Potential as Photoprotective, Antioxidant and Anti-inflammatory of Flavonoid Isolate from Okra Fruit (Abelmoschus esculentus L.)

Christina Astutiningsih[1(✉)], Herni Setyawati[2], Ali Rahmat[3], Lilla Puji Lestari[4] and Faidliyah Nilna Minah[5]

[1] Semarang Pharmaceutical College, Semarang, Indonesia, 50192
christinaastutiningsih@gmail.com
[2] Universitas Anwar Medika, Sidoarjo, Indonesia, 61253
[3] Badan Riset dan Inovasi Nasional, Indonesia
[4] University Maarif Hasyim Latif, Sidoharjo, Indonesia
[5] Institute Teknologi Nasional, Malang, Indonesia

Abstract. Long-term exposure to UV radiation can induce skin disorders, such as cancer and photo-allergic reactions. Natural products are being considered for their potential as non-irritating and effective sunscreen resources, owing to their UV absorption, antioxidant, and anti-inflammatory properties. This study aimed to assess the level of quercetin and investigate the in vitro ultraviolet radiation protection, antioxidant activity, and anti-inflammatory potential of isolated flavonoids from Okra Fruit (Abelmoschus esculentus L.). The results revealed that the average level of Okra fruit flavonoid isolate was 229.26 ± 1.16 µg/ml. In UV protection testing, at a concentration of 100 ppm, the SPF value was measured at 15.08, categorizing it as providing medium protection. Furthermore, the antioxidant activity test of the okra fruit flavonoid isolate yielded an IC50 value of 25.67 ppm, indicating a very strong antioxidant effect. The study demonstrated that the isolated compound inhibited protein denaturation at 50 ppm, with an inhibitory efficacy of 50.65%. Based on these findings, it can be concluded that the isolated compound possesses anti-inflammatory activity. There is a strong positive correlation between SPF effectiveness, antioxidant activity, anti-inflammatory effects, and flavonoid content.

Keywords: Photo protective, Antioxidant, Anti-inflammatory, Flavonoid, Okra fruit.

1 Introduction

The wavelengths of sunlight range from ultraviolet light through infrared light to visible light. The most harmful to the skin[1]. The 95% of UVA solar radiation can reach the earth because UVA wavelengths are longer than UV B and UV C but UV B radiation is light that can be absorbed by the ozone layer [2]. According to [3] UVA is approximately a thousand times more efficient than UVB in providing an immediate tanning effect. This effect is caused by the darkening of melanin in the epidermis. According to

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[4] study, chronic exposure by UVA radiation can cause damage to dermal tissue and accelerate the aging process of the skin. According to [5] UV A radiation effect on the skin sagging rather than wrinkle. According to [6], exposure to UVA can damage the structural components of DNA, suppress the immune system, and induce cancer.

The system responsible for producing melanin in the skin is activated when exposed to ultraviolet (UV) rays. However, this naturally occurring reaction is sometimes insufficient to fully neutralize the potentially damaging effects of the free radicals created by UV rays. Consequently, several biological responses occur within the body, including photoaging, inflammation, a decreased immune response, altered epidermal homeostasis, and a range of skin disorders. Photoaging refers to the premature aging of the skin caused by exposure to ultraviolet radiation. Some of these skin problems include erythema, edema, thinning of the dermis and epidermis, DNA damage, and light-induced skin aging [7,8].

The presence of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, is induced by UVB light exposure. According to [9]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 [10].

Furthermore, oxidative stress promotes the increased activity of enzymes involved in the metabolism of membrane phospholipids. This process leads to the formation of additional groups of lipid mediators, including endocannabinoids. Endocannabinoids primarily function by activating G protein-dependent membrane receptors that play a role in regulating reactive oxygen species (ROS) and levels of pro/anti-inflammatory cytokines. The release of inflammatory mediators, such as histamine, occurs as a result of interactions between ROS and mast cells in the dermis. Consequently, this induces blood vessel dilatation.

