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Morelloflavone and Molecular Docking from Stembark of *Chisocheton lasiocarpus* and Its Cytotoxic Activity Against Breast Cancer Mcf-7 Cell Lines

Nurlelasari[1]*, Desi Harneti[1]., Rani Maharani[1,2], Darwati[1], Tri Mayanti[1], Kindi Farabi[1], Muhammad Hanafi[3], Unang Supratman[1,2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia
[2]Central Laboratory, Universitas Padjadjaran, Jatinangor 45363, Indonesia
[3]Pusat Riset Kimia BRIN, Jl. Raya Puspiptek, Serpong, Kota Tangerang Selatan, Banten, Indonesia

*Corresponding author email: nurlelasari@unpad.ac.id

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ABSTRACT. *Chisocheton* is a plant of the Meliaceae family which has been known as a source of limonoids, triterpenoids, steroids, alkaloids, and phenolics. This plant is the second largest in the Meliaceae, with 53 species widely distributed in tropical and subtropical regions including Indonesia. From this genus, compounds that have interesting activities have been found, including anti-inflammatory, antimalarial, cytotoxic, antitumor, and anticancer. One of the species that has the potential to discover new compounds is *Chisocheton lasiocarpus* because there are still few phytochemical studies. This study aims to obtain compounds that are active against MCF-7 breast cancer cells using the insilico method against ER-α receptors. The morelloflavone compound was isolated from the bark of C. *lasiocarpus* using extraction and partitioning techniques which were then separated using column chromatography using the eluent *n*-hexane: dichloromethane: ethyl acetate (2:3:5). The pure isolates were then tested for their potential anticancer activity against MCF-7 cells and in silico testing for ER-α receptors (PDB code: 3ERD), ER -β (PDB code: 1QKM) and HER-2 (PDB code: 3PP0).

Keywords: *Chisocheton lasiocarpus*, cytotoxic, ER-α, ER-β, HER-2, morelloflavone.

INTRODUCTION

Meliaceae has approximately 53 genera and 650 species. This family has been known as plants that produce various secondary metabolites and have interesting activities (Hidayat et al., 2018; Hilmayanti, et al., 2022; Laino et al., 2021; Nurlelasari et al., 2021). One of the genera from this family plant is *Chisocheton* which is widely distributed mainly in the tropic such as Malaysia, Thailand, Filipina, India, China, Papua New Guinea, Nepal, Myanmar, and Indonesia (Shilpi et al., 2016). *Chisocheton* plants traditionally have been used as a traditional medicine to treat several ailments including stomachache, backache, kidney complaints, fever, rheumatism, and malaria (Chan et al., 2012; Wang et al., 2021; Zhang et al., 2012).

It has been widely reported that the *Chisocheton* plants can produce compounds that have activities as anticancer, and cytotoxic (Katja et al., 2017; Nagoor et al., 2011; Nurlelasari et al., 2017; Retnowati et al., 2021; Salam et al., 2021), antitumor (Inada et al., 1993; Rahmayanti et al., 2021), anti-inflammatory (Hilmayanti et al., 2022), antimalarial (Nugroho et al., 2021; Nurlelasari et al., 2021) and antimicrobial (Sari et al., 2022). Based on phytochemical studies of the *Chisocheton* plants containing compounds including limonoids (Chong et al., 2019; Fitriana et al., 2021; Hilmayanti, et al., 2022; Katja et al., 2016; Nurlelasari et al., 2017, 2021; Rahmayanti et al., 2021; Salam et al., 2021; Supratman et al., 2020; Tambunan et al., 2021), terpenoids (Retnowati et al., 2021; Sari et al., 2020; Supratman et al., 2019), steroids (Katja et al., 2017; Sari et al., 2022; Sari et al., 2020), phenolic (Katja et al., 2017; Nurlelasari et al., 2021), and flavonoids (Fajar et al., 2021; Hidayat et al., 2018).

One of the species whose phytochemical studies are still rare is *Chisocheton lasiocarpus*. This plant has been used by the community in food processing and is used as traditional medicine. C. *lasiocarpus* whose leaves are used to wrap sago and other foods in cooking (Hilmayanti et al., 2022; Katja et al., 2023; Zhang et al., 2012), and in one study its leaves and twigs extract showed strong inhibition as antifeedant activity against *Peridroma saucia* cutworms (Hilmayanti et al., 2022; Mabberley et al., 1995; Shilpi et al., 2016).

