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Original Research Article

A Phenylpropanoid Compound from the Seeds of *Sterculia quadrifida* and its Cytotoxic Activity

Rollando Rollando[1,2]*, Eva Monica[1], Muhammad Hilmi Afthoni[1], Warsito Warsito[2], Masruri Masruri[2], Nashi Widodo[3]

[1]*Pharmacy Department, Faculty of Science and Technology, Ma Chung University, Malang 65151, Indonesia*
[2]*Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang 65145, Indonesia*
[3]*Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang 65145, Indonesia*

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ABSTRACT

Sterculia quadrifida has been empirically used as a traditional plant for medicinal purposes. The objective of this research is to isolate secondary metabolites present in the seeds of *S. quadrifida*. Extracts of *S. quadrifida* seeds were prepared using 80% methanol, and then separated into various fractions using liquid-liquid partition with solvents such as n-hexane, chloroform, ethyl acetate, n-butanol, and an insoluble n-butanol fraction. Preparative HPLC was used to purify the compounds, and their structures were identified through spectroscopic analyses. One phenylpropanoid compound, (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, was isolated from the chloroform fraction and tested for in vitro cytotoxicity using the MTT assay. The compound demonstrated significant cytotoxic effects, with IC_{50} values of 2.29, 9.93, 18.09, and 12.12 µg/mL in 4T1, MCF-7, MDA-MB-435, and T47D breast cancer cell lines, respectively. Therefore, the phenylpropanoid compound isolated from *S. quadrifida* has potential as a cytotoxic agent.

***Keywords*:** Breast cancer, cytotoxic, phenylpropanoid, seeds, *Sterculia quadrifida*

Introduction

Sterculia quadrifida is a tree native to Southeast Asia and Australia.[1] It is commonly known as the Chinese star anise or *wu wei zi*. The tree can grow up to 20 meters tall and has large, glossy leaves with small, greenish-yellow flowers that bloom in spring.[2] Its fruits are woody capsules containing four to six seeds surrounded by a fleshy, reddish-brown aril with a sweet and sour flavour.[3] The tree has a long history of use in traditional Chinese medicine, where its fruits and seeds have been used to treat various ailments, including cough, asthma, and digestive disorders. It is also a flavouring agent in foods and beverages and in several traditional Chinese medicine preparations. The tree's wood is used for carving and making furniture, and its bark is used to make paper.[4]

S. quadrifida seeds contain various compounds, including flavonoids, alkaloids, terpenoids, and steroids.[5] Several studies have identified and isolated bioactive compounds from *S. quadrifida* seeds, such as sterculic acid, cycloart-25-ene-3*β*,24-diol, sterculinaldehyde, stigmast-5-en-3-ol, and *β*-sitosterol.[6] These compounds have demonstrated various biological activities, including anti-inflammatory, antidiabetic, antioxidant, and cytotoxic properties. Moreover, recent studies have shown that *S. quadrifida* seeds contain potent cytotoxic compounds, which have the potential to inhibit the growth of cancer cells. For example, a study conducted by Rukayadi *et al*.[7] identified a novel cytotoxic compound, namely 3,3',5,5'-tetramethoxybiphenyl-2,2'-diol, from the methanol extract of *S. quadrifida* seeds. The compound exhibited cytotoxic activity against various cancer cell lines, including human breast adenocarcinoma (MCF-7), human colon adenocarcinoma (HT-29), and human lung adenocarcinoma (A549) cells.

*Corresponding author. E mail: ro.llando@machung.ac.id
Tel: +6282220379864

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Materials and methods

General

A JEOL JNM-ECZR 500 MHz instrument was used to collect 1D and 2D NMR data, with DMSO as the internal standard. The IR spectra were recorded using a Jasco FT/IR-6800 type A instrument. LC-MS/MS data was obtained from a Shimadzu LCMS-8045. The compound was isolated using a Sykam S 723 HPLC Preparative system with an ACE®-C18 column (10x250 mm) at a flow rate of 1.5 mL/min. TLC was performed on a precoated silica gel 60 F_{254} (Merck) plate, which was then analyzed under UV light. Acetonitrile, methanol, n-butanol, chloroform, ethyl acetate, and n-hexane of Merck and HPLC grades were used as solvents.

Plant material

In January 2023, *S. quadrifida* plant samples were collected from Kupang City, East Nusa Tenggara, Indonesia. The plant samples were authenticated by botanist Dr Budi Sumatra, and a voucher specimen (FA:032-MACHUNG-2023) was deposited in the Pharmacognosy Laboratory of the Department of Pharmacy at Ma Chung University.