The skin can be protected from UV radiation by using sunscreen. When selecting a sunscreen, it's essential to choose one that also possesses antioxidant and anti-inflammatory properties, enabling it to synergistically protect the skin. Quantitative parameters used to measure a sunscreen's ability to delay erythema include the Sun Protection Factor (SPF) [11]. However, sunscreens containing active chemical ingredients may have undesirable effects on the skin, such as inflammation, infection, hypersensitivity, and even an increased risk of skin cancer. Therefore, it's crucial to explore sunscreen options derived from natural ingredients to mitigate the potential side effects associated with chemical sunscreens [12,13].

Natural ingredients containing compounds that can offer sunscreen activity and function as antioxidants are typically organic compounds with chromophore groups. These compounds have the capacity to absorb UV light by undergoing electronic transitions within the sunscreen molecule, where the energy of the transition matches that of UV light [14].

Phenolic compounds are a type of natural compound that possess chromosphere groups and exhibit potential as both sunscreens and antioxidants. This phytochemical substance possesses the capability to prevent molecular damage by sequestering and

endeavoring to eliminate reactive oxygen species (ROS) generated by biological systems, pollution, inhalation, and medications.

According to [15] polyphenols are a noteworthy natural product category in the field of dermatology due to their absorption spectrum, which effectively filters UV radiation and diminishes the probability of radiation penetrating deeply into the skin layer. Phenolic compounds such as flavonoids have antioxidant activity [16] antibacterial activity [17] and anti-inflammatory activity [18].

The okra fruit contains a high concentration of these phenolic chemicals. Examination of the ethyl acetate fraction from the okra fruit revealed flavonoid and other phenolic content levels of 127.6178 mg/g and 251.0116 mg/g, respectively, along with an IC50 value of 40.2254 ppm. The ethyl acetate fraction exhibits natural sunscreen properties, making it beneficial for sun protection. The fact that the SPF value exceeds 30 at 400 ppm demonstrates its exceptionally high protective capabilities[19] In this study, flavonoid compounds from okra fruit will be isolated and analyzed with UV-Vis spectrophotometers, FTIR spectrophotometer and LCMS. Then the isolates which are flavonoid derivate will be tested for UV protective, antioxidant and anti-inflammatory activities.

2 METHODS

The samples used were Okra fruit (Abelmoschus esculentus L.), collected in June 2022 from Purwodadi Regency, Central Java Province, Indonesia (Fig. 1). The plant materials were authentically identified by the Department of Biological Sciences at Semarang Pharmaceutical College, Semarang, Indonesia.

Fig. 1. Pictures of Okra (Abelmoscus esculentus) fruit.

2.1 Extraction and Fractionation

The preparation of a plant sample, okra fruit, involved cleaning to eliminate dust and undesirable components, followed by drying to reduce water content to prevent microbial growth. The fruit was then ground into a fine powder, hereafter referred to as the 'fine powder sample.' Approximately 200 g of the fine powder sample was placed in an Erlenmeyer flask, and 1 liter of 80% ethanol was added, ensuring that all the sample was fully covered by the ethanol. The sample was remacerated for five days. This process was repeated until approximately 150 g of viscous extract was obtained. The resulting viscous extract was stored at 4°C until analysis. Next, 25 g of the viscous extract was dissolved in 100 mL of distilled water, and 50 mL of n-hexane was added to create a solution, which underwent liquid-liquid partitioning until a colorless extract (n-hexane fraction) was produced. The remaining sample was combined with 50 mL of ethyl acetate and subjected to liquid-liquid partitioning until it yielded a colorless extract (ethyl acetate fraction). The final remaining portion was referred to as the water fraction.