In previous paper, a compound was found from stembark of C. *lasiocarpus* as a lignan derivative named lasiocarpone (Hidayat et al., 2018) which has

activity against MCF-7 breast cancer cells and also steroids, stigma-4-en-3-on (Nurlelasari et al., 2017). From the seeds, a new limonoid compound has been obtained, namely lasiocarpines and one known limonoid, 14β,15β-epoxynimonol (Fitriana et al., 2021), toonaciliaton F, 6α-(acetoxy)-14β,15β-epoxyazadirone, dysobinin, 7α-acetylneotricilenon, 6α-O-acetyl-7-deacetylnimosinol, and 7α-hydroxy-neotricilenon from the fruit C. *lasiocarpus* (Hilmayanti et al., 022; Katja et al., 2023).

This study aims to obtain a new compound from C. *lasiocarpus* which then tests its potential cytotoxic activity against MCF-7 breast cancer cells *in vitro* and *in silico*. Anticancer research is urgently needed to date because cancer treatment has many shortcomings because it not only kills cancer/tumor cells but also normal cells, and chemotherapy treatment can make cancer cells resistant to chemotherapeutic agents (Chang & Singh, 2017; Deng et al., 2021; Thrane et al., 2013).

Meanwhile, the International Agency for Research on Cancer stated that the number of cancer prevalence in 2020 was 13,587,202 cases of cancer in all genders and all ages, with 9,958,133 deaths. One type of cancer, namely breast cancer, occupies the first position in the number of prevalence in women, namely 2,261,419 cases and is the first highest cause of death in women with 684,996 cases. These numbers are predicted to increase every year (Sung et al., 2021). So that the search for natural compounds that have cytotoxic properties against cancer cells, one of which is breast cancer is very necessary.

In this paper, the isolation, structure determination, and cytotoxic activity of the flavanone-(3→8″)-flavone biflavonoid (morelloflavone) and their inhibition of the target protein ER-α (PDB code: 3ERD), ER-β (PDB code: 1QKM), and HER-2 (PDB code: 3PP0) were described. In this experiment, doxorubicin was used as a positive control. Morelloflavone were first discovered in this species. The chemical structure of morelloflavone can be shown in **Figure 1**.

