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Cytotoxicity Studies of Xanthorrhizol and Its Mechanism Using Molecular Docking Simulation and Pharmacophore Modelling

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ABSTRACT

Xanthorrhizol (XNT) is one of major compounds from temulawak`s rhizome and its activity in several cancer cells is known. The aim of this study was to identify mechanism of xanthorrhizol from temulawak`s rhizome as an hERα inhibitor against breast cancer human cell lines. The cytotoxicity of XNT from temulawak`s rhizome on T47D human breast cancer cells lined by sulforhodamine B (SRB) method has been carried out, while molecular docking simulation and pharmacophore modelling methods were employed to predict mechanism of xanthorrhizol as hERα inhibitor. Cytotoxicity studies showed that XNT of the isolated and standard had an IC_{50} 100 and 55.50 µg/mL in T47D cells, respectively. Subsequently, molecular docking interaction showed that XNT might be able to compete with estradiol (E2) as a potential ERα inhibitor with the calculated binding free-energy of -8.2 kcal/mol, even the compound superimposed with tamoxifen (4-OHT). XNT formed hydrogen bonds with Arg394 and Glu353 as mention E2 and tamoxifen also formed same interaction with same residue and interacted hydrophobic bonds similar to 4-OHT with: Leu387, Leu384, Leu391, Phe404, L349, Leu346, Met388, and Leu525 of estrogen alpha Ligan Binding Domain (LBD), although 4-OHT indicated stronger hydrophobic when the tail of tamoxifen interacted with Tyr347, Asp351, Trp383 and Leu428. XNT missed two chemical features into HipHop models pharmacophore thus may result in reduced inhibitory activity against T47D compared than 4-OHT. The xanthorrhizol mechanism as a hERα inhibitor is postulated as partial estrogen antagonist, is justifiable based on its competitive characteristic versus tamoxifen (OHT-200) which was located on the active side of HER-α.

INTRODUCTION

Temulawak (*Curcuma xanthorrhiza Roxb.*) is one of the kinds of herbs that have been used for thousands of years as traditional medicinal herbs by some Asian countries. The identification of essential oil of temulawak's rhizome showed that it consisted of several sesquiterpene derivative components, such as: artumeron, isofuranogermacene, kurlon, p-tolilmethylcarbinol and Xanthorrhizol (XNT). Essential oil fraction of temulawak was reached 3-12%, in that fraction the percentage of XNT was reached 11.6% (Sidik and Wardoyo, 1992; Hwang *et al.*, 2000). XNT and alicyclic or tumeron aromatic (ar) will shows higher R_f value than the timol standard (Wagner *et al.*, 1984). Based on the previous research, it is known that XNT restrained the growth of skin cancer by inhibiting the expression of ornithine decarboxylase, cyclooxygenase-2 and by inducting nitric oxide synthase through the activation of mitogen protein kinase and nuclear factor-KB (Chung *et al.*, 2007).

XNT has been known to curb the proliferation of liver cancer by inducing apoptosis through bax control and p53 in a cell (Ismail *et al.*, 2005). Furthermore, XNT has also been known to possess antiproliferative characteristics against breast cancer MCF-7 (Cheah *et al.*, 2006).

Breast cancer is the main cause of death in cancer cases in Asia and over the past few years it has been the most common cause of death in some developing countries in Asia surpasses even that of cervix cancer (Agarwal *et al.*, 2007). Human estrogen α receptor is a 66 kDa hormon-inducted transcription factor protein that can respond positively or negatively in controlling the expression of genes involved in the growth of and the differentiation of tissues.

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Furthermore, human estrogen receptor-α is a part of a bigger core receptor family that shares structural similarity with human estrogen receptor-β, and other core receptors (Lupien *et al.*, 2007). These two receptors are known to act as a medium for the estrogen signals. The function of this estrogen is to be a transcription factor that depends on ligands that interact with it (Lin *et al.*, 2004).