Screening Flavonoid [20–22]**.**

a. Wistater's reagent: when the sample is mixed with concentrated HCl and magnesium powder, a yellow color will result from the presence of flavonoids that are positive.
b. If positive flavonoids are present, the color orange will appear when the sample is mixed with reagent containing 10% sodium hydroxide.
c. Bate-Smite-Metcalfe reagent: Adding the sample to concentrated H2SO4 and putting it in a water bath will cause positive flavonoids to become red.
d. The TLC (thin-layer chromatography) test for the identification of flavonoids: the stationary phase will be silica GF 254, and the mobile phase will consist of n-butanol, acetic acid, and water at a ratio of 4:1:5. Following elution, the plate is inspected using a UV lamp 254nm. Flavonoid stains after steaming ammonia vapor show a brownish-yellow color.

2.2 Separation Flavonoid Compounds for Okra Fruit by TLC Preparative (TLC-P)

The viscous ethyl acetate fraction was dissolved in 1 mL of methanol p.a at room temperature and subsequently eluted using stationary phase silica GF 254 with the mobile phase consisting of n-butanol: acetic acid: water (4:1:5) as the eluent. The separation results were observed under a 254nm UV light lamp and then sprayed with ammonia vapor. The presence of brownish-yellow stains, as described by [23] indicates the possible presence of flavonoids. The stained tape, similar to quercetin, was extracted and dissolved in ethyl acetate p.a, followed by centrifugation for 10 minutes at a rate of 1500 rpm. The separation result was recrystallized [24] and its melting point was tested. Further identification was carried out using TLC with multiple eluents and analysis with UV Spectrophotometer, IR Spectrophotometer, and LCMS.

Determination of flavonoid contents [25]**.**
[26] conducted an inquiry utilizing mobile phase consisting of n-butanol: acetic acid: water (4:1: 5) and silica GF 254 as stationary phase. The method employed for the measurement of samples use of TLC densitometry (CAMAG TLC Scanner). The plate was eluted and subsequently examined using UV lamps 254 nm and 366 nm. The quantification of each standard was performed in triplicate using calibration curves that correlated the peak area of the chromatogram with the mass of the standard applied in the form of a band.

Sunscreen Activity Test.
[27] 28 utilized a spectrophotometer to evaluate the in vitro efficacy of sunscreen in their research. The spectrophotometer was utilized to analyze the concentrations of isolates at 25, 50, 75, 100, and 150 ppm. The spectral range utilized for analysis spanned from 290 to 375 nm, with an incremental step size of 5 nm. The determination of the Sun Protection Factor (SPF) is carried out by applying the Mansur equation.

ABTS radical scavenging activity
Determination of antioxidant activity was carried out by means of 4.0 mL of ATBS solution inserted in a test tube, added with 0.2mL of solution from isolates for concentration of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and 100 ppm respectively. Next, the mixture is homogenized with vortex 1 minute and allowed stand according to the operating time of each test solution in a dark place. The absorbance of the solution is read at the maximum wavelength. The same steps were taken to measure the concentration of quercetin standard series the ATBS solution control absorbance reading.

In Vitro Test of Anti-inflammatory Potential [28,29]
Making an isolate solution, 50 mg isolate dissolved 50 mL methanol obtained an concentration series so that it became an isolate solution with concentrations of 1000 ppm then made a concentration series so that it became a test solution with concentration of 1000 ppm, 100 ppm and 10 ppm.

Making a positive control solution, 100 mg of diclofenac sodium dissolved in 25 mL of methanol obtained a concentration of 4000 ppm then made a concentration series so that it became a test solution with concentration of 4000 ppm, 2000 ppm, 1000 ppm, 500 ppm, 250 ppm and 125 ppm.

50 µL of each solution concentration (isolate solution and positive control solution) are taken, then a 0.2% BSA solution is added until the volume reaches 5 mL from the mixture will produce concentrations of 0.1 ppm, 1 ppm, 10 ppm for isolate concentration and positive concentration are 1.25 ppm; 2.5ppm; 5 ppm; 10 ppm; 20 ppm and 40 ppm.

(c) A total of 50 µL methanol solvent added 0.2% BSA solution to the measuring flask until the volume reaches 5 mL.

All solution namely isolate solution, positive control solution and negative control solution were incubated at 25^0C for 30 minutes then heated for 25 minutes at 23^0C.