Extraction and isolation

To extract the compounds from the *S. quadrifida* seeds, 5.50 kg of the seed powder was macerated in 20 L of 80% methanol. After removal of solvent, 893.93 g of crude extract was obtained. The extract was then dissolved in 80% methanol and separated into fractions by solvent-solvent extraction using n-hexane, ethyl acetate, chloroform, and n-butanol. The fractions were screened for cytotoxic activity using the bioassay-guided isolation method. It was found that the chloroform fraction was the most potent. Further purification of the chloroform fraction (250 g) was carried out using Preparative HPLC with a flow rate of 1.0 mL/min and 2000 psi, using a mixture of MeOH and ACN in a ratio of 90:10. This resulted in the separation of 10 compounds, with fraction 1 (20.98 mg) being the most abundant. Fraction 1 was selected for chemical structure determination and further tested for its cytotoxic activity.[8]

In vitro cytotoxicity assay

Four different types of cancer cells (4T1, MCF7, MDA-MB-435, T47D) and normal Vero cells were grown in culture media containing

various ingredients. Then, the cells were placed in an incubator with controlled temperature, humidity, and CO_2 levels. After 24 hours, a small number of cells were placed in each well of a 96-well plate along with different concentrations of the isolated compound dissolved in a solution used for the assay.[9] The plate was incubated again for 24 hours.[10] After incubation, the cells were washed with a solution and a reagent was added to each well. The plate was then incubated again, and finally, the absorbance was measured using a microplate reader. The data was used to calculate the concentration of the compound needed to reduce the viability of the cells by 50% (IC_{50}).[11] The viability of the cells was calculated as a percentage using a formula:

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

$A_{control}$ is the absorbance of the non-treated cells, A_{medium} is the absorbance of medium, 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 exists as a yellow powder with a weight of 24.4 mg. Analysis using a UV/Vis spectrophotometer revealed a maximum absorbance at a wavelength of 334 nm, indicating the presence of a diphenylpentadienone group.[12] The compound has a molecular formula of $C_{17}H_{14}O$ with a molecular weight of 234.29 ($M+H^+$= 235.20 m/z; $M+Na^+$= 257.00 m/z) (Figure 1).

The infrared spectrum analysis of the compound revealed two sharp bands with weak intensity at wave numbers of 3052.76 and 3025.76 cm^{-1}, indicating the presence of benzene groups. The sharp and strong absorption vibration at a wave number of 1648.84 cm^{-1} indicated the presence of a carbonyl group. Additionally, a sharp vibration with strong intensity at a wave number of 1588.09 cm^{-1} indicated the presence of a double bond carbon from an aromatic compound.[13] Furthermore, two sharp vibrations with strong intensity at wave numbers 848.525 and 757.888 cm^{-1} indicated the presence of C-H bending bonds (Figure 2).

The results of the ^{1}H-NMR spectrum analysis for compound indicate the presence of sp^2 methine groups outside the benzene ring at δ_H 7.366 (2H, d, *J*=16.5 Hz, H-2 and H-4) and δ_H 7.805 (2H, d, *J*=16.5 Hz, H-3 and H-5).(14) In addition, sp^2 methine groups are part of the benzene ring at δ_H 7.461 (1H, m, H-2', H-6', H-2", and H-6"), δ_H 7.789 (1H, m, H-4' and H-4"), and δ_H 7.810 (1H, m, H-3', H-5', H-3" and H-5") (Table 1). It can be concluded that the compound has fourteen protons, all of which consist of methine protons (Figure 3).

The results of the ^{13}C-NMR spectrum analysis (Figure 4), DEPT 135 (Table 1), and HSQC (Table 1) for the compound indicate the presence of seventeen carbon atoms in eight signals. There is one carbon atom signal from the carbonyl group at δ_C 188.539 (C-1), two signals from sp^2 methine carbon atoms of the conjugated double bond outside the benzene group at δ_C 125.677 (C-2 and C-4) and δ_C 142.792 (C-3 and C-5).(15) Then, there are two signals from sp^2 quaternary carbon atoms at δ_C 134.534 (C-1' and C-1") and three signals from sp^2 methine carbon atoms that are part of the benzene group at δ_C 128.567 (C-4' and 4"), 128.989 (C-3', C-5', C-3", and C-5"), and 130.534 (C-2', C-6', C-2", and C-6").[16]

COSY and HMBC analyses were performed to verify the proposed structure (Figure 5). The COSY analysis observed correlations between the protons H2-H3-H4, H-2'-H3', H5'-H6', H2"-H3", and H5"-H6", supporting the presence of a benzene structure and an α,β-unsaturated carbonyl (Table 1).

