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Antioxidant, Photoprotectitive and Antiinflammatory activity of Okra (*Abelmoschus esculentus* L.) Fruit Extract

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ABSTRACT: Abelmoschus esculentus L. is a vegetable that contains secondary metabolites. This study tested antioxidant activity with IC50 parameters, the ability of this extract as a sunscreen with SPF, erythema and pigmentation as parameters, and an anti-inflammatory test. Abelmoschus esculentus fruit was extracted with 80% ethanol and obtained a yield of 30.68%. Screening phytochemicals to prove that Abelmoschus esculentus fruit extract contains saponins, flavonoids, steroids, tannins, and alkaloids. Compounds that have the potential to be antioxidants, sunscreens and anti-inflammation agents are phenols, especially flavonoids, so the quantitative amount is analyzed. The total amount of phenolic compounds in Abelmoschus esculentus fruit extract was 113.4751 mgQA/g, and the total amount of flavonoids was 61.1284 mgQE/g. For testing antioxidant activity with the DPPH method, the IC50 was obtained at 53.5303 mcg/mL, which is a strong category. As for testing sunscreen, the SPF value of ethanol extract in vitro using a UV-Vis spectrophotometer at levels of 100 mcg/mL of 1.1295 and 200 mcg/mL of 1.5748 means that concentration has not been able to become a sunscreen because the SPF value is still below the minimum protection category, but 400 mcg/mL levels of 2.2610 can provide minimal protection. The results of the anti-inflammatory test gave results of IC50 is 153.837 mcg/L.

Keywords: *Abelmoschus esculentus;* antioxidant; anti-inflammatory

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INTRODUCTION

Due to the depletion of the ozone layer, sunlight tends to become very intense. If the skin is exposed to sunlight for 6–20 hours, it can develop erythema sooner or later, which causes tanning, also known as tanning (Lucas et al., 2019). Tanning is quickly visible one hour after the skin is exposed to sunlight and then disappears again within 4 hours (Asai et al., 2021). This may happen because unstable semiquinone free radicals in melanin undergo oxidation reactions. Parts of the skin that are exposed to sunlight for a long time can experience an increase in free radicals, which can cause skin changes (Liu et al., 2023).

Exposure to sunlight can also trigger the emergence of reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, hydroxyl radicals, and others. These free radicals are reactive due to the presence of unpaired electrons (Amar & Kumar, 2019). According to Salminen (2022), it is these ROS that are responsible for damaging epidermal structures, including DNA, RNA, lipids, and proteins. Furthermore, UVB exposure can lead to local inflammation in the skin, which can either promote or inhibit immunosuppression. Prolonged exposure to an inflammatory environment significantly increases the risk of skin cancer and metastases, as indicated (Nica-Badea & Udristioiu, 2014). The interactions between ROS and mast cells in the dermis lead to the release of inflammatory mediators (Dudeck et al., 2019).

The effects of excessive UV radiation, namely sunburn or erythema, phototoxicity, photoallergies, photosensitivity and even skin cancer, make protection from UV radiation necessary (Jones et al., 2021). The body neutralizes solar radiation by synthesizing melanin. But with the current condition of high sunlight intensity, of course, protection is still needed for the skin against UV radiation, one of which is sunscreen (Rigel et al., 2022). Sunscreen is expected to absorb erythema, is not photolabile, is non-toxic, does not dissolve in sweat and certainly does not cause irritation and allergies. Although the problem now is that most sunscreen preparations with active ingredients of chemical compounds often have an irritating impact, so many are sought after natural active ingredients that can be used as sunscreen.