After cooling, the solution was vortexes and absorbance measurements were carried out with UV-Vis spectrophotometry at a wavelength of 660 nm[30,31].

3 Results and Discussion

The extraction of okra fruit (Abelmoschus esculentus L.) resulted in a viscous extract weighing 251.34 grams, with a yield of 31.42%. This viscous extract was fractionated using n-hexane and ethyl acetate, yielding three types of fractions: the n-hexane fraction with a total yield of 13.6944%, the ethyl acetate fraction with a total yield of 28.7696%, and the water fraction with a total yield of 46.4804%. The difference in yield indicates that the choice of solvent significantly influences the extracted compounds, which is related to differences in polarity levels, with nonpolar solvents favoring nonpolar compounds [32] The polarity of the solvent and the extracted substance are interconnected with high dissolving power. The yield of secondary metabolite chemicals that can be extracted is affected by the solvent and extraction technique employed. The three types of fractions were subsequently used to identify flavonoid compounds through chemical reactions and TLC, and the results are presented in Table 1.

Table 1. Results of Identification of Flavonoid Compounds

Fraction	Chemical Identification			TLC Identification
	Wistater's	10% NaOH	Bate Smite-Metcalfe	
n-hexane	-	+	-	-
ethyl acetate	+	+	+	3 staints at Rf 0.34; 0.66 and 0.92
water	+	+	+	1 staint at Rf 0.50

The separated components were evaporated until they became an amorphous solid, which was then crystallized and washed with solvents capable of dissolving contaminants to obtain clear crystals. Isolates in the form of yellow amorphous solid were produced with a yield of 0.18%. The compounds were characterized using a melting point determination test. The flavonoid isolates began to melt at a temperature of 307.5°C and completely melted at 309.0°C. This temperature range was slightly lower than the melting point of the quercetin standard, which is typically 316–318°C [33]The compound exhibited a narrow melting range (± 20°C), indicating that the isolated compound is pure. Identification was also performed using the TLC method with multiple eluents, and the results for each eluent produced a single stain, as shown in Fig. 2.

n-hexane: ethyl acetate (4:1) Rf spot = 0.60 | n-hexane: chloroform (3:1) Rf spot = 0.60 | n-butanol: acetic acid: water (4:1:5) Rf spot = 0.62

Fig. 2. Result of Identification of Okra Fruit Flavonoid Isolates with Multieluent TLC

According to [34] one of the steps involved in the process of identifying flavonoid isolate compounds is using a UV-Vis spectrophotometer to determine the absorbance values of the compounds at their respective maximum wavelengths. This step is essential to complete the identification process. The obtained spectra are believed to contain flavonoids. Interpretation as a compound of the flavonol group is supported by a spectral pattern almost identical to that of flavonols, with band I at a wavelength of 349 nm and band II at 253 nm [35,36]. The spectra of flavonoid isolates are identical to the quercetin standard spectra when scanned with a UV-Vis Spectrophotometer at wavelengths ranging from 200 to 400 nm. Fig. 3 displays the UV-Vis spectrum of the flavonoid compound found in okra fruit:

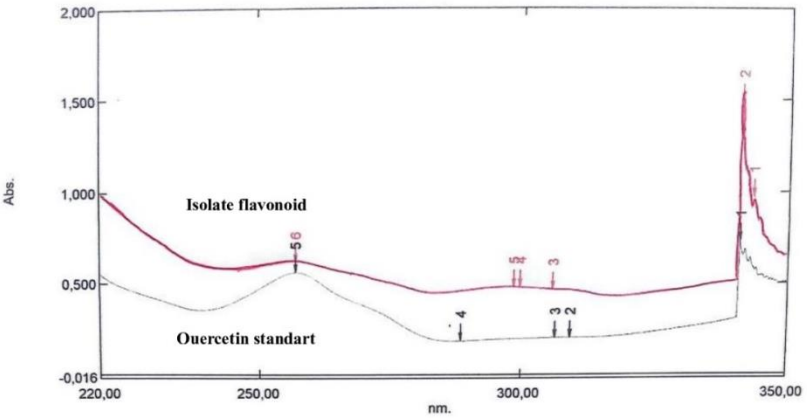

Fig. 3. Spectrum of Okra Fruit (*Abelmoschus esculentus* L.) by UV-Vis Spectrophotometry

The identification of the isolate then did use an IR spectrophotometer. The infrared spectrum results of isolates of fraction with number 4000-450 cm-1 are presented in Fig. 4. The blue line represented the quercetin standard and the red line represented flavonoid isolates.