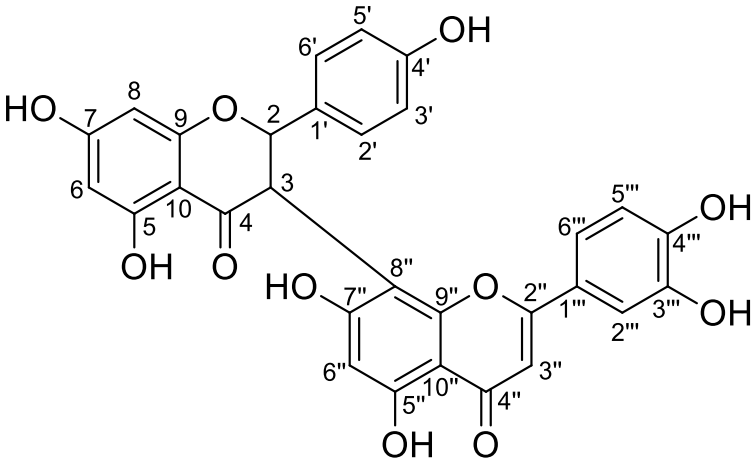

Figure 1. Chemical structure of compound 1

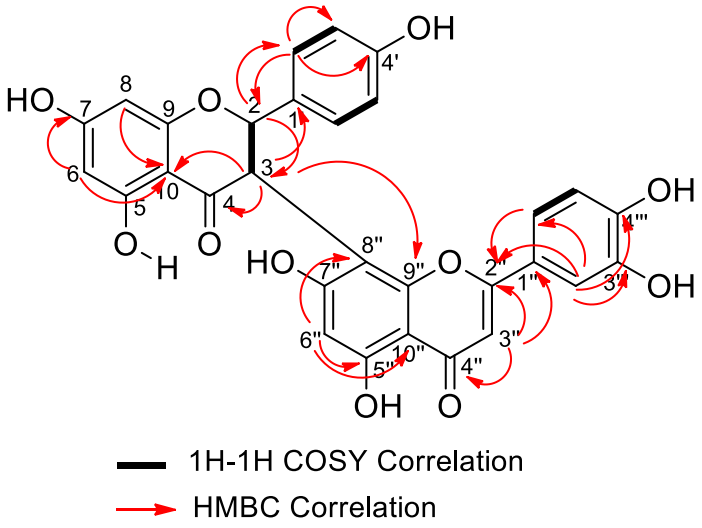

Figure 2. 1H–1H COSY and key HMBC correlations

EXPERIMENTAL SECTION

Materials

The stembark of *C. lasiocarpus* were collected from Bogor Botanical Garden, Indonesia. Plant identifications were made from Bogoriense Herbarium, Indonesia and voucher specimen (VII. G. 168). The material for in silico we used three target protein: ER-α (PDB code: 3ERD), ER-β (PDB code: 1QKM) and HER-2 (PDB code: 3PP0) was obtained from Protein Data Bank (https://www.rcsb.org/, accesed on 12 January 2022). The tasted ligand is morelloflavone from *C. lasiocarpus* stembark (Compound **1**, ID: 5464454) and ligan as positive control are genistein (ID 5280961) and doxorubicin (ID 31703) was obtained from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/).

Instrumentation

NMR spectra of ^{1}H, ^{13}C, ^{1}H-^{1}H COSY and HMBC were obtained using a Bruker AM 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). Separations and identification were conducted with liquid chromatograph by Merck Si gel 60 (230-400 mesh), and thin-layer chromatograph (TLC) on aluminum plates coated with Merck Si gel 60 F_{254} and thickness of 0.25 mm, stain was observed on UV light and heated on the hotplate after spraying with 10% H_2SO_4 in ethanol.

Extraction and Isolation

Dry powder of 1.7 kg sample was macerated with *n*-hexane, ethyl acetate and methanol subsequently each for 3 days. The extract of ethyl acetate (28.0 g) was vacuum liquid chromatographed (VCL) using silica gel as adsorbent and eluted with *n*-hexane: ethyl acetate: methanol (1:0:0~0:0:1) stepwise 10% to yield ten fraction (A-J). Fraction H (785.4 mg) was column chromatographed using silica gel G60 (230-400 mesh) with eluent *n*-hexane:ethyl acetate (4:6) and produced six subfractions (H1-H6). Subfraction H3 (115,2 mg) was further separated by column chromatography using silica gel G60 (230-400 mesh) with eluent *n*-hexane: dichloromethane: ethyl acetate (2:3:5) to afford compound **1** (26 mg). The purity of compound was tested by TLC analysis using various solvent system.

Cytotoxic Assay

The cytotoxic assay compound **1** against breast cancer MCF-7 cell lines was determined by the method of Haryanti and Widiyastuti (2017). MCF-7 cells was incubated using EMEM medium containing 10% FBS and 100 μg/mL penicillin-streptomycin in incubator with 5% CO_2 condition at 37 ºC for 48 hours. The cell suspension was transferred into all wells on microplate with density of 8000 cells/well in EMEM medium then incubated for 48 hours. Furthermore, Samples with various concentration was measured by transferred into well on the microplate containing the incubated cell, then re-incubated for 48 hours. After that, 100 μL 0,5% MTT salt in EMEM medium was added to each well and incubated at 37 ºC for 3 hours until the purple crystal formazan was seen, thus the crystal was deluted for overnight by 10% SDS in 0.01 N HCl as a stopper reagent. Furthermore, the colored formazan that has been produced was measured its absorbance using ELISA reader at wavelength of 595 nm. The IC_{50} value were taken from plotted graph of the percentage of live cells compared to the control (%).

Methods for Docking

Prepared of receptor and ligan.

Three target protein (ER-α, ER-β and HER 2) had prepared to remove water molecule and prepared between receptor and native ligand by Biovia Discovery Studio 2016 Client® and saved in PDB format (Aertgeerts et al., 2011; Pike et al., 1999; Shiau et al., 1998), this can be seen in the **Table 1** . Morelloflavone (**1**), doxorubicin A and genistein as a ligand test was obtained from PubChem (SDF format) and converted to PDB format using Chem3D Pro 12. Receptor has prepared with Autodock Tolls 4.2 by adding kollman charge and hydrogen polar only. Meanwhile, all ligand had prepared with Autodock Tolls 4.2 by adding Gasteiger charge, hydrogen and marge non-polar. Receptor and ligan was saved in pdbqt format (Desdiani et al., 2020; Kelutur & Mustarichie, 2020).

