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Original Research Article

Antibacterial, Antioxidant, and Cytotoxic Flavonoid Compound from *Sterculia Quadrifida* Leaves

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ABSTRACT

Sterculia quadrifida is empirically used as a medicinal plant. The leaves of the plant are reported to have anti-infective, antioxidant, and anti-cancer activities. This study aims to identify active compounds from the plant leaves by bioassay-guided isolation. *S. quadrifida* leaves were extracted with 80% methanol, then partitioned with n-hexane, chloroform, ethyl acetate, n-butanol, and insoluble n-butanol fractions by liquid-liquid partition. Purification of compounds was done using preparative HPLC, identification and structure elucidation were by comprehensive spectroscopic analyses. The *in vitro* antioxidant assay of the isolated compound was investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), nitric oxide (NO), and hydrogen peroxide (H_2O_2) radical scavenging methods. The *in vitro* antibacterial assay was done using the microdilution method. The *in vitro* cytotoxic effect of the compound was investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The isolation results from the n-butanol fraction found a new aurone compound named (2E)-2-[(3,4-dihydroxy phenyl)(hydroxy)methylidene]-4,6-dihydroxy-2,3-dihydro-1-benzofuran-3-one. The antioxidant assay results showed IC_{50} values of 46.36, 38.41, 37.85, and 20.50 µg/mL in the DPPH, ABTS, NO, and H_2O_2 assays, respectively. The antibacterial assay results showed IC_{50} values of 23.38, 26.22, 58.45, 100.92, 103.14, and 193.98 µg/mL against *P. aureginosa*, *H. pylori*, *S. bovis*, *S. aureus*, *S. thypi*, *E. coli*, respectively. The cytotoxicity assay showed IC_{50} values of 4.05, 12.53, 15.38, and 25.91 µg/mL against breast cancer cell lines (4T1, MCF7, MDA-MB-435, and T47D). The aurone compound could be developed as a potential antibacterial, antioxidant, and cytotoxic agent.

***Keywords*:** *Sterculia quadrifida*, aurone, antibacterial, antioxidant, cytotoxic.

Introduction

The genus Sterculia generally grows in the tropics and sub-tropics.[1] the genus had many secondary metabolites such as alkaloids, saponins, triterpenoids, tannins, and phenolics.[2] Several species in this genus are traditionally used to treat ulcers, diarrhoea, fever, stroke, diabetes, and inflammation.[3] *Sterculia quadrifida*, a plant called "Faloak" in Indonesia, has a significant role in folk medicine, especially for infectious diseases, as anti-hepatitis, and anticancer.[4] However, studies of *S. quadrifida* plants are still limited. Only a few studies have been conducted in the last decade investigating the phytochemical content of *S. quadrifida* plants and isolating triterpenoids and anthraquinone compounds.[5,6] Some of these compounds show pharmacological effects, such as anti-hepatitis[7], immunostimulant, antioxidant, anti-cancer, and antibacterial activities.[7-9] Among all compounds, flavonoids have the potential to be used as anti-infective, antioxidant, and anti-cancer agent.[10] Therefore, this study aimed to isolate the active antibacterial, antioxidant, and anti-cancer compounds from the methanol leaves extract of *S. quadrifida* using chromatographic techniques.

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Materials and Methods

General
1D and 2D NMR data are collected on JEOL JNM-ECZR 500 MHz instruments with DMSO as internal standard. The IR spectra were recorded on Jasco FT/IR-6800 type A. LC-MS/MS data were obtained using Shimadzu LCMS-8045. Isolation of the compound was done using Sykam S 723 HPLC Preparative with ACE®-C18 column (1cm-10x250 mm), 1.5 mL/min. The TLC was carried out on precoated silica gel 60 F_{254} (Merck), then the plate was analyzed under UV light. Merck and HPLC grades solvents used were acetonitrile, methanol, n-butanol, chloroform, ethyl acetate, and n-hexane.

Plant material
Sterculia quadrifida was collected from Kupang City, East Nusa Tenggara, Indonesia in January 2020. Botanist Dr Budi Sumatra authenticated the plant samples. Voucher specimen (FA:023-MACHUNG-2020) was deposited in the Pharmacognosy Laboratory, Department of Pharmacy, Ma Chung University.