One commodity that is thought to be efficacious as a sunscreen is a plant that contains large phenolic compounds because secondary metabolite compounds from this group are potent enough to be developed as sunscreen (Bondam et al., 2022). *A. gombiformis* had a high capacity for absorbing UV radiation with an SPF of 37.78 ± 0.85 and significant anti-inflammatory activity with a percentage inhibition of 75.38%, which is close to that of diclofenac and ketoprofen. Tests using LC–ESI–MS found 17 phenolic compounds, mainly cirsiliol, silymarin, quercitrin (quercetin-3-O-rhamnoside), and kaempferol. The methanolic extract of *Linaria scariosa* possessed moderate antioxidant activity and had the ability to inhibit thermally induced protein denaturation in a dose-dependent manner with a percentage of 40.98% at 500 μg/ml. Moreover, this plant extract significantly shortened the clotting time compared to the control group and had a higher capacity to absorb UV radiation with a sun protection factor estimated at 38.46 ± 0.22 (Mouffouk et al., 2020).

In several studies it has been known that secondary metabolite compounds contained in *Abelmoschus esculentus* fruit are phenolic compounds so that *Abelmoschus esculentus* fruit can be used for various therapeutic activities, one of which is its potential as a sunscreen (Patwadhan and Bhatt, 2016; Astutiningsih and Anggraeny, 2023).

Phenolic compounds are natural compounds that are protective and also capable as antioxidant due to the presence of aromatic rings (Ambriz-Pérez et al., 2016). Conjugated bonds in the phenolic group cause these natural ingredients to be used as active ingredients in sunscreen (Abdiana & Anggraini, 2017). One plant that is wealthy in phenolic content, especially flavonoids, is *Abelmoschus esculentus* fruit. Secondary metabolites in the form of flavonoids, alkaloids, tannins, saponins, and steroids/triterpenoids were found in ethanol extract from *Abelmoschus esculentus* fruit (Tandi et al., 2020) and the quercetin compounds have been isolated from *Abelmoschus esculentus* fruit (Astutiningsih, 2021). So that in this study, *Abelmoschus esculentus* fruit extraction will be carried out followed by phytochemical screening to ascertain the type of secondary metabolites and quantitatively analyze the content of total phenolic compounds and total flavonoids, antioxidant activity with the DPPH method, their ability as sunscreen, and in vitro anti-inflammatory testing carried out by inhibiting protein denaturation methods using bovine serum albumin.

METHODS

Equipment and materials

The research equipment used includes a glass plate, a rotary evaporator (Heidolph Hei-Vap Advantage), UV spectrophotometer (Shimadzu), a hotplate (IKA C-MAG), a pH meter (Trans), an analytical balance (Ohaus), waterbath (Faithful).

Research materials include Abelmoschus esculentus fruit obtained from the Toroh area, Purwodadi; technical ethanol (Bratachem), DPPH (Sigma), NaOH (Merck), Mg powder (Merk), concentrated sulfuric acid (Bratachem), Silica Gel GF 254 (Merck), ammonia (Merck), concentrated HCl (Bratachem), n -butanol (Merck), glacial acetic acid (Merck), methanol pa (Merck), Bovine Serum Albumin (Sigma).

Okra Fruit Extraction

Dried okra fruit powder 200 g was extracted with 1 liter of 80% ethanol by remaceration method for 5 x 24 hours. Every day it is filtered and the solvent used is replaced. The results of macerate were concentrated in a rotary evaporator with a temperature of 50°C for 60 minutes for 500 mL of liquid extract untul a concentrated extract is obtained ±50 mL and continued tickening using a waterbath of the same temperature until it becomes a thick extract.

Phytochemical Screening.

Flavonoids identification: the extract 0.5 g was added with 10 mL aquadest then filtered. Filtrate was added 0.1 g of magnesium metal, 2% HCl 1 mL and 2 mL amyl alcohol. If it is positive to contain flavonoids, red, orange or yellow colors are formed on the amyl alcohol layer (Harborne, 1987).

Saponins were identified by shaking the extract 0.5 g andhot aquadest 10 mL in a test tube for 10 seconds. This produced foam, and one drop of 1% HCl formed a stable foam is positive saponins (Hanani, 2015).