Validation method.

The docking process used Autodock Tools 4.2, grid box parameter (**Table 1**) obtained had used for ligand test and genetic algorithm parameter which had set only number of GA 100 (100x). Its aimed to find the best position or conformation when receptor and ligan bind. The validation result that must be obtained is the RMSD value of redocking ≤ 3Å compared to the crystallographic results (Desdiani et al., 2020; Kelutur & Mustarichie, 2020).

Docking ligand test and receptor.

The docking process used Autodock Tools 4.2 and carried out like validation. The molecular docking results obtained free energy bond (ΔG). Visualization of orientation ligand in 2D and 3D bond macromolecules using Biovia Studio 2016 Client® (Desdiani et al., 2020; Kelutur & Mustarichie, 2020).

Table 1. Coordinate of central grid point of ER-α, ER-β and HER 2

Protein	PDB ID	Grid Box Dimension	Coordinates of Central Grid Point (x; y; z)
Estrogen Receptor Alpha (ER-α)	3ERD		45.704; 12.631; -14.553
Estrogen receptor beta (ER-β)	1QKM	90x90x90	38.591; 24.773; 43.464
HER-2	3PP0		10.209; -5.706; 14.700

RESULTS AND DISCUSSION

The compound obtained, compound **1** (**Figure 1**), is a pale-yellow solid, was found to have a molecular formula of $C_{30}H_{20}O_{11}$. However, in the ^{1}H NMR spectrum recorded in acetone-d_6 at room temperature, the major peaks were accompanied by corresponding less intense peaks with close chemical shifts, indicated that the compound presented as two conformers. In solution, the ^{1}H and ^{13}C NMR signals of the major conformer were unequivocally assigned and listed in **Table 2** since the minor conformer signals were generally of low intensity. The ^{1}H NMR spectrum of compound **1** showed an oxymethine proton at δ_H 5.86 (H-2) and methine proton at 4.99 (H-3) with the same coupling constant of 12.06 Hz and correlation in ^{1}H-^{1}H COSY indicated the presence of flavanone unit. Also ^{1}H NMR spectrum revealed *p*-disubstituted phenyl moiety at δ_H 7.24 (2H, d, *J*= 8.3 Hz, H-2′, H-6′) and 6.53 (2H, d, *J*= 8.3 Hz, H-3′, H-5′) and there is a singlet signal at δ_H 6,02 for 2H (H-6 and H-8) on meta position of ring A flavanone. The data also indicate the presence of flavone unit by showed signals due to 1,3,4-trisubstituted phenyl moiety, as supported by the hydrogen signals at δ_H 7.50 (1H, s, H-2′′′), 7,02 (1H, d, *J*=8.4 Hz, H-5′′′) and 7.52 (1H, d, *J*=8.4 Hz, H-6′′′). Additionally, two unsaturated methane protons were determinded by signals at δ_H 6.47 (1H, H-3′′) and 6.30 (1H, H-6′′) were observed in the ^{1}H NMR spectrum.

The ^{13}C NMR spectrum of compound **1** (**Table 2**) revealed resonances of 30 carbons that could be attributes to two saturated methane, eleven unsaturated methane, seventeen quaternary carbons including two carbon carbonyl based on DEPT spectrum. The above observations indicated that compound **1** should be a biflavonoid analog

Table 2. Comparison of ^{1}H NMR and ^{13}C NMR values of compound **1** and morelloflavone (Jamila et al., 2014)

