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Quercetin Compound of Okra Fruit (*Abelmoschus esculentus* L.) as Potential of Antioxidant and Antimicrobial

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Abstract. *Respiratory tract infections can be caused by various microbes including Staphylococcus aureus bacteria and Candida albicans fungus. The treatment can use antibiotics or medical plants. Okra plants especially the fruit, are plants with antioxidant and antimicrobial activity caused by one of the flavonoids namely quercetin compounds. The purpose of this study was determine the total flavonoid, identify the isolate, and test the antioxidant and antimicrobial activity of the quercetin compound isolated from okra fruit. The result of the measurement of the total flavonoid content of the diethyl ether fraction obtained an average of 54.8723 ± 0.8059 mg QE/g and the ethyl acetate fraction obtained an average of 99.3155 ±1.4518 mgQE/g. The isolated flavonoid from okra fruit had antioxidant power with IC50 of 31,4 ppm, antibacterial activity against Staphylococcus aureus with inhibition zone diameter of 10,372mm; 13,964 mm; and 15, 392mm (1%:5%:10%), and the antifungal Candida albicans with inhibition zone diameter of 9,104mm; 284mm; and 11,380 mm (1%:5%:10%). Flavonoid isolates from Okra Fruit, namely quercetin compounds had strong antioxidant and antimicrobial activity.*

Keywords: *antioxidant, antimicrobial, Candida albicans, Quercetin, Staphylococcus aureus*

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INTRODUCTION

The common cold is a prime example of acute infections of the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi. Antibiotics are often used to treat and control respiratory infections. The majority of Upper Respiratory Tract Infection (URTI) are caused by viruses, with rhinovirus being the most common cause (along with influenza, coronavirus, adenovirus, parainfluenza, and respiratory syncytial virus) (Alsayari et al., 2021). People have long recognized that certain medical plants are effectively treatinfectious disorders. Research on the prevention and treatment of URTI utilizing herbs, plant extracts, and isolated plant molecules is ongoing, although clinical trials have often shown contradictory (Sarker et al., 2022). When grown with target cells and URTI causative agents such as the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and other adenoviruses, quercetin, an antioxidant flavonoid abundant in fruits and vegetables, has been demonstrated to display substantial anti-viral activity (Mehrotra & Jadhav, 2021).

Flavonoids play a number of critical

roles in plant defense and prevent a wide range of human pathologies with various mechanisms, such as neurological diseases, cardiovascular diseases, infectious diseases, and diabetes. They do this by reducing oxidative stress, modulating cell signaling responses, controlling the expression of specific genes, or interacting directly with proteins and membranes. It has been extensively investigated and well-documented that polyphenols or flavonoid (or their synthetic counterparts) have the potential to be efficient antibacterial and antiviral medicines with a targeted mode of action, few side effects, and the capacity to prevent the generation of bacterium toxins, and biofilm format.

Phenolic or flavonoid compounds from various plants have been shown to exhibit antibacterial action against several pathogenic microbes in addition to their antioxidative functions. Due to the rise in antibiotic resistance, there is growing interest in using medicinal plants as an alternative to manufactured medications, notably for the treatment of microbial agents. Therefore, it has become essential to look for novel antimicrobial agents such phenolic or flavonoid compound (Almuhayawi,. 2020).

One of the most prevalent flavonoids, quercetin, effectively slows the growth of a variety of drug-resistant Gram-positive and Gram-negative bacteria, fungi, and viruses due to the destruction of bacterial cell walls and membranes, the intercalation of quercetin with DNA and inhibition of nucleic acid and protein synthesis, the reduction of virulence factor expression, the inhibition of the activities of essential virulent enzymes, and the prevention of biofilm formation. However, it is necessary to conduct research related to the isolation of quercetin and test its activity as an antioxidant and antimicrobial needed when there is an infection due to bacteria or viruses (Nguyen & Bhattacharya, 2022).