Alkaloids: the extract 0.5 g was added to 10 ml of HCl solution (1:10) divided into 3 test tubes and then added with 2-3 drops of precipitating settling reagent of different classes. The test solution contains alkaloids if at least a precipitate is formed using the two experimental classes used (Departemen Kesehatan RI, 1980)

Tannins: the extract 0.5 g is dissolved in 10 mL of aquadest and divided into 2 tubes. Tube 1 is added 2 drops of $FeCl_3$ where color green black or blue black can form, and the other tube adding NaCl and gelatin solution where a precipitate forms if it contains tannins.

Terpenoids: adding the extract 0.5 g with 3 drops Liebermann-Burchard reagent. Then concentrated 5 drops H_2SO_4 was added, shaken and observed. If a red or purple color is formed, it indicates the presence of triterpenoids. Meanwhile, if it is formed in green, it indicates the presence of steroid compounds (Departemen Kesehatan RI, 1995).

TLC test

Three chambers were prepared and each chamber was filled with eluent to identify alkaloids, flavonoids, tannins and saponins and then saturated with the system used as table 1

Table 1. TLC system of Extract Okra Fruit with Silica Gel GF 254

Sample	Mobile phase	Detection
Flavonoids	Butanol: acid acetate:water(4:1:5) (Harborne, 1987)	Ammonia vapor formed yellow or yellow-brown color (Yuda dkk., 2017)
Alkaloids	Ethyl acetate:methanol:water (100:13,5:10) (Harborne, 1987)	Dragendorf formed brown or orange color (Marliana, 2007)
Saponins	Chloroform: methanol: water (64:50:10) (Hanani, 2015)	Anisaldehyde – H2SO4(p) formed in blue, purple, yellow colors (Hanani, 2015)
Tannin	Methanol : water (6:4)(Hanani, 2015)	FeCl3 5% formed green or blackish blue color (Rahayu dkk., 2012)
Terpenoids	n-hexane : ethyl acetate (5;5) (Hanani, 2015)	Anisaldehyde – H2SO4(p) steroid positive if purple color is formed and triterpenoid positive results when blue color is formed (Hanani, 2015)

Determination of Total Phenolic

Determination of total phenolic content was carried out by making concentration series of Gallic acid (4, 5, 6 ,7, 8; 9, and 10 mcg/mL) as a standard solution. Folin-Calteau reagent was added as much as 1.5 mL (1:10) for every 1 mL of sample (extract, n-hexane fraction, ethyl acetate fraction, and fraction ethanol. Then 1.2 mL of 7.5% Na_2CO_3 was added, and 10 mL distilled water was added (Ghazi, 2022). The solution was incubated at the temperature room for 120 minutes. The solution was measured at a wavelength of 775 nm using a UV-Vis spectrophotometer.

Determination of Total Flavonoid

The stock solution was prepared by dissolving 50 mg of standard quercetin in 50 mL of ethanol. The stock solution was pipetted in 1 mL and then made to 10 mL with ethanol to obtain a concentration of 100 mcg/mL. From the 100 mcg/mL quercetin solution standard, several concentrations were prepared, namely 20 mcg/mL, 80 mcg/mL, and 100 mcg/mL. From each concentration of quercetin solution standard, pipetted 4 mL, then added 0.1 mL of 2% $AlCl_3$, 2.8 mL of distilled water, and 0.1 mL of 1 M sodium acetate (Baba & Malik, 2018), added ethanol in 10 mL volumetric flask. Samples were incubated for 30 minutes in a temperature room. The absorbance was determined using the UV-Vis spectrophotometry method at a maximum wavelength of 436.5 nm. Measurement of extract and fraction samples was carried out by weighing a 10 mg sample dissolved in 10 mL of a volumetric flask for produced 10,000 mcg/mL.

DPPH Method Antioxidant Activity Test

All extracted samples were dissolved in methanol and made at various concentrations of 10 mcg/mL, 20 mcg/mL, 30 mcg/mL, 40 mcg/mL, 50 mcg/mL, and 60 mcg/mL. 10 mL solution served as the basis for measuring antioxidant activity. All extract samples were put in the test tubes and added with 4,0 mL of DPPH 0.1mM for each concentration. Next, the mixture is homogenized with a vortex for 1 minute and allowed to stand according to the operating time of each test solution. The absorbance of the solution is focused at the maximum wavelength. The same steps are taken to measure the concentration of the quercetin standard series in the absorbance reading (Jadid et al., 2017; Wołosiak et al., 2021).