	Morelloflavone (Acetone-d6) (300 MHz)		Compound 1 (Acetone-d6) (500 MHz)	
	δC	δH(Multiplicity, J(Hz)	δC	δH(Multiplicity, J(Hz)
2	81.0	5.86 d (12 Hz)	82.42 d	5.86 s (12.06)
3	48.4	4.98 d (12 Hz)	50.09 d	4.99 d (12,06)
4	196.3		197.29 s	
5	161.8		165.68 s	
6	95.4	6.01 br. s	99.63 d	6.02 s
7	163.6		162.39 s	
8	96.3	6.01 br. s	97.19 d	6.02 s
9	166.6		164.39 s	
10	101.6		103.09 s	
1′	128.2		130.20 s	
2′	128.6	7.23 d (7.7 Hz)	129.38 d	7.24 d (8.3 Hz)
3′	114.5	6.52 d (7.7 Hz)	115.54 d	6.53 d (8.3)
4′	157.4		158.46 s	
5′	114.5	6.52 d (7.7 Hz)	115.54 d	6.53 d (8.3)
6′	128.6	7.23 d (7.7 Hz)	129.38 d	7.24 d (8.3)
2′′	163.8		164.90 s	
3′′	102.3	6.46 s	103.86 d	6.47 s
4′′	181.7		183.19 s	
5′′	160.6		162.64 s	
6′′	98.7	6.29 s	96.09 d	6.30 s
7′′	162.9		167.23 s	
8′′	100.6		105.01 s	
9′′	155.3		156.89 s	
10′′	103.2		101.73 s	
1′′′	121.1		123.53 s	
2′′′	113.4	7.49 m	114.23 d	7.50 s
3′′′	145.7		146.41 s	
4′′′	149.8		150.23 s	
5′′′	116.2	7.01 d (7.7 Hz)	116.74 d	7.02 d (8.4)
6′′′	119.4	7.49 m	120,64 d	7.52 d (8.4)
5 OH		12.31 s		12.32 s
5′′ OH		13.15 s		13.15 s

consisting of a flavanone unit and a flavone unit. In the ^{1}H-^{1}H COSY experiment (**Figure 2**) the correlation of saturated proton H-2/H-3 and H-2′/H-3′ combined with the HMBC correlations (**Figure 2**) of H-2/C-2′, C-6′, C-3′, C-5′ and H-3/C-1′, C-4, C-10 confirmed that unit I should be a flavanone unit. the unsaturated singlet proton at δ_H 6.47 (1H, s, H-3′′) as well as the HMBC correlations of H-3″/C-4′′, C-2′′, C-1′′′ and H-6′′′/C-2′′ concluded unit II should be a flavone unit.

In the HMBC spectrum, the correlation of H-3/C-9′′ were observed, suggesting that the linkage position of unit I and II was at C-3 and quaternary carbons C-8′′. Therefore, the planar structure of compound **1** was elucidated to be a flavanone-(3→8′′)-flavone biflavonoid, and with those data and comparison literature (Darwati et al., 2021; Jamila et al., 2014) could identify that structure of compound **1** as morelloflavone (**Table 3**)

In this study, compound **1** was also tested for its cytotoxicity against breast cancer MCF-7 sell lines. The results indicated compound **1** considered as weak category with IC_{50} >100 μM that is 610 μM (Kuete & Efferth, 2015). In summary, Compound **1** is first reported biflavonoid isolated from *C. lasiocarpus*.

Doxurubicin and genistein are commonly used in treatment breast cancer. Genistein can enchane chemotherapeutic afficacy and overcome chemoresistance in breast cancer (Xue et al., 2014). The long duration treatment of breast cancer with doxorubicin can causes cancer cell resistance and has serious adverse effect to heart damage (Lovitt et al., 2018). Therefore, the development of anti-breast cancer is needed.

All protein were docked with morelloflavone (**1**), genistein and doxorubicin. According to the docking result, Morelloflavone has a highest activity on HER-2 protein with binding activity of -7.58 Kcal/mol. In ER-α and ER-β, which has the highest activity is genistein with binding activity are -9.32 and -10.25, respectively. But when compared with doxuribicin, morelloflavone compounds have higher activity (**Table 3**). Binding affinity is the energy of intermolecular interaction between receptor and ligand. If the binding affinity value was lower, it's meant that ligand had more significant potential to interact with receptor and indicates a good activity (Kelutur & Mustarichie, 2020; Riyana et al., 2020).

The interaction of amino acid residues between receptor and ligand was in hydrogen and Van der Waals bond (**Table 4-6** and **Figure 3-5**). The similarity of residue amino acid showed that the ligand had the same binding pocket (Herdiyati et al., 2020). The Similar residue of amino acid of ER-α, ER-β and HER-2 proteins with all ligand test are Lys 529, Val 534, Met 528; Val 485, Asp 489, Cys 481, Thr 299, Val 487, Tyr 488, Met 479; and Ser 855, Tyr 803, Gly 818 respectively.

