50 and of the DPPH free radical. The IC_{50} values are ranged from 0.05 to 24.29 mg/mL. Ethyl acetate fraction showed low IC_{50} (0.05 mg/mL) compared to that EE (0.21 mg/mL), BF (0.31 mg/mL), PEF (22.52 mg/mL) and WF (24.29 mg/mL). Based on the IC_{50} value, it can be interpreted that EAF having free radical scavenger activity higher than EE, BF, PEF, and WF. When compared to α-tocopherol as a positive control with ethyl acetate fraction. It was found that ethyl acetate fraction showed lower scavenging activity than α-tocopherol.

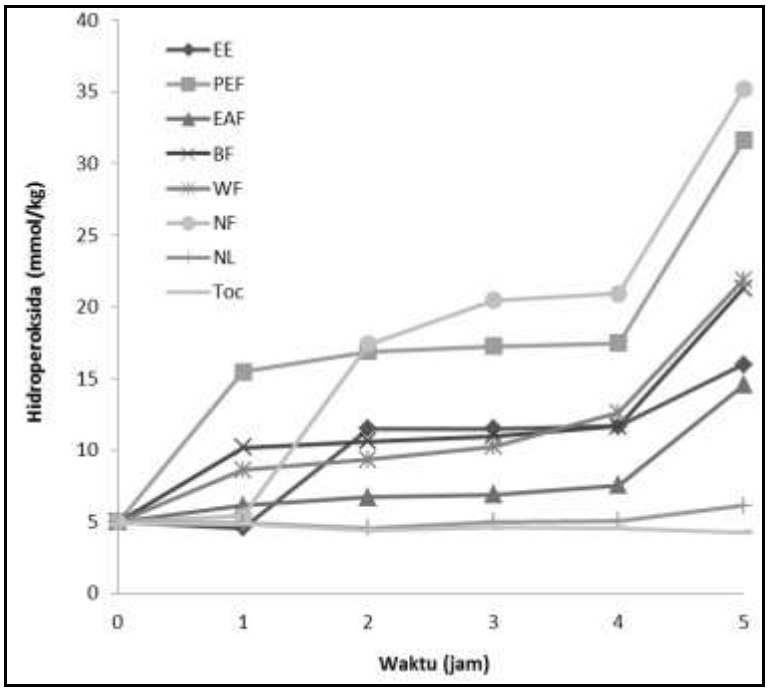


Fig. 3: Effect of 200 µg/mL ethanol extract and solvent fraction on oxidation of singlet oxygen in linoleic acid for 5 hours (NF: no fraction, NL: no light).