Sunscreen Activity Test

The sunscreen activity test utilized a spectrophotometer to evaluate the in vitro efficacy of sunscreen in their research. The spectrophotometer was utilized to analyze the The concentrations of the extract used were 100, 200, and 400 mcg/mL. The spectral range utilized for analysis spanned from 290 to 375 nm, with an incremental step size of 5 nm (Mutmainah et al., 2020).

Antiinflamation

As much as 50 ⌠L of each solution concentration sample was taken, then a 0,2% BSA solution is added until the volume reaches 5 mL from the mixture, which will produce a concentration sample in the positive control. All samples were incubated at 25°C for 30 minutes and then heated for 25 minutes at 23°C. After cooling, the solution was vortexed, and absorbance measurements were carried out with UV-Vis spectrophotometry at a wavelength of 660 nm (Akhil & Prabhu, 2013; Murthuza & Manjunatha, 2018; Djova et al., 2019; Hamoudi et al., 2021).

Data Analysis

For physical characteristic data, the average value of each reported parameter was used. The linear regression equation Y = bx + a was used to test the antioxidant activity of okra fruit serum. Study data are shown as the mean ± standard deviation (SD) determined from the results of three replicates in each test. Data using a one-way ANOVA. The results differ significantly when the p-value is less than 0.05 (<0.05).

RESULT AND DISCUSSION

Extraction and Phytochemical screening

The process of extracting *Abelmoschus esculentus* L. fruit using 80% ethanol is carried out based on previous research. The main compounds to be taken are phenol and flavonoid compounds, which in general have a high level of solvency for solvents such as a mixture of water and ethanol with a certain ratio. The yield of the extract obtained was 30.68%. The tested extract is ethanol-free to maintain the stability of the extract during storage and so as not to affect subsequent test results. Test results showed the extract was free of ethanol. The ethanol extract obtained was identified for its secondary metabolite content by phytochemical screening using the tube method and Thin Layer Chromatography (TLC). The test results can be seen in table 2. The test results obtained are the same as the research (Nurfatwa, 2018; Tandi et al., 2020).

Table 2. Results of Phytochemical Screening and TLC Tests of Okra Fruit Ethanol Extract

Class	Extract	
	Chemical identification	TLC identification
Phenolic	Blue black	1 spot with Rf 0.33 and colour is blue black
tannins	Blue black	1 spot with Rf 0.33 and colour is blue black
Flavonoids	Orange at amyl alcohol	1 spot with Rf 0.73 and colour is yellow
Saponins	+ Stable foam	2 spot with Rf 0.39 and 0.4 with colour are blue and purple
Steroids	+ purple	1 spot with Rf 0.31 colour is purple
Alkaloids	+black brown precipitate with dragendrof + white precipitatw with Mayer + red brown precipitate with bourcardat	1 spot with Rf 0.74 colour is brown orange

Determination of Total Phenolic, Total Flavonoid and Antioxidant Activity Testing

It was shown through phytochemical screening and TLC that the extract contains phenolic and flavonoid compounds. The amounts of these two compounds are then found. The total phenolic content of *Abelmoschus esculentus* fruit extract is obtained from the regression equation Y = 0.015x + 0.0967 which shows a strong correlation with the value of r = 0.9997 as shown in table 3. At these stages, gallic acid standards are used to ensure the total phenolics of *Abelmoschus esculentus* fruit as natural phenolics derived from hydroxybenzoic acid. Phenolic compounds in determining levels by the Folin-Ciocalteu method under alkaline conditions with the addition of Na_2CO_3 are able to react with Folins so as to convert phenolics into phenolics due to proton dissociation (Carmona-Hernandez et al., 2021).