Extraction and isolation
The dried leaf powder (8.45 kg) was extracted using 32 L of 80% methanol. The crude extract was concentrated, and 798.21 g of extract was obtained. The extract was dissolved in 80% methanol and partitioned by solvent-solvent extraction using n-hexane, ethyl acetate, chloroform, and n-butanol. Each fraction was tested for antibacterial, antioxidant, and cytotoxic activity using the concept of bioassay-guided isolation. The test results showed that the n-butanol fraction was the most active. Then, the n-butanol fraction (150 g) was purified

using Preparative HPLC (MeOH:CAN; 80:20, the flow rate 1.5 mL/min, 2000 psi). The separation resulted in 14 fractions, and further purification of fraction 7 (23.4 mg) which was the major fraction yielded the pure compound. The isolated compound was subjected to spectroscopic analyses.

Antibacterial assay

The method used for the *in-vitro* antibacterial test was the microdilution method.[11] Into a 96-well plate were mixed 50 µL of Muller Hinton media, 50 µL of the test bacteria, and 100 µL of the isolated compound with concentrations of 800; 400; 200; 100; 50; 25; 12.5; and 6.25 µg/mL. For the control, 100 µL of the compound solution for each concentration was mixed with 100 µL of Muller Hinton media without bacteria. Observations were made after incubation at 37°C for 24 h. Optical density was calculated using a microplate reader instrument with measurements at wavelength of 595 nm. Percent cell viability was calculated using the following equation:

$$\%\text{Cell viability} = \frac{Asampel}{Acontrol} x100\%$$

$A_{control}$ is the absorbance of the non-treated cells and A_{sample} is the absorbance of the samples at 595 nm.

DPPH radical scavenging assay

The isolated compound (2 mg) was dissolved in methanol and made up to 2000 µL. The solution was transferred into a test tube as 10; 20; 40; 80; 120; 160; and 200 µL. Furthermore, methanol was added successively to each test tube, as much as 190; 180; 160; 120; 80; 40, and 0 µL, respectively. To each test tube was added 3.8 mL of 10 ppm DPPH. The positive control used was Trolox at concentrations of 100, 75, 50, and 25µg/mL. The mixture was allowed to stand for 30 minutes; then, the absorbance was measured with at a wavelength of 712 nm.[12] The blank used in the absorbance measurement was methanol. The DPPH radical scavenging capacity (%inhibition) was calculated as:

$$\%\text{Inhibition} = 1 - \frac{Asampel}{Acontrol} x100\%$$

ABTS radical scavenging assay

ABTS (7.1 mg) and potassium persulfate (3.5 mg) were dissolved seperately in 5 mL of ethanol. Each solution was incubated for 16 hours in a dark room. The two solutions were mixed and made up to 25 mL with ethanol. The test compound solutions were made at 50, 100, 200, 400, 600, 800, and 1000 µg/mL. Trolox (positive control) solution at concentrations of 5, 10, 20, 40, and 60 µg/mL was prepared. The ABTS solution and the test compound solution were put into a 96-well microplate at a ratio of 1:1. The absorbance of the solution was measured with a microplate reader at 734 nm.[13] Trolox as a positive control was treated the same as the test sample. The ABTS radical scavenging capacity (%inhibition) was calculated as:

$$\%\text{Inhibition} = 1 - \frac{Asampel}{Acontrol} x100\%$$

NO radical scavenging assays

The test solutions of the isolated compound were made at concentrations of 50, 100, 200, 400, 600, 800, and 1000 µg/mL. Griess reagent was prepared by mixing equal amounts of 1% sulfanilamide in 2.5% phosphoric acid and 0.1% naphthalene diamine dihydrochloride in 5% phosphoric acid immediately before use. A total of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffer solution was mixed with 1 mL of different concentrations of the fraction and isolated compound solution (50-1000 g/mL) and incubated at 25°C for 180 min. Then, the test solution was mixed with an equal volume of freshly prepared Griess reagent. A total of 150 µL of the solution was transferred to a 96-well microplate. It was allowed to stand for 5 min and measured at 546 nm.[14] The NO radical scavenging capacity (%inhibition) was calculated as:

$$\%\text{Inhibition} = 1 - \frac{Asampel}{Acontrol} x100\%$$

Peroxide assay

Hydrogen peroxide solution (40 mmol/L) was prepared using phosphate buffer (50 mmol/L, pH 7.5). Furthermore, the isolated compound solution was made at 50, 100, 200, 400, 600, 800, and 1000 µg/mL. Then, 150 µL of the isolated compound solution and 50 µL hydrogen peroxide were added to a 96-well microplate and scanned at 230 nm after 10 minutes. Finally, the control solution was prepared by mixing the phosphate buffer without the addition of hydrogen peroxide.[15] The radical scavenging capacity (%inhibition) was calculated as:

$$\%\text{Inhibition} = 1 - \frac{Asampel}{Acontrol} x100\%$$

In vitro cytotoxicity assay

The breast cancer cell lines (4T1, MCF7, MDA-MB-435, and T47D) and Vero cells were cultured using RPMI 1640 medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% fungizone. Then the cells were inoculated in 5% CO_2 incubator for 24 hours (37°C; 95% relative humidity). Then, 8×10^3 cells in 100 µL of media were inoculated into 96 well plates together with 100 µL of the isolated compound in assay solution (1, 10; 25; 50; 75; 100; and 200 g/mL), then incubated for 24 hours. After incubation, the cells were washed with PBS, then 100 µL of MTT reagent/well was added, and incubated for 4 hours at 37°C. Thereafter, 100 L of 10% SDS solution was added/well and incubated overnight at room temperature in the dark, then the absorbance was determined with microplate reader at 595 nm.[16] The data of single treatment was converted into percent viability and used to calculate IC_{50}. Percent cell viability was calculated using the following equation:

$$\%\text{Cell viability} = \frac{Asampel}{Acontrol} x100\%$$

$A_{control}$ is the absorbance of the non-treated cells and A_{sample} is the absorbance of the samples at 595 nm.

Statistical Analysis

Statistical analysis was performed by linear regression method using Microsoft Excel software.

Results and Discussion

Isolation and characterisation

The compound was isolated from the n-butanol fraction of *S. quadrifida* leaf. The compound (7.8 mg) was isolated as a yellow powder. The UV/Vis spectrophotometer analysis results showed maximum absorption at 367 nm (Figure 1), which indicated the presence of aurone structure.[17-19] The compound had the molecular formula $C_{15}H_{10}O_7$ with molecular weight of 302.24 ($M+H^+$ = 303.20 m/z) (Figure 2).

Infrared spectrum analysis showed a broad absorption with a strong intensity at 3257.18 cm^{-1}, indicating the presence of the hydroxy group. The broad absorption with a strong intensity at 3090.12 cm^{-1} indicated the presence benzene group. Then there was a sharp absorption with a strong intensity at 1660.41 cm^1, indicating carbonyl group. In addition, there was a sharp absorption with a strong intensity at 1605.45 cm^{-1}, indicating the presence of carbon double bonds originating from aromatic groups. Next, there was a sharp absorption at 1164.79 cm^{-1} with strong intensity, indicating the presence of C-O bonds. Then at 817.67 cm^{-1}, a sharp absorption with a strong intensity was found, which indicated the presence of methyl C-H bonds (Figure 3).

The results of the ^{1}H-NMR spectrum analysis of the compound showed the presence of an sp^2 methine group which is part of the benzene group at δ_H 7.673 (1H, d, J = 2, H-2"), δ_H 6.880 (1H, d, J = 2, H-5"), δ_H 7.536 (1H, dd, J = 8.5;7.5, H-6"), δ_H 6.182 (1H, d, J = 1.5, H-5"'), and δ_H 6.403 (1H, d, J = 2, H-7"'). In addition, there were

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hydroxy protons at δ_H 12.492 (1H, s, 1'-OH), δ_H 10.784 (1H, s, 4"-OH), δ_H 9.596 (1H, s, 6"'-OH), δ_H 9.368 (1H, s, 3"-OH), δ_H 9.315 (1H, s, 4"'-OH) (Table 1). Therefore, it can be concluded that the compound has ten protons (Figure 4).

The results of the ^{13}C-NMR, DEPT 135, and HSQC spectrum analysis of the compound showed the presence of fifteen carbon atoms in the fifteen signals (Figure 5). There was one carbon atom signal from a carbonyl group at δ_C 175.85 (C-3), five signal atoms from methine sp^2 from the benzene group at δ_C 115.061 (C-2"), δ_C 115.608 (C-5"), δ_C 119.976 (C-6"), δ_C 98,187 (C-5'"), δ_C 93,795 (C-7'"). Then there were nine sp^2 quaternary carbon signals at δ_C 147.706 (C-3"), δ_C 145,067 (C-4"), δ_C 160.735 (C-4'"), δ_C 163.830 (C-6'"), δ_C 135,764 (C -2), δ_C 156.134 (C-1'), δ_C 121, 953 (C-1"), δ_C 103.015 (C-3a), and δ_C 146.795 (C-7a) (Table 1).