The medical plant widely used to be developed is the okra plant (*Abelmoschus esculentus* L.) (Khan et al., 2022). The flavonoid contained in okra fruit is a quercetin compound. Quercetin is the largest flavonoid about 60-75% of the total flavonoid compounds in Okra fruit (Suganthy et al., 2016). It provides biological effects such as treating asthma symptoms, bronchial, hyperactivity, and inflammation of the respiratory tract (Jafarinia et al., 2020).

Based on previous, in this study, the okra fruit was macerated with 80% ethanol and continued by fractionation using n-hexane, diethyl ether, and ethyl acetate as solvents to separate active compounds based on their polarity. The determination of flavonoid levels was carried out as it is suspected to be efficacious as a antioxidant and antimicrobial. The flavonoid was separated by the preparative thin layer chromatography method with silica gel as the stationary phase and n-butanol: acetic acid: water (4:1:5) (Suresh et al., 2018) as the mobile phase followed by the recrystallization process. The results of the separation were identified by measuring the melting point, interpreting the structure of the compound using a UV-Vis Spectrophotometer, and identifying the separation of the compound by TLC Densitometry. The remaining major challenge in this research was testing flavonoid isolates which quercetin compounds from Okra fruit as antioxidants using the DPPH method (Jarial et al., 2018) and the same time can also be antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Romulo et al., 2018).

MATERIALS AND METHODS

The research was carried out from March 2019 – June 2020 at the Phytochemical Laboratory and Microbiology Laboratory of the College of Pharmacy Sciences Semarang

Pharmacy Foundation.

Sample Extraction Fractination and Phytochemical Screening

Green okra fruit powder was macerated with 80% ethanol solvent as much as 1000 ml for one vessel so that the simplicia was completely submerged. The extract was dissolved in aquadest and then liquid-liquid partitioned by adding n-hexana, diethyl ether, and ethyl acetate. The screening procedure went through the TLC method after previously going through the tube method

Determination of Total Flavonoid Content

In calculating the total flavonoids, the operating time and standard curve with quercetin were determined. Various ingredients such as 0.2 ml of sodium acetate, 3 ml of methanol, and 0.2 ml of 10% AlCl3, and 0.2 ml of sodium acetate were mixed were determined. Furthermore, a spectrophotometer was used to measure the absorbance at a wavelength of 415 nm (Chang et al., 2002).

Isolation and Identification of Quercetin Compound

The viscous fraction was dissolved in 1mL of methanol, smeared on the stationary phase GF60 and diluted with n-buthanol:acetic acid:water (4:1:5). The same band stain with quercetin standard was scraped off and dissolved in ethyl acetate pa then centrifuged. The isolate was then crystallized (Klitou et al., 2022; Rossi et al., 1986). The purity of the isolated compound was tested by chemical reaction, melting point test, multieluent TLC stain pattern, structure identification by UV-Vis Spectrophotometer and Densitometry TLC.

Antioxidant Activity Test

The isolates of 0,2 mL with various concentrations were added to 4.0 mL DPPH 0.1 mM, vortexed and left for 30 minutes and the absorbance was measured at 517nm wavelength with UV-Vis Spectrophotometer (Shimadzu Tipe UV-1700). The same was done for the blank measurement. Antioxidant activity test results than compared with standard quercetin (Jarial et al., 2018).

Antifungal Activity Test Using *Candida albicans*

With a sterile round ose needles, cultured of *Candida albicans* were taken from stock, then inoculated into tubes containing 5 mL of sterile liquid Nutrient Broth media (NB), then incubated into tubes containing 5 mL of sterile liquid NB media, then incubated for 3 x 24 hours at a temperature of 250C. The suspension was added with NB media or fungal colonies until 0.008-0.100 absorption was obtained on a UV spectrophotometer (wavelength 625 nm) in accordance with the ½ Mc Farland.