Table 3. The Result of Setting The Total Phenolic, The Total Flavonoid and IC_{50} Antioxidant of *Abelmoschus esculentus* L. Fruit Extract

Parameters	**Ethanol Extract**
Total Phenolic	113.4751±4.2619 mg GAE/g
Total Flavonoid	61±2.500 mg QE /g
IC_{50} Antioxidant	33.5303±2.3316 mcg/mL

The formula obtained a linear regression equation Y = 0.0624x + 0.067 with a very strong correlation with the R = 0.999976 as a result of quercetin calibration as explained table 3. Total flavonoids and quercetin standards reacted with $AlCl_3$ reagents which form complexes with $AlCl_3$ compounds that cause a shift in visible waves so that the yellow color of the sample was also changed by adding sodium acetate mixed so that the waves remained visible. Phenolic content tends to have higher levels than total flavonoids because total phenolic levels are a combination of flavonoid compounds plus other phenolic compounds and phenolic compounds are the main part contained in plants (Harborne, 1987).

Antioxidant will cause a decrease in the color intensity of DPPH. Measurements of antioxidant activity were taken at 517nm for 12 min (Munteanu & Apetrei, 2021). Free radicals can be neutralized through hydrogen abstraction reactions due to antioxidants in DPPH which neutralize free radicals from DPPH with hydrogen atom donation to stabilize DPPH so that DPPH-H is formed. When all electrons can find their partners, the purple solution turns yellow (Foti, 2015). If the added DPPH (free radical) forms a pair with a hydrogen atom (antioxidant), then a reduced DPPH-H will be formed, which already has nonradical properties. The ability of antioxidants is generally measured based on the value of IC_{50}, which describes the magnitude of the concentration of a compound that can inhibit free radicals by as much as 50%. If the IC_{50} value is getting smaller then the antioxidant ability is greater activity (Molyneux, 2004). The IC_{50} ethanol extract of *Abelmoschus esculentus L.* fruit belongs to the category of powerful antioxidants because IC_{50} at concentration less than 50 mcg/mL. IC_{50} from ethanol extract of *Abelmoschus esculenthus* L. fruit can be seen in table 3.

Phenolic compounds and flavonoids contribute directly to the antioxidant effect (Selvaraj et al., 2014). Increased antioxidant activity is characterized by high phenolics and flavonoids (Erukainure et al., 2011). The IC_{50} value has a negative relationship with a strong correlation with total phenolic levels at the value of (-0.89529) and total flavonoids at -0.90018, so if total phenolics or total flavonoids increase, the IC_{50} will decrease. As for total flavonoid levels and total phenolic levels, they have a positive relationship with correlation (0.993223) so that if flavonoids increase, phenolics will increase. Futher, the relationship between IC_{50}, total phenolics and total flavonoids is $Y = 0.01669\ X1 - X2 + 73.38984$ so that if the phenolic value increases by one unit, the IC_{50} value will decrease by 0.001559 and if the flavonoids increase, the IC_{50} value will decrease by 0.028668. Based on the R-squared value of 0.7788 or 77.88%, IC_{50} value is influenced by total phenolic and total flavonoid levels, while the rest is determined by other variables. The concentration and chemical form of phenolics and flavonoids exert a significant effect on antioxidant activity. So, exposure to UV rays, which triggers the formation of free radicals can be handled with phenolics and flavonoids in *Abelmoschus esculentus* L. fruit extract. This happens because of the electronic transition in sunscreen molecules proportional to the energy of UV light.

Photoprotective Activity Test

This stage focuses on three things, namely the SPF (Sun Protection Factor) value, % erythema and % pigmentation as a real picture of the effect of photoprotection or sunscreen. The SPF value can be used as an indicator of the effectiveness of a substance as a protector from UV rays. The higher the SPF value, the greater its ability to provide protection against sun exposure (Dutra et al., 2004), but for percent erythema and percent pigmentation on the contrary, the more effective the compound as a sunscreen, the smaller percent value of erythema and the percent pigmentation. These parameters can becalculated using mathematical equations (Dutra et al., 2004; Mansur et al., 1986). The test results of *Abelmoschus esculentus* L. fruit extract as a sunscreen with parameters SPF, % erythema and % pigmentation can be seen in table 4. Okra fruit extract has a fairly low SPF value at a concentration of 400mcg/mL this is still a weak category for photoprotective The ability of the extract as sunscreen is due to the fact that the sample contains secondary metabolite compounds such as phenols and flavonoids. Phenols and flavonoids are compounds that absorb UV radiation, which gives them photoprotective capabilities. The absorption of UV light by flavonoids causes changes in their structure.