Only recently has α estrogen inhibitor been developed as a new type of medicine in preventing breast cancer. The slowing mechanism of this drug against estrogen has been researched and classified as: (a) estrogen partial antagonists such as tamoxifen; and as: (b) estrogen pure antagonists such as fulseverant (Jordan, 1994).

In this research, the mechanism possibility of temulawak's XNT as an inhibitor to human receptor estrogen alpha (hERα) was scrutinized by combining both the *in vitro* test or cytotoxic test and then *in silico* test using the molecular docking simulation and pharmacophore modelling The cytotoxicity test was done by using the *Sulforhodamine* B (SRB) method.

The cytotoxicity test was done into essential oils and isolate of xanthorrhizol which isolated from *Curcuma xanthorrhiza*. Subsequently, XNT was docked into estrogen alpha receptor (hERα) with PDB code: 3ERT (Shiau *et al.*, 1998) and compared to tamoxifen (OHT) and also E2 to clarify the mechanism of XNT. The aim of this study was to analyze the mechanism of xanthorrhizol from temulawak`s rhizome as an hERα inhibitor in breast cancer human cell lines.

MATERIAL AND METHODS

Materials

Materials of plants: The material of temulawak`s rhizome used (Curcuma xanthorrhiza Roxb.) obtained from from Balitro, Lembang, West Java in April 2008. Voucher specimen (no. 54/HB/04/2008) was deposited in Herbarium of Department of Biology, Universitas Padjadjaran.

Isolation of essential oil

The temulawak`s rhizome dried (83 gram) were distilled with steam-distillation to obtain 5 mL essential oils in Monaco Lembang, West Java, for 3 hours to isolate the essential oil fraction. Essential oil stored at -20°C after the addition of sodium sulfate.

Extraction of Temulawak`s rhizome

The temulawak`s rhizome powder (5945.32 g) was extracted by methanol solvent 3 x 24 hours in that stirring was done intermittently. At the total extract was evaporated in vacuo at 40-50°C at 80 rpm. The extracts (902, 30 gram) were partitioned using mix solvent water and ethyl-acetate (EtOAc) with 1:1 ratio.

Isolation xanthorrhizol from Temulawak's Rhizome

Isolation of xanthorrhizol was referred to Hwang *et al.* (2000). Subsequently, isolate compounds have been compared by using TLC (Fig. 1) and FT-IR (Fig. 3).

Phytochemical Screening

The phytochemical screening was done to simplisia, methanol extract and ethyl acetate fraction by using (Farnsworth, 1966).

Antiproliferative Activity Test

The cytotoxicity test was done by using Sulforhodamine B (SRB) method (Vichai and Kirtikara, 2006) by measuring proteins in the cells using ELISA plate reader. From this test the percentage of surviving cells could be counted and the value of IC50, that is, the concentration of essential oil, temulawak's rhizome isolate and standard xanthorrhizol needed to inhibit 50% of human breast cancer T47D cells, could be determined.

Data Analysis

The antiproliferative activity data were analyzed using ANAVA one-way test ($\alpha = 0.05$). The analysis is done to the percentage of survival cells, the IC50 value of essential oil, isolate, and xanthorrhizol standard.

Interaction Study of Xanthorrhizol against Human Estrogen Receptor-α by Molecular Docking Simulation

The crystal structure of protein ERα complexed with E2, E2 (PDB id 1G50) and ERα complexed with 4-OHT (3ERT) were used in this docking study. The 3D structure of 1G50 was reported by Eiler et al. (Eiler *et al.*, 2001) using X-ray diffraction technique with a resolution of 2.90Å. Although the resolution is quite low, this structure represents the best resolution of E2 bound to ERα. 3ERT (Shiau *et al.*, 1998) is an antagonist form of ERα which different from 1G50 at the region of loop-534. Water molecules and ligands were removed from the protein and the polar hydrogen were assigned for the protein by using AutoDockTools 1.5.4 (Sanner, 1999). Non-polar hydrogen atoms of E2, 4-OHT and xanthorrhizol were merged and rotatable bonds were assigned.