Sabouraud Dextrose Agar media was Poured into sterile Petri dishes and allowed to solidify as a base layer, then placed 5 cylinder cups at distances. The fungi are then inoculated by pour plate on Sabouraud Dextrose Agar media that has been attached to the cylinder cup. After the upper media solidified, the cylinder cup was taken and each well was filled with 50µL of n-hexane fraction, diethyl ether and ethyl acetate fraction. Nystatin as a positive control solution and DMSO as a negative control solution. Each group was replicated five times. The Petri dishes were incubated at 250C for 3x24 hours (Astutiningsih & Kristianti, 2022).

Antibacteria Activity Test Using *Staphylococcus aureus*

Staphylococcus aureus bacterial suspension incubated at 370C for 24 hours mea-

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sured absorbance at 625nm, equivalent to the comparison absorbance of a standard solution of ½ Mc Farland (absorbance 0.08-0.1) to estimate the number of bacteria. The mannitol salt agar medium was measured in a sterile petri dish and allowed to solidify. The bacteria are then inoculated by pour plate on MSA media that has been attached to the cylinder cup. The isolates with concentrations of 2%, 4% and 8% of positive control (Amoxicillin) and negative control (DMSO) were poured into 50 L in the wellbore then incubated for 24 hours at 370C. Observation and diameter measurement of the barriers formed was conducted using a caliper (Suganthy et al., 2016).

Data Analysis

Antioxidant data was taken from the interpretation of the absorbance results of isolates using a UV-Vis Spectrophotometer to calculate the IC50 value. The IC50 were then determined with SPSS 21.Antibacterial activity test data in the form of inhibition zones were measured using a caliper and analyzed by T-test using SPSS 21.

RESULTS AND DISCUSSION

The process extraction of Okra with 80% of ethanol obtained an 11.80% yield. The results of fractionation with various solvents obtained yield as follows: n-hexane of 11.98%, diethyl ether of 6.54%, and ethyl acetate solvent of 5.36%. Phytochemical screening was carried out through chemical color tests and TLC method. The result of the chemical color test can be seen in Table 1. The okra fruit fraction positive for flavonoids was only present in the diethyl ether and ethyl acetate fractions, meaning that the type of flavonoids in okra fruit is a glycoside which tends to be polar so it was only found in both solvents and was not present in the n-hexane fraction which tends to be nonpolar.

Table 1. Results of the color reaction of Okra flavonoids (*Abelmoschus esculentus* L.)

Reaction	Fraction		
	n-hexane	Diethyl Ether	Ethyl Acetate
NaOH	green	yellow	yellow
Wilstater	green	orange	orange
Bate Smite-Metcalfe	green	red	red
TLC mobile phase n-butanol: acetic acid: water (4:1:5) and stationary phase silica gel GF 254	No stains	Yellow stain Rf 0.65	Yellow stain Rf 0.62

According to the results of this study, the ethyl acetate fraction yeilds nearly twice as much flavonoid as the ether fraction. The okra fruit's ethyl acetate fraction yielded an average result of 99.3155 ± 1.4518 mgQE/g, while the assessment of the total flavonoid content of the diethyl ether fraction yielded an average result of 54.8723 ± 0.8059 mgQE/g (Table 2). According to Deghima et al., (2021) ethyl acetate, a solvent with semipolar characteristics, is more efficient in extracting phenol than ethanol, n-hexane, and aquadest. Flavonoids, tannins and simple phenolics are phenolic substances that could be found in the ethyl acetate fraction. Flavonoid isolation is only done from the ethyl acetate fraction due to the higher overall flavonoid levels in this fraction.

The isolation of flavonoid compounds from okra fruit used the preparative TLC

method. On the preparative TLC plate, three spots were obtained with an Rf value of 0.34; 0.66, and 0.92. The stains were then scraped and crystallized to obtain the yellow warrant powder of0.18% and with a melting point of 307-309.5ºC.

Table 2. Results of the color reaction of Okra flavonoids (*Abelmoschus esculentus* L.)