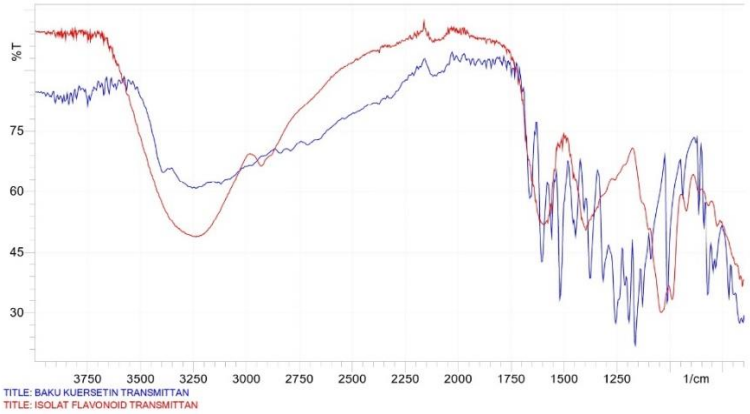

Fig. 4. Spectrum of Okra Fruit Flavonoid Isolates (*Abelmoschus esculentus* L.) with Infrared Spectrophotometer

Here are the results of the analysis of the function group of the flavonoid isolate of okra fruit, standard quercetin, and comparison with standard quercetin in Table 2. Indicates that the isolate contains several functional groups that lead flavonoid compounds

Table 2. IR Spectra Results

Isolate flavonoid Okra Fruit cm^{-1}	Quercetin standard cm^{-1}	Functional Group
3291, 3306, and 3329	3407.322	OH vibration on phenol
1716.5	1665. 82	C=O aril ketone
1662 and 1651	1610	C=C aromatic bond
1459.03	1382	OH-phenol
1018 and 1114	1012	C-O alcohol
719	795.83	C-H aromatic
2922.04; 2851.78 and 1376.3		C-H aliphatic

In study, the identification of flavonoid isolates was continued using the LCMS instrument. LCMS is a combination of Liquid Chromatography (LC) used for sample separation and followed by Mass spectrophotometers (MS) which detect and identify because LCMS can obtain information identity of specific compounds as information about the molecular weight and structure of identified compounds [37] The results of separation and identification using LCMS can be seen in Fig. 5 below:

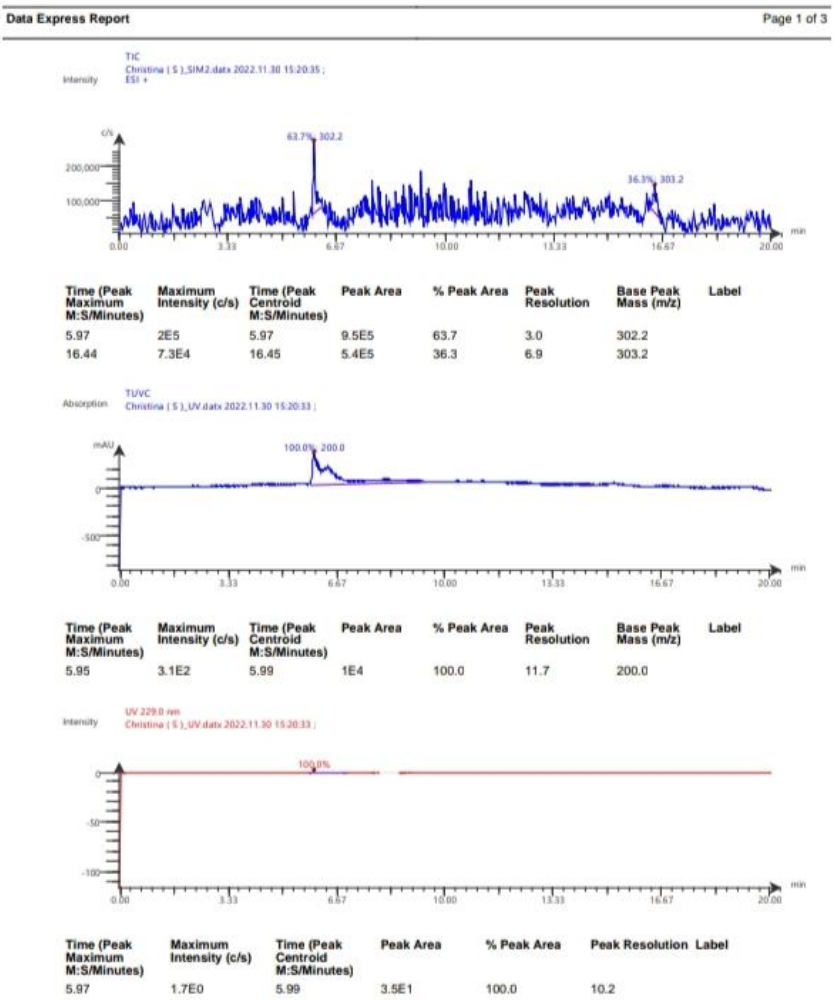

Fig. 5. Results of Separation of Okra Fruit flavonoid Isolate (*Abelmoschus esculentus* L.) with LCMS

The identification results using LCMS revealed that the compounds within the ethyl acetate fraction were predominantly those with a molecular weight (WM) of 302.2, accounting for 63.7% of the total, with a retention time of 5.97. Additionally, compounds with a WM of 303.2 constituted 36.3% of the total, with a retention time of 16.44. These two compounds appear to be identical due to their very small WM difference, suggesting that they are the same compounds. This finding aligns with prior research, which demonstrated that avocado leaf extract contains quercetin compounds with a retention time of 5.83 minutes and an m/z range of 300.50 - 301.5047. Other studies aimed at identifying quercetin compounds using LCMS have reported quercetin

compounds with a molecular weight of m/z 302.042748. According to the literature, the molecular formula for quercetin compounds is C15H10O7, with a molecular weight of 302.236 g/mol [38,39].

Subsequently, flavonoid isolate levels were determined using densitometry TLC. Quantitative analysis was conducted to provide information about the composition and concentrations of active components present in the sample. In the assessment of okra fruit flavonoid isolate levels, the identified TLC plates were scanned using a densitometer. A standard curve was constructed from five standard series, and the Rf value of quercetin was determined for each sample and standard. The data obtained, in the form of the area and mass of quercetin standard solutions, were then used to generate a linear area determination curve with the equation y = 1.225 x 10-8 + 3.976 x 10-4. This calibration curve is considered reliable, as it possesses a regression coefficient of 0.997. Fig. 6 displays the calibration curve.

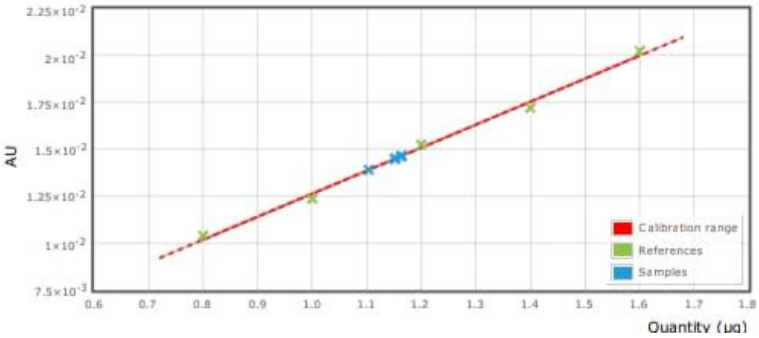

Fig. 6. Calibration Curve

The sample rate is calculated based on the standard curve series. The measured sample content on the densitometer is obtained in the Table 3.