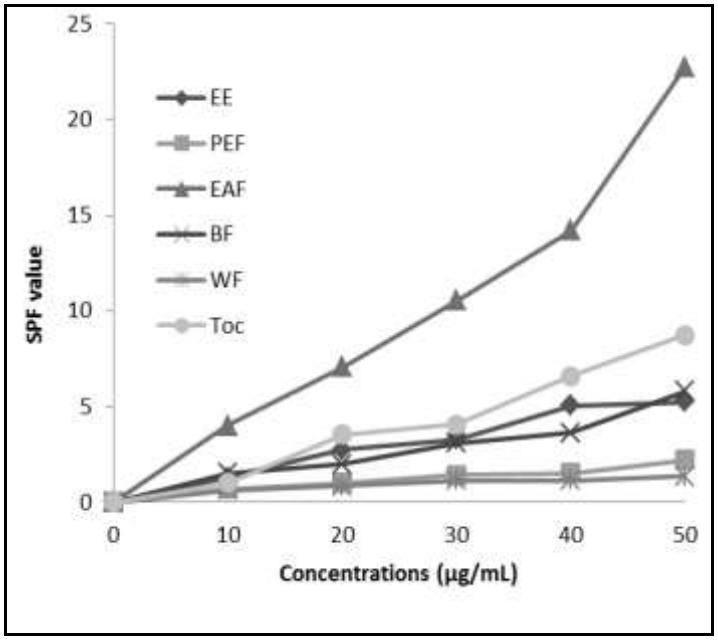
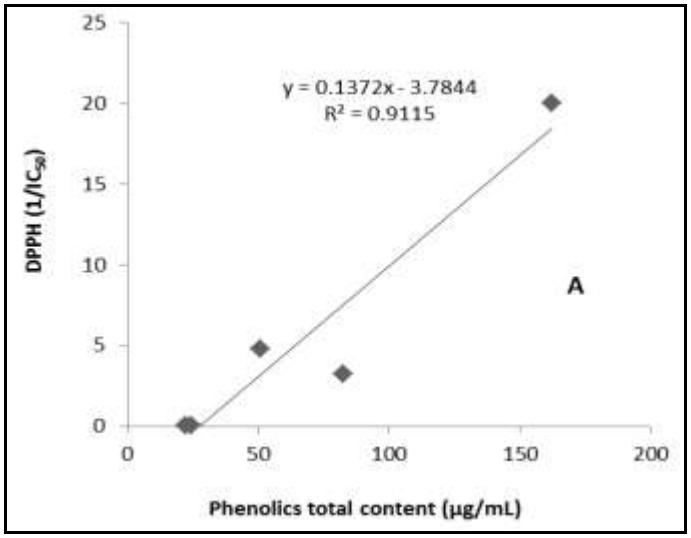
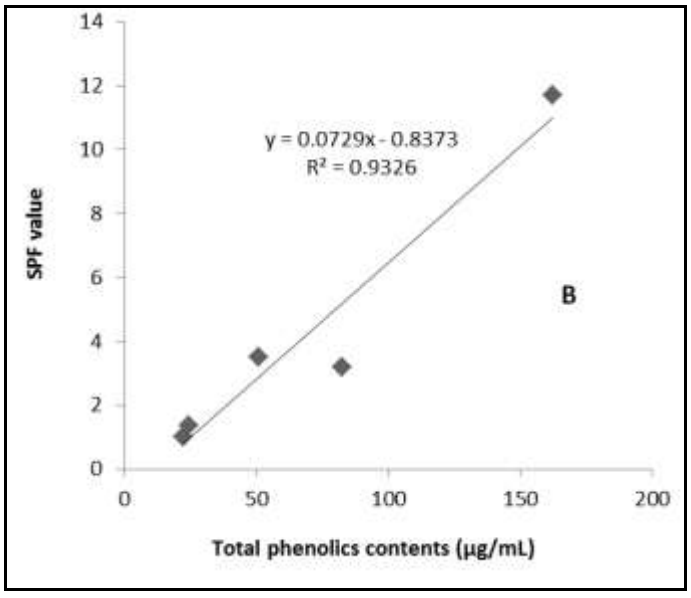


Fig. 4: SPF values of ethanol extract and solvent fractions of corn cob.





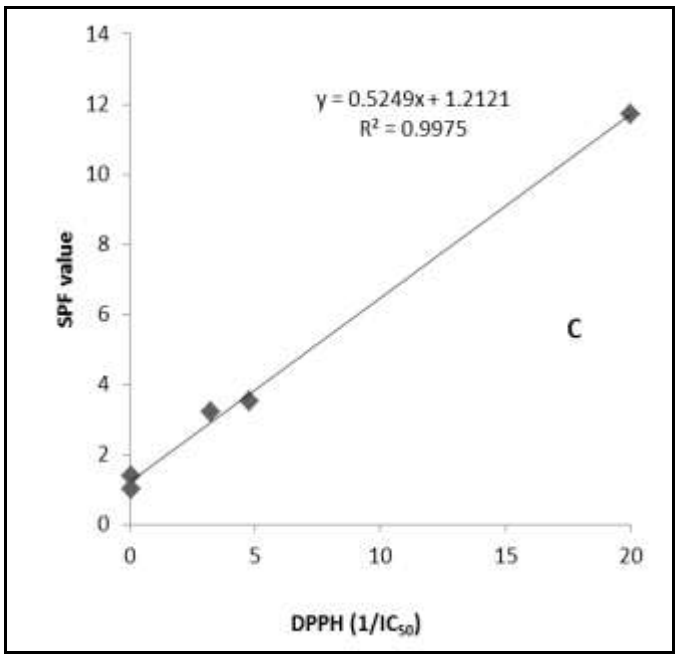


Fig. 5: Correlations between total phenolics and antiradical activity by DPPH assay and photoprotective activity of crude extracts and solvent fractions, A. DPPH radical scavenging activity, B. Photoprotective activity (SPF values), and C. DPPH radical scavenging activity and Photoprotective activity.