COSY and HMBC analyses were performed to verify the structure obtained. In the COSY analysis, there was a correlation between protons H_5"-H_6" and H_5'"-H_7'", which supports the presence of benzene structure in the basic structure of aurone.[17-19] (Figure 5). HMBC analysis showed a correlation between the proton signal of methine sp^2 at δ_H 7.673 (H-2") to carbon atoms at δ_C 147.706 (C-3"), δ_C 145.067 (C-4"), and δ_C 119.976 (C-6"). Then there was a correlation between the proton signal of methine sp^2 at δ_H 6.880 (H-5") to carbon atoms at δ_C 121.953 (C-1"), δ_C 147.706 (C-3"), and δ_C 145.067 (C-4"). In addition, there was a correlation between the proton signal of methine sp^2 at δ_H 7.536 (H-6") to carbon atoms at δ_C 147.706 (C-3") and 115.608 (C-5"). The correlation between the protons of methine sp^2 with the carbon atom, indicates the presence of benzene group composed of C-1", C-2", C-3", C-4", C-5", and C-6". There was a correlation between protons at δ_H 6.182 (H-5'") to carbon at δ_C 103.615 (C-3a), δ_C 160.735 (C-4'"), δ_C 163.830 (C-6'"), and δ_C 93.359 (C-7'") which indicates the benzene group. Then there was a correlation between protons at δ_H 6.403 (H-7'") to the carbon at δ_C 156.134 (C-1'), δ_C 103.015 (C-3a), δ_C 175.850 (C-3), δ_C 98.187 (C-5" '), and δ_C 163.830 (C-6'") which indicates the presence of heterocyclic benzene with carbonyl group at the C-3 position. In addition, there was correlation between the proton of the hydroxy group at δ_H 12.492 (1'-OH) to the carbon atom at δ_C 103,015 (C-3a), δ_C 160.735 (C-4'"), δ_C 98,187 (C-5'"), and δ_C 163.830 (C-6'") which indicates the presence of hydroxy group attached to carbon atom at the C-1' position (Figure 6). The results of the NOESY analysis showed that structurally the isolated compound did not correlate with the spaces of each proton it possessed. This could be due to the protons in the compound structure being perpendicular to the plane.[20] From the spectral information, the structure of the compound was elucidated as (2E) 2-[(3,4 dihydroxyphenyl)(hydroxy)methylidene]-4,6-dihydroxy-2,3-dihydro-1 benzofuran-3-one.