Table 3. Binding affinity of the compounds against ER-β, ER-α and HER-2

Compound	Binding Affinity (Kcal/mol)		
	ER-α	ER-β	HER-2
Morelloflavone (**1**)	-7.51	-7.71	-7.58
Genistein*	-9.32	-10.25	-6.61
Doxorubicin *	-6.83	-6.82	-7.13

**positive control*

Table 4. Result of molecular docking of ER-α with test ligand

Ligand Test	Amino Acid Residue Interactions	
	Hydrogen Bond	Van der Waals Bond (Hydrophobic)
Morelloflavone (1)	**Lys 529**, **Val 534**, Val 533	Asn 532, **Met 528**, Leu 541, Ala 340
Genistein*	His 524, Leu 346, Leu 387, Arg 394	**Met 528**, Leu 349, Glu 353, Met 388, Leu 384, Gly 521
Doxorubicin*	Tyr 537, Val 533, Asn 532, **Val 534**, Leu 536	Asn 348, Ala 340, **Lys 529**

*positive control

Table 5. Result of molecular docking of ER-β with test ligand

Ligand Test	Amino Acid Residue Interactions	
	Hydrogen Bond	Van der Waals Bond (Hydrophobic)
Morelloflavone (1)	**Val 485**, **Asp 489**, **Cys 481**	**Thr 299**, **Val 487**, **Tyr 488**, **Met 479**
Genistein*	Leu 339, Glu 305, Arg 346, Gly 472, His 475, Leu 298	Met 340, Leu 380, Ile 376, Ile 373, **Met 479**, Met 295, **Thr 299**, Leu 301
Doxorubicin*	**Asp 489**, Pro 486, **Val 485**, **Tyr 488**, **Val 487**, **Cys 481**	Val 484, Leu 476, Met 473, Trp 335, **Met 479**