Effect of Extract and Solvent Fraction on Photooxidation of Linoleic aAcid Emulsion

Effect of concentration at 200 µg/mL of ethanol extract and solvent fractions such as hexane fraction (PEF), ethyl acetate fraction (AEF), butanol fraction (BF) and water fraction (WF) on peroxide value for 0.03 M of linoleic acid in ethanol for expose to light at room temperature (Figure 5). The hydroperoxide values were increased in linoleic acid that addition erythrosine as a photosensitizer. A probable explanation was that erythrosine may produce singlet oxygen from triplet oxygen when it was exposed to light. The formation of singlet oxygen by photosensitizer accelerated lipid peroxidation. Therefore, erythrosine effectively acts as a photosensitizer for accelerate of linoleic acid oxidation in a model system to expose to light[8]. This result agrees with the previous report on the photosensitizing effect of erythrosine to accelerate the oxidation of soybean oil in acetone model system under the light storage[8]. The other research showed that erythrosine photosensitized oxidation of ascorbic acid in an aqueous system at the pH range from 4 to 7[3]. Data of hydroperoxide value in linoleic acid were relatively similar for 5 hours under light without sensitizer (WS) and dark (NL). However, without the presence of sensitizer in linoleic acid oil or in dark system, triplet oxygen could not be converted to singlet oxygen.

The ethyl acetate fraction showed very strong anti-photooxidative (singlet oxygen quenching) activity on erythrosine-sensitized photooxidation of linoleic acid for 5 hours exposure to fluorescent light ($p<0.05$). On the contrary, petroleum ether fraction indicated decreasing antiphooxidative effect. This is probably due to the effect of nature sensitizer can act synergistically with erythrosine to generate singlet oxygen so that oxidation rate of hydroperoxide formation in increase. Endo et al.[34] had reported that nature sensitizer as chlorophyll and its derivate can promote lipid oxidation for storage. In addition, the phenolic compound content in petroleum ether fraction was lower. This might be caused by the effect of singlet oxygen quenching from petroleum ether fraction.

Phenolic compounds are the most prevalent antioxidant phytochemicals in the plant kingdom and reportedly possess both anti-photooxidative activity and radical scavenging activity[35]. Ethyl acetate fraction contained the highest total phenolic (163.57 µg/mL). The petroleum ether and water fraction contained the least amount of total phenolics, showing 23.71 and 23.57 µg/mL. The ethyl acetate fraction showed the highest anti-photooxidative activity contain, hence large quantity of total phenolic. This result suggested that phenolic compound in ethyl acetate fraction is the major components for singlet oxygen quenching activity. However, petroleum ether and water fractions showed antiphotooxidative activity, had the least quantity of total phenolic. It has been reported that phenolic phytochemicals had strong antiphotooxidative activity[36]. This result clearly

indicated that phenolic phytochemicals in ethyl acetate fraction are the major components of supporting the all antiphotooxidative. The antioxidant phytochemicals in food plant contain various antioxidant components such as flavonoids, anthocyanins, carotenoids, amines, and vitamins. These components have also been known to possess anti-photooxidative activity. Thus, extracting and fractionating procedure used in the experiment were favorable conditions for the obtaining phenolic compounds in the ethyl acetate fraction, active components in ethyl acetate fraction was greatly dependent on the polarity of the extracting solvents. As the polarity of the extracting solvent increased, the anti-photooxidative activity of the extract also increased. This result indicated that the anti-photooxidative component in ethyl acetate has strong semi polar properties and are easily extracted with semi polar solvent as ethyl acetate. Effect of ethyl acetate fraction in model system showed significant that different with 200 µg/mL α-tocopherol ($p<0.05$). Alpha tocopherol (Toc) is nature compound that used to inhibit in lipid oxidation for food and reported as a quencher of singlet oxygen in soybean oil[37].