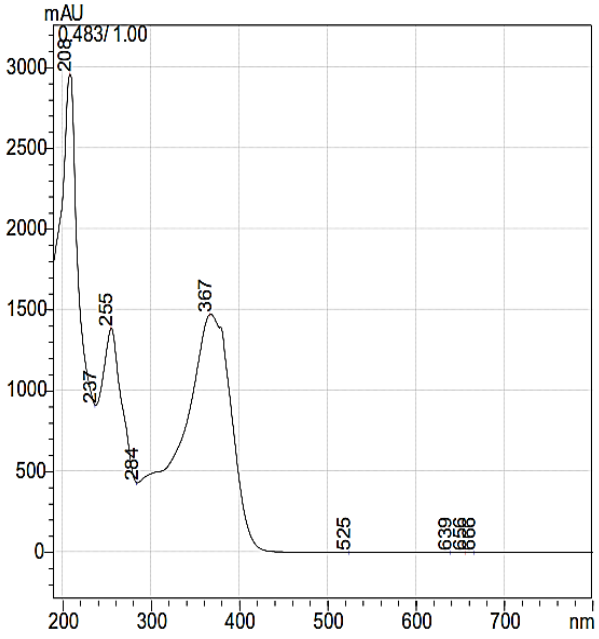

Figure 1: UV/Vis spectra of compound in methanol *In vitro antioxidant assay*

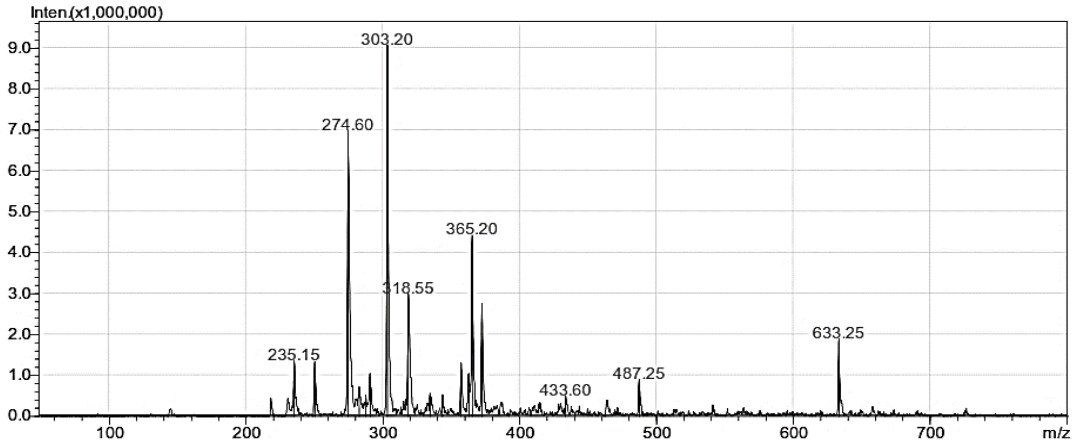

Figure 2: Mass spectrometry with Q1 scan mode (E+) of compound

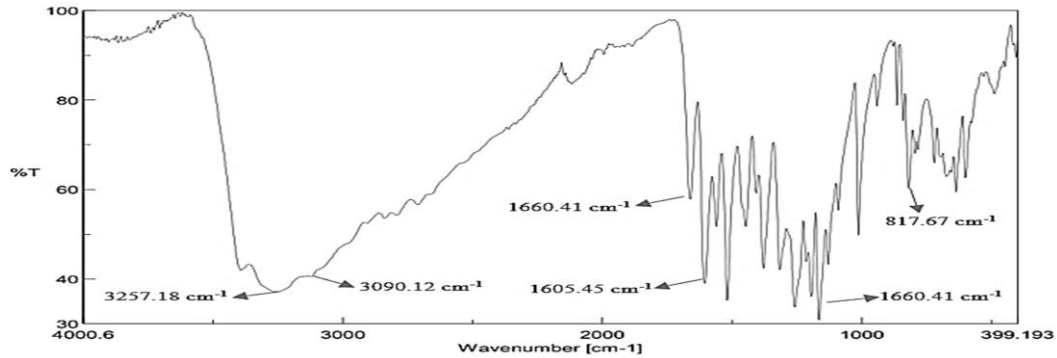

Figure 3: FT-IR spectrum of compound

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Table 1: ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), DEPT 135, COSY, HSQC, HMBC, NOESY spectrum data of compound

Position	δ_C (Multiplicity)	DEPT 135	δ_H (ΣH, Multiplicity, J=Hz)	COSY	HSQC	HMBC	NOESY
2	135.764 (s)	-	-	-	-	-	-
3	175.850 (s)	-	-	-	-	-	-
1’	156.134 (s)	-	-	-	-	-	-
1”	121.953 (s)	-	-	-	-	-	-
2”	115.061 (s)	115.062	7.673 (1H, d, 2)	-	C-2”	C-3”, C-4”, C-6”	-
3”	147.706 (s)	-	-	-	-	-	-
4”	145.067 (s)	-	-	-	-	-	-
5”	115.608 (s)	115.608	6.880 (1H, d, 8,5)	H-6”	C-5”	C-1”, C-3”, C-4”	-
6”	119.976 (s)	119.976	7.536 (1H, dd, 8,5;7,5)	H-5”	C-6”	C-3”, C-5”	-
3a	103.015 (s)	-	-	-	-	-	-
4”’	160.735 (s)	-	-	-	-	-	-
5”’	98.187 (s)	98.187	6.182 (1H, d, 1,5)	H-7”’	C-5”’	C-3a, C-4”’, C-6”’, C-7”’	-
6”’	163.830 (s)	-	-	-	-	-	-
7”’	93.359 (s)	93.359	6.403 (1H, d, 2)	H-5”’	C-7”’	C-1’, C-3a, C-3, C-5”’, C-6”’	-
7a	146.795 (s)	-	-	-	-	-	-
1’-OH	-	-	12.492 (1H, s)	-	-	C-3a, C-4”’, C-5”’, C-6”’	-
3”-OH	-	-	9.368 (1H, s)	-	-	-	-
4”-OH	-	-	10.784 (1H, s)	-	-	-	-
4”’-OH	-	-	9.315 (1H, s)	-	-	-	-
6”’-OH	-	-	9.596 (1H, s)	-	-	-	-