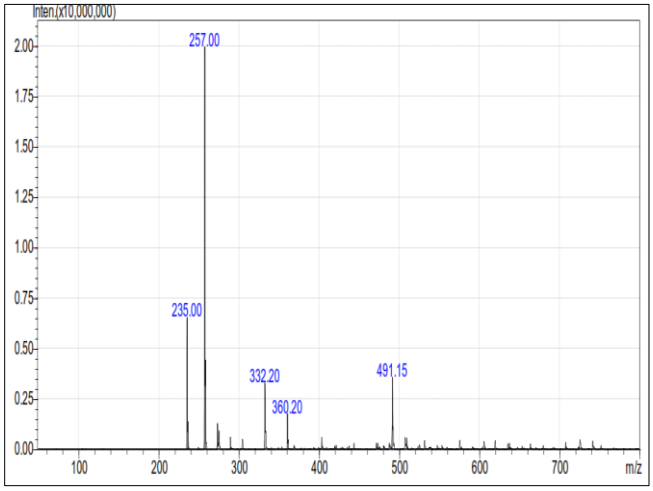

Figure 1: Mass spectrometry with Q1 scan mode (E+) of compound

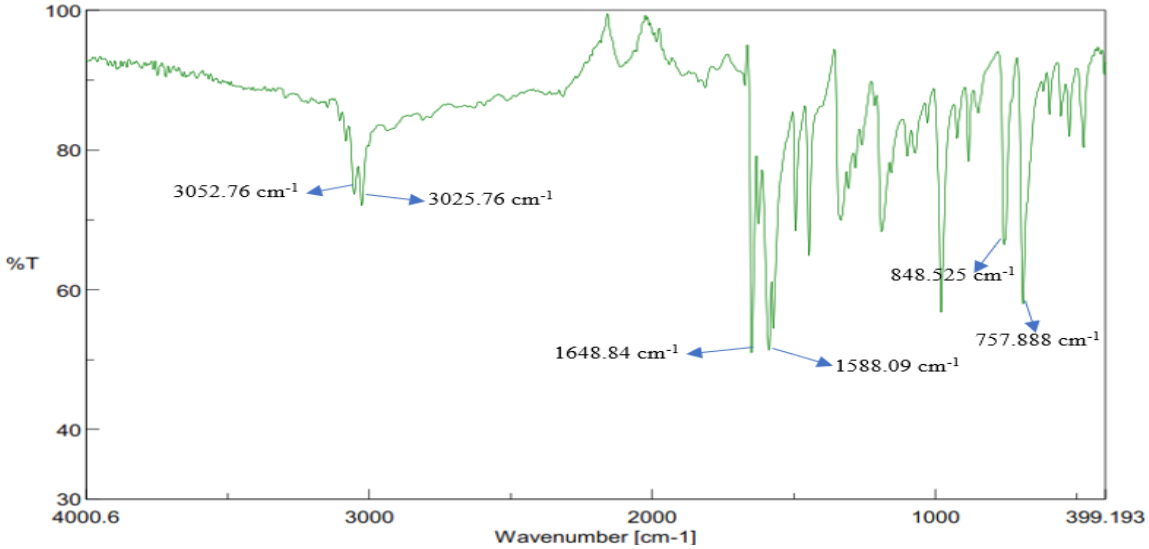

Figure 2: FT-IR spectrum of compound