Sample		Weight (gram)	Absorbance	Total Flavonoid Content (mgQE/g)	Average Content ± SD (mg/g)
Diethyl ether fraction	1	0.0506	0.345	52.5039	53.8723 ± 0.8059
	2	0.0512	0.360	54.1953	
	3	0.0510	0.361	54.5618	
	4	0.0508	0.355	53.8474	
	5	0.0510	0.359	54.2529	
Ethyl acetate fraction	1	0.0505	0.636	97.9812	99.3155 ± 1.4518
	2	0.0515	0.668	100.9709	
	3	0.0510	0.650	9.1814	
	4	0.0509	0.640	97.8301	
	5	0.0509	0.658	100.6139	

The identification of the flavonoid isolate compounds was then carried out using a UV-Vis spectrophotometer to determine the absorbance value of the compound at the maximum wavelength. The UV-Vis spectrum of the okra flavonoid compound can be seen in Figure 1. The typical flavonoid spectrum consisted of 300-560 nm for band I and 230-290 for band II. The spectrum results obtained were two maximum wavelengths that occur due to the absorption of the benzoyl ring and cinnamoyl ring on the flavonoid structure. Absorption on the benzoyl ring occurs at a wavelength of 256 nm forming band II and absorption in the cinnamyl ring occurs at a wavelength of 373 forming band I.

The result identification quercetin using the TLC densitometry method then obtained 374nm quercetin wavelength while theoretically, the quercetin wavelength is 380nm. The result of the isolates identification showed an Rf value was identical to the standard quercetin when identified by TLC densitometry as seen in Figure 2. Qualitative analysis with TLC Densitometry was carried out by comparing the retardation factor (Rf) of the analyte with the standard. The compound was identical to the standard because the standard Rf value of quercetin was 0,065 and the sample Rf was 0.062 with the difference ≤0,05.

Antioxidants' ability to donate hydrogen or their radical scavenging impact on DPPH radical's activity of scavenging. When a DPPH solution is combined with a material that can donate a hydrogen atom, diphenyl picryl hydrazine is produced in its reduced form, losing its violet color (Molyneux, 2004).

The antioxidant activity test showed that the okra fruit quercetin isolate had an IC50 of 31.4 ppm while the standard antioxidant activity of quercetin had an IC50 of 24,6 ppm, which is categorized as a strong antioxidant (Table 3). The results showed that the test group had a statistically significant difference by the unpaired T-test < 0.05.

The mechanism of action of antioxidant activity is influenced by the concentration and chemical structure, especially the flavonoid group.There are several mechanisms of Quercetin as an antioxidant such as scavenging free radicals directly (Oh et al., 2019) by the mechanism of chelating metal ions from catechol groups in the structure and inhibiting lipid peroxidation (Zhang et al., 2021). The mechanism of quercetin as an antioxidant

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in vivo is by regulating glutathione levels to increase antioxidant capacity, and increasing the work of several antioxidant enzymes, such as glutathione transferase and aldo-ketoreductase (Li et al., 2016). Quercetin has a protective effect on granulosa cells by increasing the expression of several genes associated with oxidative stress (Rashidi et al., 2019). Quercetin increases the regulation of Nrf2 expression and nuclear transfer by activating the intracellular MAPK p38 pathway, increasing intracellular GSH levels, and influencing the activity of antioxidant enzymes so that it can increase the the capacity of cells to work as antioxidants (Kobori et al., 2015).

The ability of quercetin isolates to inhibit *Staphylococcus aureus* can be seen in Figure 3 and Table 4. In the well method, the result of positive control group was greater than the concentration of the quercetin isolate. The greater the clear zone produced, the greater the antibacterial power produced. The results of the T-test showed $p< 0.05$ indicating that the test group had a statistically significant difference with the unpaired T-test. However, the 10% concentration was not significantly different from the positive control.