Table 3. Results of Quercetin Levels in Sample

Type	Area	Quercetin levels (µg/ml)	Average±SD
raw 1	0.01041		
raw 2	0.01237		
raw 3	0.01525		
raw 4	0.01721		
raw 5	0.02024		
Sample 1	0.01391	220.7	
Sample 2	0.01466	233.0	
Sample 3	0.01463	232.4	229.26±0.04
Sample 4	0.01452	230.1	
Sample 5	0.01449	230.1	

Based on the results of determining levels, it is known that the average level of Okra fruit flavonoid isolate 229. 26 ± 1.16 µg/ml

The Sun Protection Factor (SPF) of the sample was determined using UV spectrophotometry in the spectral range of 290 to 320 nanometers. One commonly used technique for calculating SPF is the Mansur equation. SPF is a numerical measure of sunscreen's effectiveness in reducing the harmful effects of UV radiation on the skin, including sunburn and other types of skin damage. The higher the SPF, the more effectively the sunscreen neutralizes these harmful effects. According to [40] evaluating SPF in vitro serves as an additional measure alongside in vivo evaluation. Consequently, in vitro SPF evaluation is a crucial component of product development screening testing. The isolate flavonoid demonstrated a medium level of protection against UV B rays at a concentration of 100 ppm, as indicated by an SPF rating of 15.0765, falling into the medium protective category. According to [41] SPF values are used to categorize different levels of protection against ultraviolet (UV) radiation, which are typically divided into four categories: low (SPF 2-15), medium (SPF 15-30), high (SPF 30-50), and highest (SPF>50). Table 4 presents the results of SPF analysis conducted on the isolated flavonoids from okra fruit at various concentrations.

Table 4. SPF Value of Isolate Flavonoid Okra Fruit

Concentration (ppm)	SPF Level	Protection Category
25 ppm	3. 5643	Low
50 ppm	5. 9782	Low
75 ppm	8. 3217	Low
100 ppm	15. 0765	Medium
Commercial sunscreen 100 ppm	20. 3452	Medium
Standard quercetin 100 ppm	31. 3456	High

The SPF value of the isolate at a concentration of 100 ppm showed a decrease compared to the control. However, as the concentration of the isolate increased, so did the SPF value. This is attributed to the compound's content that possesses the ability to absorb light, and as the concentration increases, there is an increase in absorbance. The potential of flavonoids as sunscreens is attributed to the presence of chromophore groups, which are conjugated aromatic systems that enable the absorption of strong rays within the UV light wavelength range, including both UVA and UVB rays [42].

The flavonoid isolates from okra fruit, in addition to their ability to protect against UV radiation, also exhibit potential as antioxidants. Therefore, this study proceeded to assess antioxidant activity. The antioxidant activity was evaluated using the ATBS method. In ATBS experiments, an inverse relationship exists between antioxidant activity and the IC50 value, where a higher concentration of the sample results in lower absorbance. The observed decrease in the absorbance value of the test solution indicates an increase in the content of antioxidant compounds. This decrease in absorbance value of the test solution can be attributed to the action of antioxidant chemicals, which transfer hydrogen or electrons, leading to a detectable change in the color of ATBS. The results of the antioxidant testing using ATBS techniques are presented in Table 5.

Table 5. Test Results of Quercetin Standard Antioxidant Activity and Quercetin Isolate Okra Fruit with ATBS Method

Concentration (ppm)	Absorbance	% Inhibition	Linear Regression	IC50
Standard				
101.30	0.228	69.81		
81.33	0.268	64.50		
60.77	0.311	58.81	Y = 23.713x - 20.149	19.26 ppm
42.46	0.363	51.92		
21.32	0.468	38.01		
Isolate				
102.6	0.199	74.32		
82.0	0.228	70.58		
61.56	0.284	63.35	Y = 17.087x + 5.4547	25.67 ppm
41.04	0.329	57.54		
20.52	0.412	46.84		