In vitro antioxidant assay
The test results showed that the compound had radical scavenging activity in the range of 20.50–46.36 µg/mL. Trolox as a positive control had strong radical scavenging activity (3.11–8.12 µg/mL). The test results using the DPPH method showed that the compound had an IC_{50} value of 46.36 µg/mL. Then, the test results using the ABTS method showed an IC_{50} value of 38.41 µg/mL. In addition, the test results using the NO method showed an IC_{50} value of 37.85 µg/mL. Finally, with the H_2O_2 assay, the IC_{50} was 20.50 µg/mL (Table 2).

In vitro antibacterial assay
The results of antibacterial testing showed that the compound had the highest inhibitory activity on *P. aureginosa* with an IC_{50} of 23.38 µg/mL, followed by *H. pylori* (26.22 µg/mL), *S. bovis* (58.45 µg/mL), *S. aureus* (100.92 µg/mL), *S. thypi* (103.14 µg/mL), and *E. coli* (193.98 µg/mL). The positive control of streptomycin showed a range of IC_{50} values in the test bacteria between 5.22-23.78 µg/mL (Table 3).

In vitro cytotoxicity assay
Test results on breast cancer cell lines showed that the compound had the highest activity in inhibiting 4T1 cells with an IC_{50} value of 4.05 µg/mL. Then the isolated compounds were able to inhibit the growth of MCF7 cells with an IC_{50} of 12.53 µg/mL, then MDA-MB-435 cells (15.38 µg/mL) and T47D cells (25.91 µg/mL). In addition, the selectivity index of the test compound showed that the compound had SI values in 4T1 cells of 10.68, MCF cells (3.45), MDA-MB-435 cells (2.81), and T47D cells (1.67).
Flavonoid compounds have been reported to have antioxidant activity. First, Deng *et al*.[21] reported four flavonoid compounds namely; maringin, rhoifolin3-hydroxy-3-methylglutaryl and melitidine from the *Citrus grandis* L. Osbeck plant with high radical scavenging activity (Table 4).

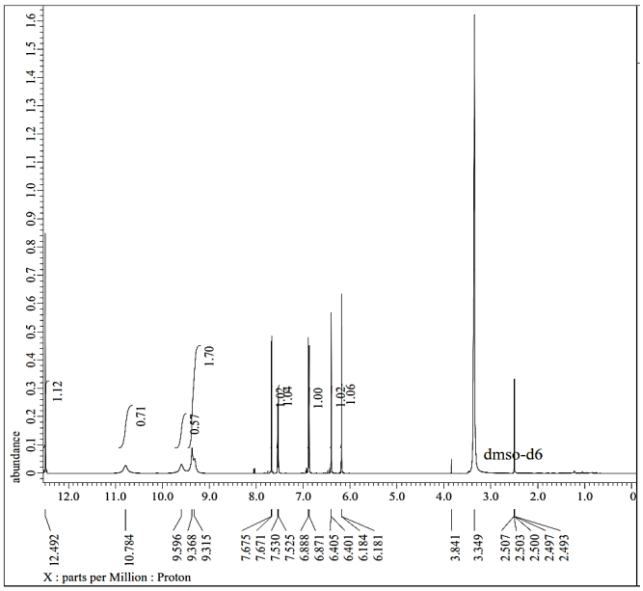

Figure 4: ^{1}H-NMR spectrum of compound (δ 0.000 – 12.000) (500 MHz)