The results of the test for the inhibition of quercetin isolates on *Candida albicans* can be seen in Figure 4 and Table 5. The results of the unpaired T-test showed $p< 0.05$ meaning that the test group had a statistically significant difference. It showed that the quercetin isolates were found at various concentrations with different abilities to inhibit the *Candida albicans*.

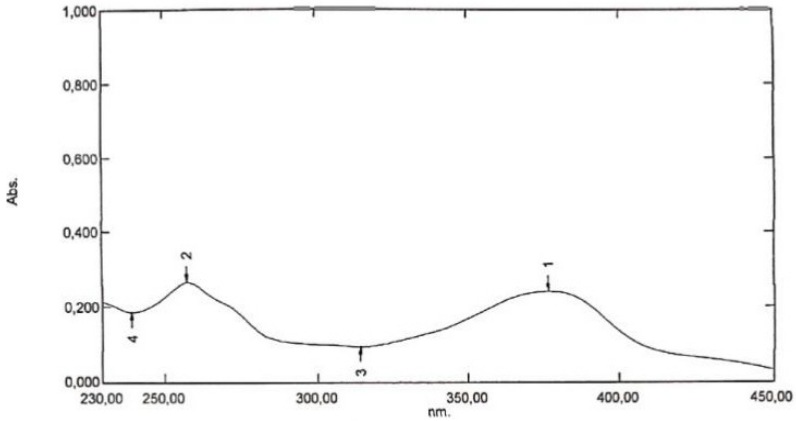

Figure 1. Spectrum of Okra (*Abelmoschus esculentus* L.) Flavonoid Isolate with UV-Vis Spectrophotometry

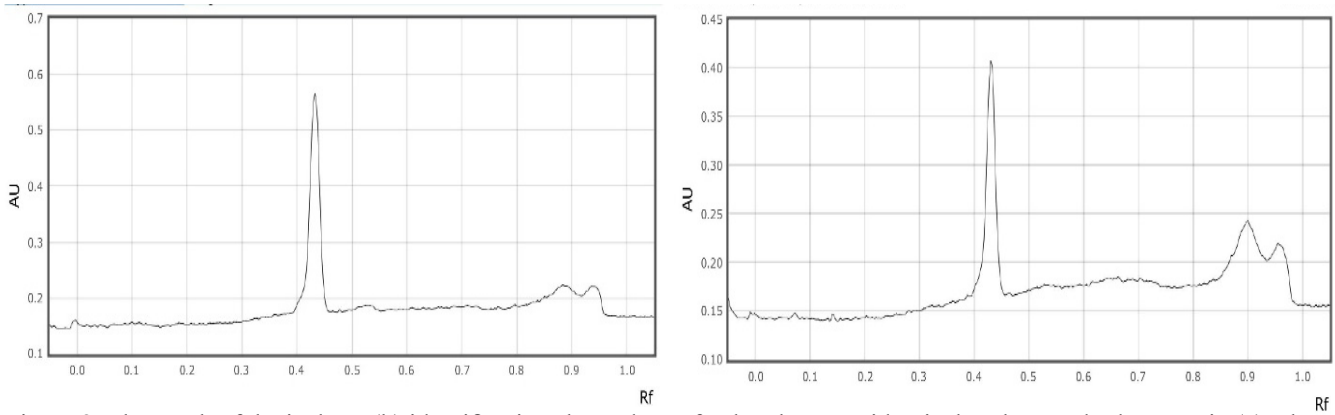

Figure 2. The result of the isolates (b) identification showed an Rf value that was identical to the standard quercetin (a) when identified by TLC densitometry