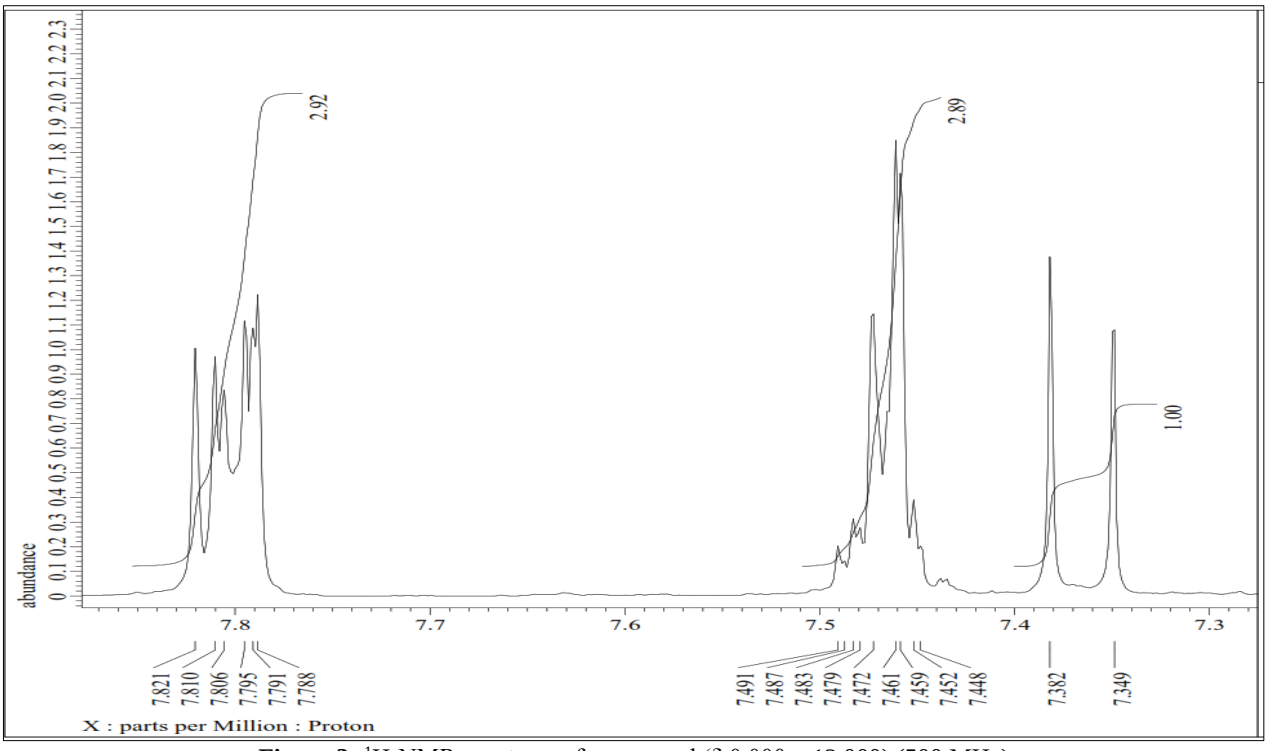

Figure 3: ^{1}H-NMR spectrum of compound (δ 0.000 – 12.000) (500 MHz)

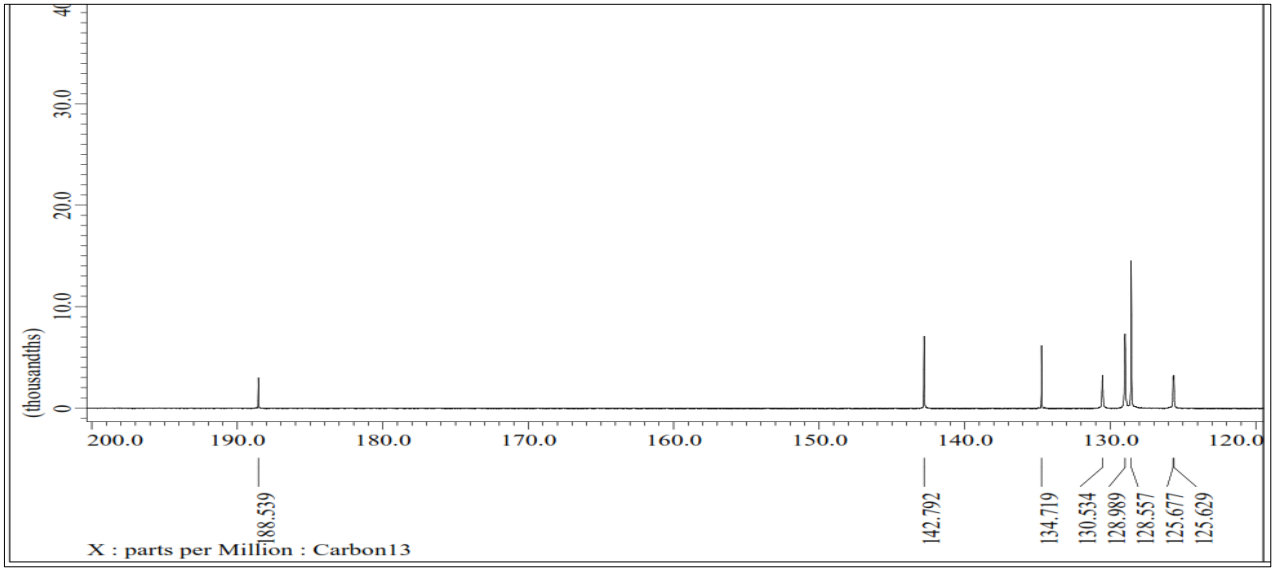

Figure 4: ^{13}C-NMR spectrum of compound (δ 120 – 200) (125 MHz)