Flavonoids may offer protection against UV-induced oxygen free radical production and lipid peroxidation. According to [43] high SPF values may be related to high flavonoid content. The higher the concentration of flavonoid isolates, the greater their inhibitory capacity, leading to reduced absorption in UV-Vis measurements. Numerous studies have demonstrated that mutations caused by the UV component of sunlight represent the initial step in the development of skin cancer[44]To prevent DNA damage resulting from UV radiation penetrating skin cells, antimutagens with UV-blocking properties are essential. One approach to reducing UV exposure is the use of antimutagens that are effective against UV-induced mutations [45]. Flavonoid isolates with UV protection and antioxidant capabilities are anticipated to function as UV-induced antimutagens.

Table 6. In vitro Anti-inflammatory Activity Test Results

No	Sample	Concentration (ppm)	% inhibition
		1.25	20.25%
		2.5	19.23%
		5.0	34.48%
1	Diclofenac Sodium	10.0	55.09%
		20.0	64.93%
		40.0	80.76%
		1.0	13.88%
2	Isolate Flavonoid	10.0	28.54%
		50.0	50.65%

The interactions between reactive oxygen species (ROS) and mast cells in the dermis can trigger the release of inflammatory mediators. Therefore, in this study, the test for anti-inflammatory activity against okra fruit flavonoid isolates was continued. Anti-inflammatory activity testing was conducted using Bovine Serum Albumin (BSA) by

observing the inhibitory effect on protein denaturation. BSA measurements were performed to reduce or eliminate the use of live specimens in the drug development process [46]. Compounds that exhibit more than a 20% inhibition of protein denaturation are considered to possess anti-inflammatory activity [47]. The test results for diclofenac sodium as a positive control and the flavonoid isolate can be seen in Table 6.

The current investigation utilized diclofenac sodium as a positive control, and it demonstrated the ability to suppress protein denaturation by 55.09% at a concentration of 10 ppm. According to the findings of this study, the flavonoid isolate exhibited an inhibitory effect on protein denaturation, starting at a concentration of 1 ppm, with a corresponding percentage inhibition of 13.88%. The inhibitory potential of the isolate increased with higher concentrations, culminating in a protein denaturation inhibition of 50.65% at a concentration of 50 ppm. The percentage of inhibition exceeded 20% and increased proportionally with concentration. Flavonoids have the ability to directly block lipoxygenase pathways, consequently inhibiting the generation of eicosanoids and inactivating free radicals, both of which are widely recognized as inflammatory mediators. The formation of free radicals in the body makes proteins susceptible to denaturation, which in turn triggers inflammatory processes by increasing the release of inflammatory mediators [48,49]. The presence of external chemicals induces proteins to undergo a process known as denaturation, during which proteins lose their tertiary and secondary structures. Due to the high likelihood of interactions or bonds between molecules in BSA and flavonoid isolate molecules, flavonoid isolates can decelerate the process of protein denaturation and, as a result, possess anti-inflammatory properties [50]. There is a positive connection between the effectiveness of the UV protection factor, antioxidant activity, anti-inflammatory properties, and flavonoid concentration. This connection is significant. The significance threshold for the relationship between SPF and flavonoid concentration was determined to be $p = 0.01$, with a correlation coefficient of 0.52553. Similarly, the correlation coefficient between antioxidant activity and flavonoid content was 0.51159, with a statistically significant p-value of 0.02. Additionally, there was a correlation coefficient of 0.50 between antioxidant activity and flavonoid content, with a statistically significant p-value of 0.019.

4 Conclusion

The conclusion of this research is that the flavonoid isolate from okra fruit exhibits photoprotective activity with an SPF value of 15.08, falling into the medium category, which is similar to commercial sunscreen. Additionally, the flavonoid isolate from okra fruit demonstrates very strong antioxidant activity. Furthermore, when tested at a concentration of 50 ppm, the flavonoid isolate exhibited an inhibition activity of 50.68% against inflammation.

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