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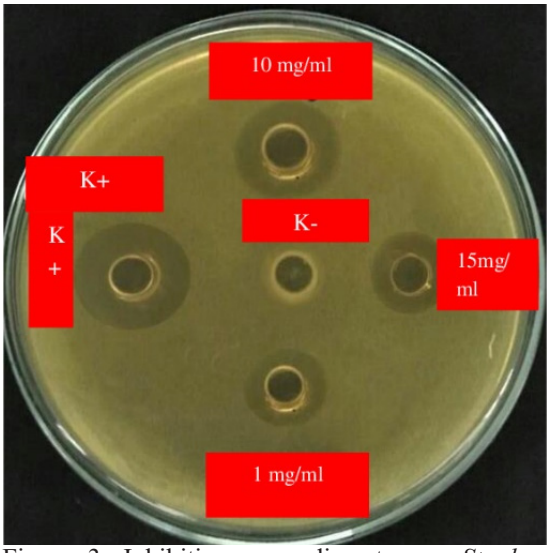

Figure 3. Inhibition zone diameter on *Staphylococcus aureus*. Description 1%, 5% and 10%: concentration of isolates; K+: 0.05% ciprofloxacin positive control; K-: DMSO negative control

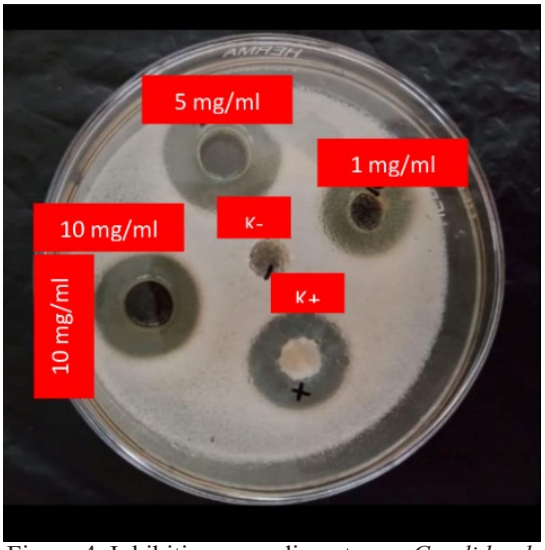

Figure 4. Inhibition zone diameter on *Candida albicans*. Description 1%, 5% and 10%: isolate concentration; K+: 0.05% nystatin positive control; K-: DMSO negative control

Table 3. Quercetin antioxidant activity and flavonoid isolate Okra fruit

Concentration (ppm)	Absorbance	% Inhibition	Linear Regretion	IC50
Standard				
108.0	0.143	75.98		
91.0	0.157	68.12		
55.4	0.176	59.87	Y = 0.234x + 3.658	24.6 ppm
25.0	0.199	51.54		
12.5	0.218	49.92		
Isolate				
115.4	0.192	79.14		
89.0	0.211	68.22		
59.0	0.263	57.33	Y = 0.016x + 3.4879	31.4 ppm
28.3	0.287	49.15		
10.2	0.301	44.11		

Table 4. Diameter of *Staphylococcus aureus* antibacteri zone of inhibition

Replication	Inhibition Zone Diameter (mm)				
	Quercetin Isolate			Control	
	1%	5%	10%	K+	K-
1	10.89	13.15	15.81	15.21	0.00
2	10.06	14.50	14.33	16.91	0.00
3	10.44	13.94	15.43	15.46	0.00
4	10.29	14.22	15.29	15.93	0.00
5	10.18	14.01	16.10	15.76	0.00
Average	10.372*	13.964*	15.392	15.854	0.000*

Description: *significantly different

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Table 5. Diameter of *Candida albicans* antifungal zone of inhibition

Replication	Inhibition Zone Diameter (mm)				
	Quercetin Isolate			Control	
	1%	5%	10%	K+	K-
1	9.54	10.32	11.54	12.96	0.00
2	9.06	10.66	10.76	13.49	0.00
3	8.12	10.49	11.19	13.98	0.00
4	8.82	9.76	11.87	12.88	0.00
5	9.98	10.19	11.54	13.73	0.00
Average	9.104*	10.284*	11.380*	13.408*	0.000*