The HMBC analysis showed correlations between the signal of the sp^2 methine proton at δ_H 7.366 (H-2 and H-4) and the carbon atoms at δ_C 188.539 (C-1) and δ_C 134.719 (C-1' and C-1"), indicating the location of the α,β-unsaturated carbonyl and conjugated double bonds at positions C-1, C-2, C-3, C-4, and C-5.[17] The correlations between the signal of the sp^2 methine proton at δ_H 7.461 (H-2', H-6', H-2", and H-6") and the carbon atoms at δ_C 134.719 (C-1' and C-1"), δ_C 128.989 (C-3' and C-3"), δ_C 128.557 (C-4' and C-4"), and δ_C 128.989 (C-5' and C-5") indicated the presence of two benzene rings, each attached to C-1' and C-1" (Table 1).[18]

The relative stereochemistry of the compound's structure was determined using NOESY analysis (Table 1 and Figure 6). The correlation between protons in space consists of a correlation between protons on the same side (alpha) and a correlation between protons on opposite sides (beta).[19] The analysis showed a correlation between protons at δ_H 7.366 (H-2/H-4) and protons at δ_H 7.805 (H-3/H-5) with an alpha orientation. This indicates that H-2/H-4 protons are oriented alpha, and H-3/H-5 protons are oriented beta. In addition, there is a correlation between protons at δ_H 7.461 (H-2'/H-6'/H-2"/H-6") to protons at δ_H 7.789 (H-2/H-4) with a beta orientation, indicating that H-2'/H-6'/H-2"/H-6" protons are oriented beta and H-2/H-4 protons are oriented alpha (Figure 6). From the process of determining information in the structure elucidation, it can be concluded that the compound is named (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one (Figure 5).

In vitro cytotoxicity assay

The study results revealed that the tested compound exhibited notable activity against breast cancer cell lines. Specifically, the compound exhibited the strongest inhibitory activity against 4T1 cells, with an IC_{50} value of 2.29 µg/mL. Additionally, the isolated compound demonstrated inhibition of MCF-7, T47D and MDA-MB-435 cells with IC_{50} values of 9.93, 12.12, and 18.09 µg/mL, respectively. Furthermore,

the selectivity index of the tested compound indicated that it exhibited SI values of 21.11, 2.67, 2.67, and 3.98 against 4T1, MCF-7, MDA-MB-435, and T47D breast cancer cell lines, respectively (Table 2).

The compound belongs to the group of phenylpropanoid compounds with a six-carbon, aromatic phenyl group and a three-carbon propene tail of coumaric acid. Phenylpropanoid compounds are produced from the shikimate pathway with the precursor of L-phenylalanine/L-tyrosine.[20] Several studies have shown phenylpropanoid compounds have anticancer activity through the induction of apoptosis. Hematpoor *et al.*[21] found two phenylpropanoid compounds, asaricin, and isoasarone, isolated from the roots of *Piper sarmentosum* that had cytotoxic activity against breast cancer cells of the MDA-MB-231 type. Both compounds can induce apoptosis by inhibiting the expression of the antiapoptotic protein Bcl-2 and inducing the proapoptotic protein Bax. Furthermore, Qi *et al.*[22] discovered four new phenylpropanoid compounds isolated from *Leptopus lolonum*, namely 3*β*-*O*-(*trans*-p-coumaroyl)-norlupane-17*β*,20-diol, 3*β*-*O*-(*trans*-p-coumaroyl)-lupane-20,28-diol, 3*β*-O-(*trans*-p-coumaroyl)-20-ol-betulinic acid, and 24-*O*-(*trans*-p-feruloyl)-3*β*-hydroxyl-olean-12-en-28-oic acid. The four compounds inhibited the growth of MCF-7 breast cancer cells by inducing apoptosis through the mitogen-activated protein kinase (MAPK) and Ak strain transforming (Akt) pathways.[23] Additionally, 1′S-1′-acetoxyeugenol acetate, isolated from the rhizome of *Alpinia conchigera* Griff, inhibited the growth of MCF-7 breast cancer cells by inducing apoptosis through the mechanism of inhibiting the expression of Bcl-2 protein and inducing Bax protein.[24]