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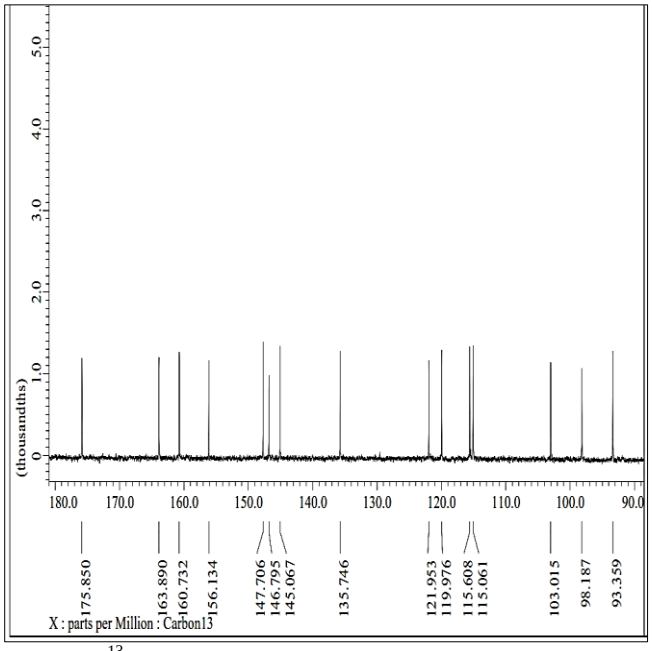

Figure 5: ^{13}C-NMR spectrum of compound (δ 120 – 200) (125 MHz)

Then, Owe *et al*.[22] in their research, reported that flavonoid compounds, namely; luteolin and apigenin in brined olive drupes, were found to have IC_{50} values of 2.34 and 3.54 µg/mL, respectively. In addition, oppong *et al*.[23] first reported two flavonoid glycosides from the plant *Sterculia lychnophora* with DPPH and ABTS radical scavenging activities with IC_{50} of 27.39 µg/mL and 3.23 µg/mL, respectively.

Each compound has different abilities and mechanisms in inhibiting bacterial growth.[24] For example, it was reported that aurone group of compounds, namely; aurantiamide acetate and tiliroside from the *Gomphrena agrestis* plant, could inhibit *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* bacteria.[25] Specifically, the research reported by Wu *et al*.[26] stated that quercetin and apigenin, which belong to the flavonoid group, can inhibit D-ala-D-ala ligase.

Compounds from the Sterculia family have been found to have antibacterial activity. For example, compounds from the phenylpropanoid group, namely; cinnamic acid, p-methoxy-cinnamic acid, 1,6-*O*-dicinnamoyl-glucose, p-coumaric acid, cis-p-coumaric acid-glucoside, trans-ferulic acid-glucoside, and 1,6-diferuloyl glucose were found in the leaves and bark of the plant *Sterculia foetida*, and were shown to inhibit the growth of the bacteria *S. aureus*, *S. thypi*, *E. coli* and *P. aeruginosa*.[27] Then compounds of the flavonoid group such as apigenin, apigenin 7-*O*-*β*-D-glucoside, apigenin 7-*O*-*β*-D-glucuronide, 6-hydroxyapigenin (scutellarein) from *Sterculia alata* leaves, and *Sterculia foetida* leaves inhibited the growth of *S. aureus* and *E. coli*.[1] Furthermore, phenolic derivatives such as p-hydroxybenzoic acid, 2,4-dihydroxy-benzoic acid, 3,4-dihydroxy-benzoic acid, 4-hydroxy-3,5-dimethoxy-benzoic acid 4-*O*-*β*-D-glucopyranosyl, and 4-hydroxy-3,5-dimethoxy benzaldehyde isolated from the leaves of *Sterculia foetida*, were shown to have antibacterial activity against *E. coli*, *S. thypi*, *S. aureus*, *P. aeruginosa*, and *S. bovis*.[1]