*positive control

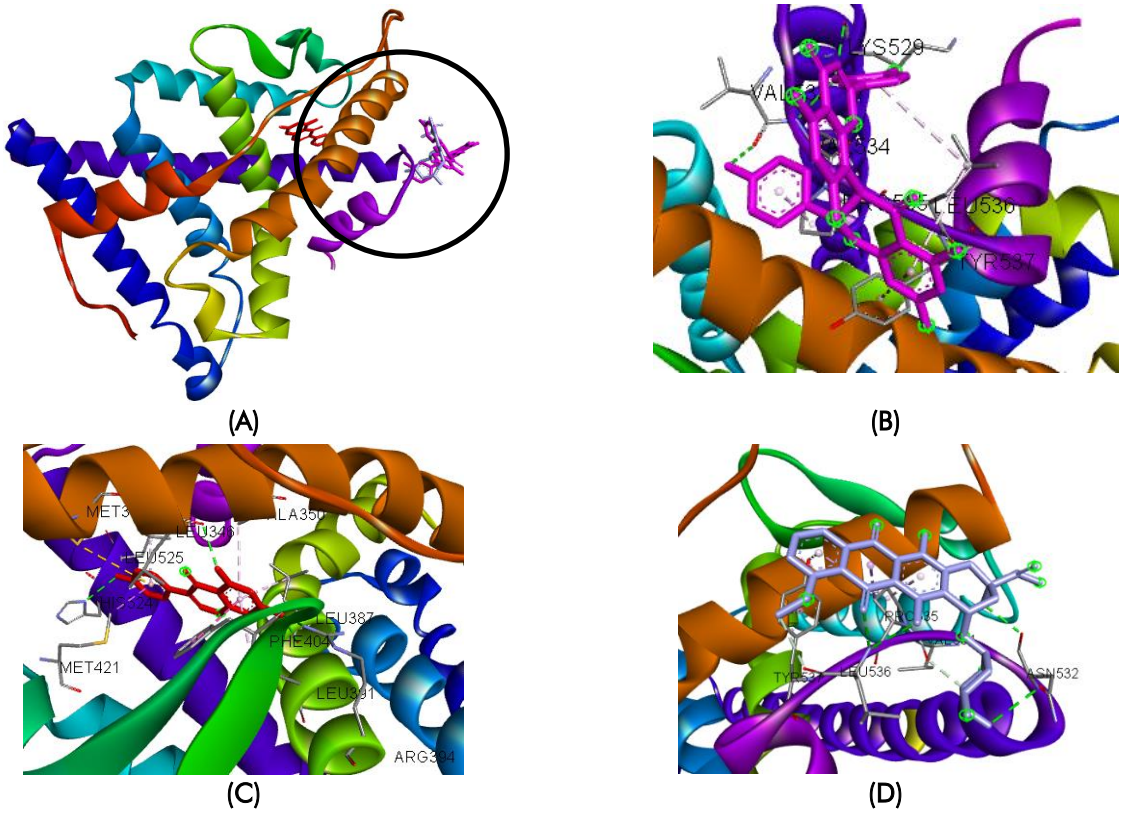

Figure 3. Interaction of ER-α with all test ligand (A), ER-α with morelloflavone (B), ER-α with genistein (C) and ER-α with doxorubucin (D).

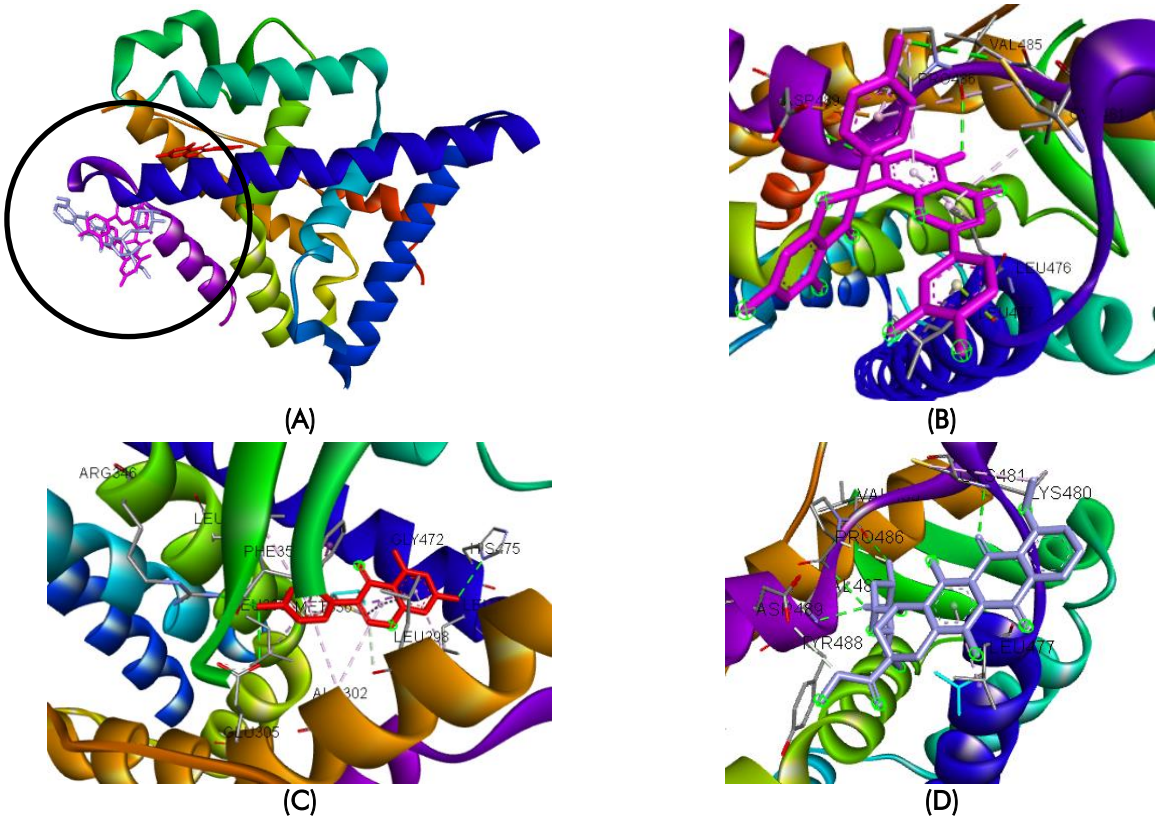

Figure 4. Interaction of ER-β with all test ligand (A), ER-β with Morelloflavone (B), ER-β with Genistein (C) and ER-β with Doxorubucin (D).