Phenylpropanoid compound, which has been found to inhibit breast cancer cell migration in several studies. Researchers from China, Tian *et al.*[25], reported that valtrate compound from *Valeriana jatamansi* plant could inhibit the migration of MDA-MB-231 breast cancer cells by inhibiting the expression of matrix metalloproteinase-2/9 (MMP2) and MMP9 through the downregulation of p-Akt (Ser 473), cyclin B1, and caspase 8, and upregulation of p21, p-cdc2, cleaved-caspase 3, cleaved-caspase 7, and poly (ADP-ribose) polymerase (PARP).[26] Moreover, Vo *et al.*[27] have isolated phenylpropanoid glycoside compounds, baimaside and isoverbascoside, from *Staurogyne concinnula* plant, which have been shown to inhibit the expression of MMP2 and MMP9. Furthermore, they have also isolated new phenylpropanoid compounds, (7S,8R)-1-(1-ethoxy-2-hydroxypropyl)-2-methoxy-3,4-(methylenedioxy)benzene, (7S,8S)-1-(1-ethoxy-2-hydroxypropyl)-2-methoxy-3,4-(methylenedioxy)benzene, and (7S,8R)-1-(1-methoxy-2-hydroxypropyl)-2-methoxy-3,4-(methylenedioxy)benzene from *Chloranthus anhuiensis* plant, which have been shown to inhibit the expression of MMP9.

Conclusion

Conclusion

S. quadrifida is traditionally used as a medicinal plant, particularly its seeds, and therefore, bioassay-guided fractionation was employed to isolate its bioactive constituents. The structural elucidation of the isolated compound revealed its identity as (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, which belongs to the phenylpropanoid class of compounds. This compound exhibits anticancer properties.

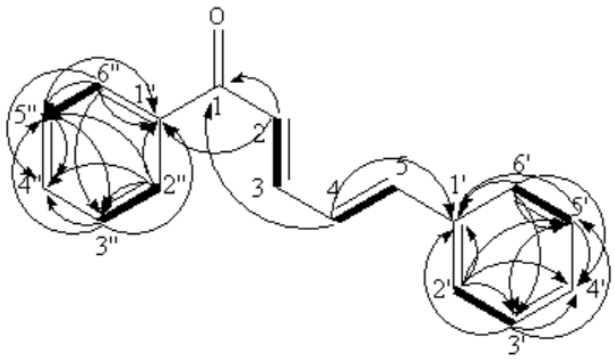

Figure 5: Correlation of COSY (▬) and HMBC (⌒) of compound

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

Position	δ_C (Multiplicity)	DEPT 135	δ_H (ΣH, Multiplicity, J=Hz)	COSY	HSQC	HMBC	NOESY
1	188.539 (s)	-	-	-	-	-	-
2	125.677 (d)	125.677	7.366 (1H, d, 16.5)	H-3	C-2	C-1, C-1”	H-3
3	142.792 (s)	142.792	7.805 (1H, d, 16.5)	H-2	C-3	-	H-2
4	125.677 (d)	125.677	7.366 (1H, d, 16.5)	H-3	C-4	C-1,C-1’	H-3
5	142.792 (s)	142.792	7.805 (1H, d, 16.5)	H-4	C-5	-	H-4
1’	134.719 (s)	-	-	-	-	-	-
2’	130.534 (s)	130.534	7.461 (1H, m)	H-3’	-	C-1’, C-3’ C-4’, C-5’	H-4
3’	128.989 (s)	128.989	7.810 (1H, m)	H-2’	C-3’	C-1’, C-4’, C-5’	-
4’	128.577 (s)	128.577	7.789 (1H, m)	-	C-4’	-	-
5’	128.989 (s)	128.989	7.810 (1H, m)	H-6’	C-5’	C-1’, C-3’, C-4’	-
6’	130.534 (s)	130.534	7.461 (1H, m)	H-5’	-	C-1’, C-3’, C-4’,C-5’	H-4
1’’	134.719 (s)	-	-	-	-	-	-
2”	130.534 (s)	130.534	7.461 (1H, m)	H-3”	-	C-1”, C-3”, C-4”, C-5”	H-2
3”	128.989 (s)	128.989	7.810 (1H, m)	H-2”	C-3”	C-1”, C-4”, C-5”	-
4”	128.577 (s)	128.577	7.789 (1H, m)	-	C-4”	-	-
5”	128.989 (s)	128.989	7.810 (1H, m)	H-6”	C-5”	C1”, C-3”, C-4”	-
6”	130.534 (s)	130.534	7.461 (1H, m)	H-5”	-	C-1”, C-3”, C-4”, C-5”	H-2