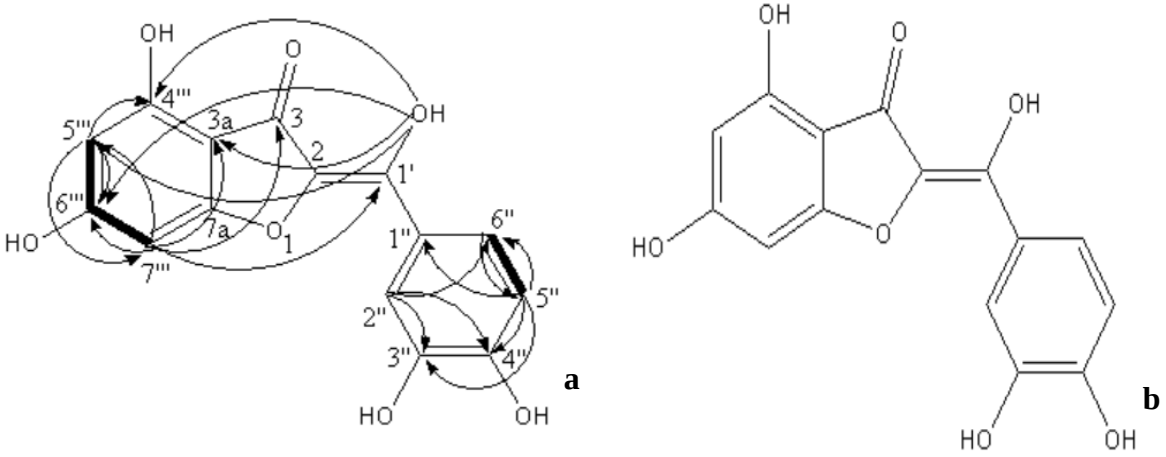

Figure 6: Correlation of COSY (⟵) and HMBC (↷) of compound (a), chemical structure of compound (b) isolated from n-butanol fraction of *S. quadrifida* leaves

The aurone is a compound resulting from the combined biosynthesis of the shikimate and acetate pathways.[28] The aurone compound has been widely studied and its activity proven as an anticancer compound. Huang *et al*.[29] in their research, isolated the Hamilton compound from the plant *Uvaria hamiltonii*, which was shown to inhibit the growth of MCF-7 and T47D breast cancer cells by increasing the expression of the p53 gene. In addition, researchers from Japan isolated a new aurone compound, isoaurostatin, produced by the fungus *Thermomonospora alba*, which could inhibit the development of MCF-7 and 4T1 breast cancer cells by inhibiting the topoisomerase enzyme.[32] Aurone compounds that have anticancer activity are not only found from the isolation process but can also be produced from chemical synthesis with raw materials or semi-synthetically from the basic structure of aurone compounds. Kumar *et al*.[28] in their research, reported the synthesis of eight aurone-derived compounds that have cytotoxic activity against MCF-7 breast cancer cells. Hassan *et al*.[31] in their research, synthesized furoaurones, (Z)-2-benzylidenefurano[3,2-f]benzofuran-3(2H)-ones which were proven to have cytotoxic activity against T47D breast cancer cells with an ATP-dependent enzyme inhibition mechanism.

Aurone compounds in several studies have also been shown to have anticancer activity against breast cancer cells. For example, research conducted by Huang *et al*.[33] reported the isolation of the compound isoaurone 4′,6-Dihydroxy-4-methoxyisoaurone from the seeds of the plant *Trichosanthes kirilowii*. These compounds were shown to inhibit the growth of T47D and MCF-7 breast cancer cells by inhibiting Cyclin-dependent kinases (CDKs). Proof of aurone compounds as anticancer is not only obtained from the

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isolation process from natural materials. It can also be obtained from chemical synthesis. Kafle *et al.*[34] in their research claim to be the first research group to carry out the total synthesis of aurone glycosides. The compound was tested on 4T1 breast cancer cells and reported to be very active with an IC_{50} value of 2.34 µM and has a mechanism of inhibiting cyclin D1 and cyclin E.

Table 2: Antioxidant activity of compound and positive control

Method	IC_{50} (µg/mL)	
	Isolated compound	Trolox
DPPH	46.36	3.11
ABTS	38.41	4.21
NO	37.85	5.92
H_2O_2	20.50	8.12

Table 3: Antibacterial activity of compound and positive control

Microorganisms	IC_{50} (µg/mL)	
	Isolated compound	Streptomycin
E. coli	193.98	5.22
S. aureus	100.92	7.89
P. aureginosa	23.38	8.98
S. thypi	103.14	9.87
S. bovis	58.45	18.12
H. pylori	26.22	23.78

Table 4: Cytotoxic activity of compound

Cells Lines	IC_{50} (µg/mL)	Selectivity Index
4T1	4.05	10.68
MDA-MB-435	15.38	2.81
T47D	25.91	1.67
MCF7	12.53	3.45
Vero	43.27	-

Conclusion

S. quadrifida is empirically beneficial as traditional medicine, especially the leaves. Therefore, *S. quadrifida* leaves were fractionated using the concept of bioassay-guided isolation. From the structural determination process, it can be concluded that the isolated compound has the name (2E)-2-[(3,4 dihydroxyphenyl)(hydroxy)methylidene]-4,6-dihydroxy-2,3-dihydro-1 benzofuran-3-one, which is a compound of the aurone class. This compound have antioxidant, antibacterial, and anti-cancer activities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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