Table 6. Result of molecular docking of HER2 with test ligand

Ligand Test	Amino Acid Residue Interactions	
	Hydrogen Bond	Van der Waals Bond (Hydrophobic)
Morelloflavone (1)	Asb 824, Gln 828, Tyr 781, Asn 857, His 858, Lys 854, **Ser 855**	**Tyr 803**
Genistein	Gln 990, Ser 819, Ile 989, **Gly 818**, Gln 820	Asp 821, Gln 984, Val 987
Doxorubicin	**Tyr 803**, Asp 1001, Leu 1000	Leu 817, **Gly 818**, Asp 821, **Ser 855**, Ser 1002, Tyr 1005

**positive control*

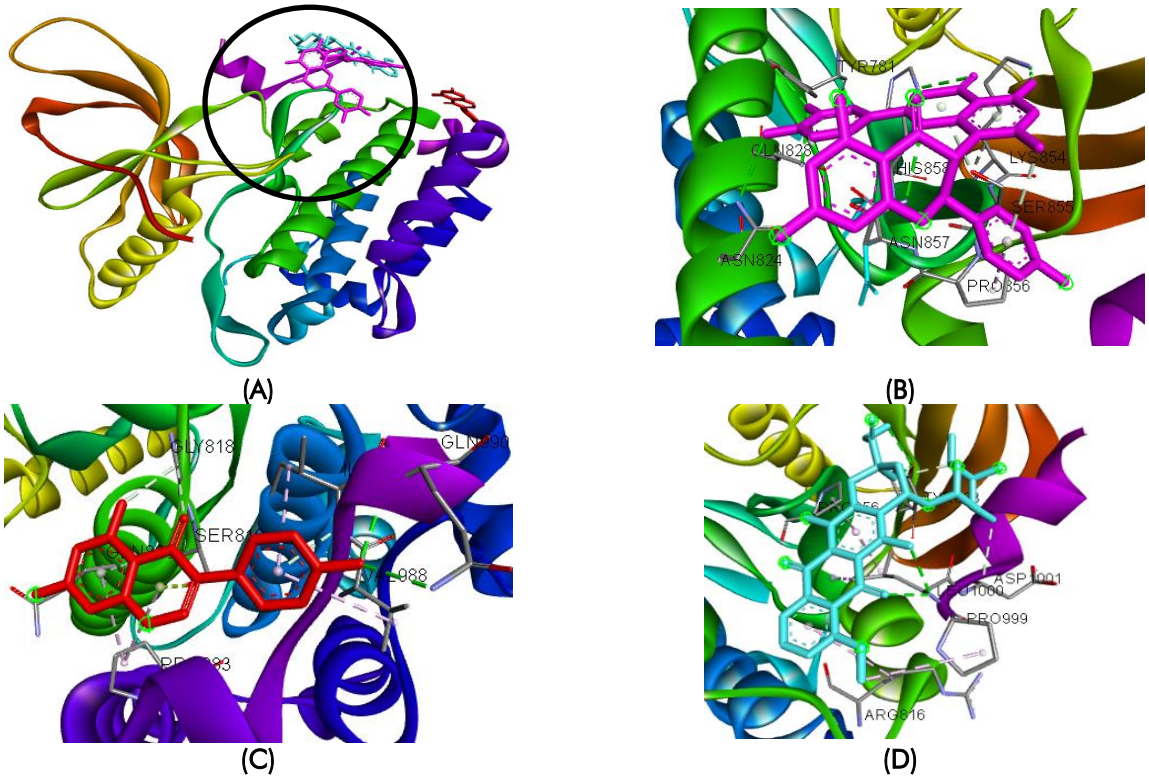

Figure 5. Interaction of HER2 with all test ligand (A), HER2 with morelloflavone (B), HER2 with genistein (C) and HER2 with doxorubucin (D).

CONCLUSIONS

Morelloflavone has been isolated for the first time from stembark of *C. lasiocaropus* and the cytotoxic activity of this compound considered as inactivity against breast cancer MCF-7 cell lines with IC_{50} value of 610 μM. The results of molecular docking analysis showed that morelloflavone had a highest *activity* on HER-2 protein with binding activity of -7.58 Kcal/mol. Whereas in ER-α and ER-β proteins, morelloflavone activity was greater than doxuribicin with binding activity are -9.32 and -10.25, respectively. Therefore, morelloflavone have a potency to develop as new anti-breast cancer agent.

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