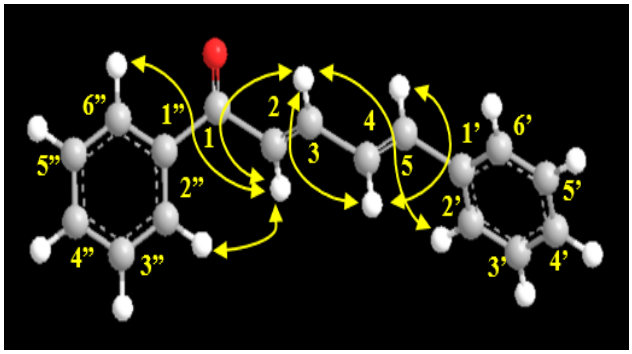

Figure 6: Correlation NOESY of compound

Table 2: Cytotoxic activity of compound

Cells Lines	IC_{50} (µg/mL)	Selectivity Index
4T1	2.29	21.11
MDA-MB-435	18.09	2.67
T47D	12.12	3.98
MCF-7	9.93	4.87
Vero	48.34	-

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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References

1. Rollando R, Monica E, Aftoni MH. *In vitro* Cytotoxic Potential of *Sterculia quadrifida* Leaf Extract Against Human Breast Cancer Cell Lines: doi.org/10.26538/tjnpr/v6i8.12. Trop J Nat Prod Res TJNPR. 2022;6(8):1228–32.
2. Rollando R, Warsito W, Masruri M, Widodo W. Potential Therapeutic Use of *Sterculia quadrifida* R.Br and *Sterculia foetida* Linn.: Review. Asian J Plant Sci. 2020;19(4):325–34.
3. Rollando R, Warsito W, Masruri M, Widodo N. Potential matrix metalloproteinase-9 inhibitor of aurone compound isolated from *Sterculia quadrifida* leaves: *In-vitro* and *in-silico* studies. Res J Pharm Technol. 2022;15(11):5250–4.
4. Rollando R, Warsito W, Masruri M, Nashi W. Antibacterial, Antioxidant, and Cytotoxic Flavonoid Compound from *Sterculia Quadrifida* Leaves. Trop J Nat Prod Res. 2021;5(11):1979–85.
5. Rollando R, Engracia M, Monica E, Siswadi S. Immunomodulatory Activity Test of Syrup Dosage Form of Combination *Phyllantus niruri* Linn. and *Sterculia quadrifida* R.Br. Extract. Int J Res Pharm Sci. 2020;11(1):191–9.
6. Akter K, Barnes EC, Brophy JJ, Harrington D, Community Elders Y, Vemulpad SR, Busra RA. Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia. 2016, Evi-B Comp and Al Med. 2016;11(2):189-192.
7. Siswadi S, Saragih GS, Rianawati H, Umroni A, Pujiono E, Setyowati R. Faloak (*Sterculia quadrifida* R.Br.) bark harvesting for curing hepatitis in Kupang City: Herbal medicine in the urban environment. IOP Conf Ser Earth Environ Sci. 2021;918(1):012014.
8. Iskandar D, Widodo N, Warsito W, Masruri M, Rollando R, Warsidah W, Antang, YPP. Proposed Functional Activity of Bioactive Compounds from *Spatholobus littoralis* Hassk in LC-MS-MS and Silico Studies. Mater Sci Forum. 2022;1061:181–6.
9. Hariono M, Nuwarda RF, Yusuf M, Rollando R, Jenie RI, Al-Najjar B, Julianus J, Putra KC, Nugroho ES, Wisnumurti YK, Dewa SP, Jati BW, Tiara R, Ramadani RD, Qodria L, Wahab HA. Arylamide as Potential Selective Inhibitor for Matrix Metalloproteinase 9 (MMP9): Design, Synthesis, Biological Evaluation, and Molecular Modeling. J Chem Inf Model. 2020;60(1):349–59.
10. Hariono M, Rollando R, Karamoy J, Hariyono P, Atmono M, Djohan M, Wiwy W, Nuwarda R, Kurniawan C, Salin N, Wahab H. Bioguided Fractionation of Local Plants against Matrix Metalloproteinase9 and Its Cytotoxicity against Breast Cancer Cell Models: In Silico and In Vitro Study. Mol Basel Switz. 2020;25(20).
11. Hariono M, Rollando R, Yoga I, Harjono A, Suryodanindro A, Yanuar M, Gonzaga T, Parabang Z, Hariyono P, Febriansah R, Hermawansyah A, Setyani W, Wahab H. Bioguided Fractionation of Local Plants against Matrix Metalloproteinase9 and Its Cytotoxicity against Breast Cancer Cell Models: In Silico and In Vitro Study (Part II). Mol Basel Switz. 2021;26(5):1464.
12. Yao Q, Kong L, Zhang F, Tao X, Li Y. Base-Promoted Tandem Reaction towards Conjugated Dienone or Chromone Derivatives with a Cyano Group: Insertion of Alkynes into C–C σ-Bonds of 3-Oxopropanenitriles. Adv Synth Catal. 2017;359(18):3079–84.
13. Fadana Y, Dinana IA, Srihardyastutie A, Rollando R, Masruri M. Screening Indonesian Pine (*Pinus merkusii* Jungh at de Vriese) Compound as an Antibacterial Agent: In Vitro and In Silico Study. Trop J Nat Prod Res TJNPR. 2023;7(3):2586–95.
14. Mathew B, Oh JM, Abdelgawad MA, Khames A, Ghoneim MM, Kumar S, Sahkar S. Conjugated Dienones from Differently Substituted Cinnamaldehyde as Highly Potent Monoamine Oxidase-B Inhibitors: Synthesis, Biochemistry, and Computational Chemistry. ACS Omega. 2022;7(9):8184–97.
15. Przydacz A, Kowalczyk R, Albrecht Ł. Brønsted-base-catalyzed remote cascade reactivity of 2,4-dienones – asymmetric synthesis of tetrahydrothiophenes. Org Biomol Chem. 2017;15(45):9566–9.
16. Nejrotti S, Mannu A, Blangetti M, Baldino S, Fin A, Prandi C. Optimization of Nazarov Cyclization of 2,4-Dimethyl-1,5-diphenylpenta-1,4-dien-3-one in Deep Eutectic Solvents by a Design of Experiments Approach. Molecules. 2020;25(23):5726.
17. Zhang S, Neumann H, Beller M. Synthesis of α,β-unsaturated carbonyl compounds by carbonylation reactions. Chem Soc Rev. 2020;49(10):3187–210.
18. Postolache R, Pérez JM, Castiñeira Reis M, Ge L, Sinnema EG, Harutyunyan SR. Manganese(I)-Catalyzed Asymmetric Hydrophosphination of α,β-Unsaturated Carbonyl Derivatives. Org Lett. 2023;25(10):1611–5.
19. Budzianowski J, Nawrot J, Nowak G. NMR data for the hydroxyl groups detected by NOESY spectra in sesquiterpene lactones. Data Brief. 2019;25:104246.
20. Parthasarathy A, Cross PJ, Dobson RCJ, Adams LE, Savka MA, Hudson AO. A Three-Ring Circus: Metabolism of the