Jung et al.[4] reported that methanol extract in edible and nonedible plant from huanglia (*Coptis japonica* Makino) and clove (*Eugenia caryopylla* Thunb.) showed stronger singlet oxygen activities (anti-photoxidative) in both chlorophyll and methylene blue sensitized photooxidation of linoleic acid. In addition to the methanol extract of *Coptis japonica* Makino was fractionated into three fractions (ethyl ether, ethyl acetate, and butanol fraction) by liquid-liquid partitioning fractionation, butanol fraction showed strongest antiphotooxidative activity in both chlorophyll and methylene blue sensitized photooxidation of linoleic acid.

Determination of SPF *in vitro*

Figure 5 shows correlation analyses between the total phenolics content, antiradical (DPPH) and photoprotective activity (SPF value). In this study, IC_{50} values were calculated by linear regression analysis will be the $1/IC_{50}$ to depicted correlation graphs between antiradical activity and total phenolic content. The results showed that there was a positive relationship between the amounts of total phenolics content in phytochemical crude extract and solvent fractions and antiradical activity (DPPH assay), indicating that phenolic compounds in crude extracts and solvent fractions were possible to contribute to the anti-radical activity in the corncob. The results suggested that total phenolic content of crude extract and solvent fractions in line with the development of antiradical characteristic which evaluated with DPPH assay. There was significant correlation coefficient (R^2) between total phenolic content with antiradical activity in crude extract and fractions of corncob is 0.9115 (Figure 5A). It meant a high correlation coefficient indicated there was a strong linear correlation between phenolic composition and antiradical activity. Many research showed that extract of edible as fruits, leaves, spice, and vegetables or medicinal plant material have positive correlation among total phenol content and antioxidant activity[27,45,46,47,48,49,50,51].

A good correlation was observed also between total phenolics content and photoprotective activities (determined by SPF value) are as shown Figure 5B. The results may be possible there was the presence of phenolic compounds in corncob crude extract and solvent fractions. There is a trend of corncob extracts to exhibit phenolic composition in line with the photoprotective activity which measured by SPF values method. Correlation between total phenolic content and photoprotective activity show a significant correlation using five points with R^2 value is 0.9326 and even better than R^2 value of correlation analyses between total phenolics content and antiradical activity. Many food and medicinal plants containing phenolic compounds such as hydroxycinnamic acid derivatives and flavonoid have been reported to possess strong antioxidant and sunscreen activity[43,44,52,53,54]. The content of phenolic and flavonoid produced by a plant is considered an important factor for protecting plants against ultraviolet radiation (Silva et al., 2014; Souza et al., 2015). Therefore, there is positive correlation between photoprotective activities in phytochemicals extract with phenolic content of secondary metabolites in plants. Figure 5C shows the relationship between the antiradical and photoprotective activity in the corncob extract crude and its solvent fractions. The results of correlation analyses between antiradical and photoprotective activity showed strong relationship with R^2 was 0.9975. This indicated that there was positive relationship between the amounts of antiradical activity and photoprotective activity in crude extract and solvent fractions. Several studies have been reported that highest SPF value (photoprotective effect) analogously free radical scavenging activity determine by DPPH assay[40,41,55]. However, In general, antioxidant effect from crude extracts and solvent fraction exhibit the ability as electron donor and can react with free radical to form a stable product, so it ends the chain of radical reaction[56].

Conclusions

The ethyl acetate fraction seems to contain highest phenolic compound and showed good free radical scavenging in free DPPH system. This study showed also that ethyl acetate fraction could act as anti-photooxidation (singlet oxygen quenching) on linoleic acid photooxidation with present erythrosine as a sensitizer. The ethyl acetate fraction possesses an active component of sunscreen activity to prevention UVB radiation. Total phenolic content and radical scavenging and photoprotective activity showed a positive correlation in crude extract and solvent fraction of corn cob.

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