Description: *significantly different

Flavonoids have greater inhibitory activity against gram-positive bacteria, including Staphylococcus aureus. It is because flavonoid compounds are part of the polar nature so it is easier to penetrate the polar peptidoglycan layer than the non-polar lipid layer, thus it may cause greater inhibitory activity of gram-positive bacteria than the gram-negative bacteria. The inhibitory activity of flavonoids in gram-positive bacteria causes the disruption of the cell wall as a function to give cell shape and protects cells from osmotic lysis. Disruption of the cell wall may cause cell lysis (Nguyen & Bhattacharya, 2022). The effect of quercetin compounds on *C. albicans* is by denaturing protein bonds in the cell membrane so that the cell membrane becomes lysed and these flavonoid compounds can penetrate into the cell nucleus. The entry of quercetin compounds into the cell nucleus can prevent the growth of *C. albicans*. The flavonoid group can act as an antifungal because it has phenols that can denature proteins and can damage the cell membranes irreversibly (Balouiri et al., 2016).

Lin et al. (2020) reported that flavonoids significantly inhibited the development of *S. aureus* and noted changes in the activity of genes involved in metabolic regulation and the processing of genetic information. Polyphenols were discovered to decrease the activities of ribosomal proteins, degrade nucleic acid synthesis, and inhibit bacterial topoisomerases (Baikar, 2010). It was discovered that flavonoids might increase *S. aureus* membranes' permeability to ions (Lin et al, 2020). It is possible that flavonoid interactions with the protein component of the biological (bacterial) membrane are the cause of the distinct effects of flavonoids on the fluidity of the surface area of cellular and liposomal membranes. The liposomal membrane rigidification effect and the antimicrobial action of flavonoids were previously discovered to be significantly correlated, and it was hypothesized that the flavonoids worked to inhibit bacterial growth by decreasing membrane fluidity (Wu et, al., 2013).

The hydroxyl group in the quercetin compound causes changes in organic components and nutrient transport that lead to toxicity to microbes. The mechanism of action of flavonoids as an antibacterial is by inhibiting cell membrane function and energy metabolism. These groups can interact with a bacterial cell membrane to alter its composition, resulting in the loss of biological components. It has been shown that these OH groups have the ability to interfere with the metabolism of microorganisms by acting at the active site of enzymes. Additionally, it has been discovered in certain research that the length of the saturated side chain and the position of the OH group in the aromatic ring of polyphenols can

both boost the antibacterial action (Admczak et al., 2019). According to Xie et al. (2017), the structure flavonoids, quercetin, rutin, and naringenin's antibacterial properties are caused by two hydroxyl substituents on C-5 and C-7 of ring A.

Because they contain phenol groups, flavonoids are a class of substances known as antioxidants that have antibacterial and antifungal properties. Proteins can also clump together when exposed to phenolic flavonoids, which can likewise lower the surface tension of microbial cells (Chang et al., 2013). In opposition to extracellular proteins that disrupt the integrity of membranes and cell walls, flavonoids can create complex molecules. Additionally, flavonoids can disrupt cellular metabolism by preventing nutrient delivery. Since flavonoids' lipophilic nature breaks microbial membranes, *C. albicans* is gradually inhibited (Hirai et al., 2010). Phenols are proteins' denaturing agents and fungistatic substances. Fungal cell walls will become brittle due to the denaturation of the proteins in them, making them more easily permeable by other fungistatic active chemicals. If the denatured protein is an enzyme protein, it cannot function, which will affect metabolism and nutrition absorption.

CONCLUSION

Based on the results of the study, flavonoid compounds have been isolated and identified from okra fruit, namely the type of quercetin and the isolate of quercetin compounds had strong antioxidant and the antimicrobial.

AUTHOR CONTRIBUTION

C.A, J.K and W. L. A conceived and designed the research , J.K and W. L.A conducted the explorations, collected and analyzed the data, interpreted the result. C.A conducted the explorations and wrote the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest.

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