Table 4. Results of Photoproptective Activity Test of *Abelmoschus esculentus* L. Fruit Extract

Concentration mcg/mL	SPF Average	% Erythma Average	% pigmentasi Average
100	1.1295±0.06030	0.9015±0.01094	0.9107±0.01146
200	1.5945±0.0509	0.7943±0.0129	0.8012±0.0127
400	2.2284±0.0737	0.6599±0.0169	0.6695±0.0108

Anti-inflammatory Activity Test

According to Nasution et al., (2019), compounds that can stabilise proteins from the protein denaturation process are compounds that have the potential to be anti-inflammatory. This is because, protein denaturation in tissue is one of the causes of inflammation. Heat can be used to influence hydrogen bonding and nonpolar hydrophobic interactions because heat increases kinetic energy and causes the molecules that make up proteins to move so fast that they mess with hydrogen bonds. In addition, heating will make the protein change its water-binding ability. Thermal energy will result in breaking noncovalent interactions that exist in the natural structure of proteins but do not break their covalent bonds in the form of peptide bonds. This process occurs over a narrow temperature range. Inhibition of protein denaturation is known by UV-Vis spectrophotometric absorption measurement (Osman et al., 2016). Compounds that inhibit protein denaturation by more than 20% are considered to have inflammatory activity. The result of the anti-inflammatory test can be seen in Table 5.

Table 5. Results of Antiinflammatory Activity Test

Concentration (mcg/mL)	**Average % Inhibition**	**IC_{50} (mcg/mL)±SD**
100	40.9075±1.4585	
80	35.3035±1.2990	
60	27.8551±0.9715	**153.837±0.3375**
40	17.7882±0.6891	
20	10.3993±0.3375	

Based on the results of statistical analysis the extract showed the anti-inflammatory activity. According to the results of phytochemical screening, okra fruit extract contains terpenoids, flavonoids, and tannins. Flavonoids are one of the bioactive compounds that have anti-inflammatory activity because these compounds are able to inhibit the lipoxygenase pathway directly in inflammation, which causes inhibition of eicosanoid biosynthesis and inactivates free radicals that can attract various inflammatory mediators (Farzaei et al., 2019). Tannin compounds are also reported to have an anti-inflammatory role (Priya et al., 2018). The formation of free radicals causes proteins in the body to denature, leading to inflammatory mechanisms by stimulating the release of immatory mediators. There may be interaction or bonds between molecules contained in Bovine Serum Albumin (BSA) and molecules contained in extracts of *A. esculentus* fruits (Acharya & Chaudhuri, 2021), so that the extract can inhibit the occurrence of protein denaturation.

CONCLUSION

The research concludes that the extract of okra fruit demonstrates powerful antioxidant activity with an IC_{50} of 33.5303±2.3316 mcg/mL; and exhibits photoprotective activity with an SPF value of 2.2284±0.0737 falling into the medium category. Furthermore, the extract of okra fruit exhibited inhibition activity against inflammation with an IC_{50} is 153.837±0.3375 mcg/mL. Taken together, these results suggest that the extract of okra fruit could be used as a skincare agent in cosmetic formulations, as an antioxidant and anti-inflammatory, allowing the treatment of skin conditions, as well as a pharmaceutical agent with multidimensional applications.

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AUTHOR CONTRIBUTION

CA: Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis.
ENA: Manuscript preparation and editing; literature search; data analysis.

CONFLICT OF INTEREST

None to declare

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