Three Proteogenic Aromatic Amino Acids and Their Role in the Health of Plants and Animals. Front Mol Biosci. 2018;5(12):29-34.
21. Rollando R, Yuniati Y, Monica E. Bioactive Potential of Cephalosporium sp. a Fungal Endophyte Isolated from Phyllanthus niruri L. Trop J Nat Prod Res TJNPR. 2023;7(4):2749–55.
22. Qi SZ, Zhang XX, Jin Y, Wang M, Long LP, Jing WH, Prita RA. Phenylpropanoid-conjugated pentacyclic triterpenoids from the whole plants of *Leptopus lolonum* induced cell apoptosis via MAPK and Akt pathways in human hepatocellular carcinoma cells. Bioorganic Chem. 2021;111:104886.
23. Meng DF, Guo LL, Peng LX, Zheng LS, Xie P, Mei Y, Sai HP. Antioxidants suppress radiation-induced apoptosis via inhibiting MAPK pathway in nasopharyngeal carcinoma cells. Biochem Biophys Res Commun. 2020;527(3):770–7.
24. Rollando R, Maulada F, Afthoni MH, Monica E, Yuniati Y, Nugraha AT. Screening *Carica Papaya* Compounds as an Antimalarial Agent: In Silico Study. Trop J Nat Prod Res TJNPR. 2023;7(5):2895–903.
25. Tian S, Wang Z, Wu Z, Wei Y, Yang B, Lou S. Valtrate from *Valeriana jatamansi* Jones induces apoptosis and inhibits migration of human breast cancer cells in vitro. Nat Prod Res. 2020;34(18):2660–3.
26. Astuti P, Rollando R, Wahyuono S, Nurrochmad A. Antimicrobial activities of isoprene compounds produced by an endophytic fungus isolated from the leaves of *Coleus amboinicus* Lour. J Pharm Pharmacogn Res. 2020;8(4):280–9.
27. Vo TH, Lin YC, Liaw CC, Pan WP, Cheng JJ, Lee CK, Fung KH. Triterpene glycosides and phenylpropane derivatives from *Staurogyne concinnula* possessing anti-angiogenic activity. Phytochemistry. 2021 ;184:112666.