Molecular Docking Simulation Parameters

Crystal structure of 1G50 and 3ERT were superimposed by their sequence similarities and important binding residues by using Accelrys Discovery Studio (DS) 2.5.5. Docking calculations were performed by the AutoDock 3.0 (Morris *et al.*, 1998). All waters and hetero-atoms attached to the proteins were removed. Hydrogens were deleted from the receptor structure but later added only for polar hydrogen atoms using the program protonate and charges assigned using kollua.amber option of AutoDock 3.0.5. 40×40×40 grid map points a spacing of 0.375Å were set for AutoGrid computation with grid centred at x; 30.215, y; -0.021, and z;27.79 to cover important residues in the binding site. Docking were performed employing Lamarckian genetic algorithm (LGA) with pseudo-Solis and Wets local search and with the following standard parameters: population size of 150; energy evaluations of 10000000 and a total of 100 docking runs for each ligand. The other parameter was default. The docking results from each of calculations were clustered based on root mean square deviation (rmsd) and on free energy of binding.

Pharmacophore Modelling

HipHop was run by using Catalyst embedded Discovery Studio 2.5. The models were generated based on some information whose consisted most-active compounds in the training set (Qin *et al.*, 2007, Gauthier *et al.*, 1997, Chao *et al.*, 2006). In HipHop1 model, the six most-active compounds (1-6) in training set were firstly selected for HipHop run to identify common pharmacophore features. Compound 1 (tamoxifen) was considered as reference molecule. The principle term and the MaxOmitFeat term were set to 2 and 0, respectively. Subsequently, the HipHop2 was built from four most-active compounds (7-10) in training set. The structure of compounds can be seen in table5 and 6.

A conformational was set for each molecule using the polling algorithm and the best energy option, based on CHARMm force field from Discovery Studio 2.5 (Brooks *et al.*, 1983). The molecules associated with their conformational models were mapped onto the pharmacophore model using the "best fit" option to obtain the bioactive conformation of each molecule.

RESULTS AND DISCUSSIONS

Phytochemical Screening

The phytochemical screening was done to determine the secondary metabolite group contained. The result of the screening can be shown in Table 1.

Table. 1: Secondary metabolite groups in simplisia, extracts and ethyl acetate fractions.

Compound Groups	Simplisia Powder	Methanol Extracts	Ethyl Acetate Fractions
Alkaloid	+	+	+
Pholiphenol	+	+	-
Tanin	-	-	-
Flavonoid	+	+	+
Monoterpene dan sesquiterpene	+	+	+
Steroid	-	-	-
Triterpenoid	+	+	-
Quinine	-	-	-
Saponin	+	+	+

Legends: (+): Detected (-): Undetected

Temulawak's Rhizome Isolate Identification

Essential Oil of Temulawak`s rhizome

The essential oils of temulawak`s rhizome was isolated with yield 6 % w/w. It was good quality as it has ideal percentage of essential oils. The standard of Materia Medica Indonesia (MMI) requires that essential oils content in temulawak's rhizome be 2 to 7 %.

Extraction and Isolation Results

MeOH extract obtained the yield of 15.18% (90.32 gram). The fraction of ethyl acetate yield 5.19% (222.48 gram). This fraction (30.32 gram) was subjected by liquid vacuum chromatography and n-hexane-ethyl acetate as an eluent having gradient polarity to obtain five fractions (A, B, C, D, and E). Fraction C was further subjected to gravitation column chromatography (GCC) with mobile-phase toluene-ethyl acetate (99:1) thus produced eight sub-fractions (C-1 until C-8). Subfraction C-7 has one spot that same Rf (toluene-ethyl-acetate; 99:1) with reference of xanthorizol as shown in Fig. 1. Subfraction C-7 was further evaluated by UV and FTIR, as shown in Fig. 2 and Fig. 3, respectively.

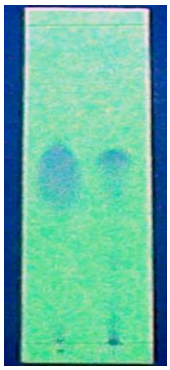


Fig. 1: C-7 isolate and standard *XNT* thin layer chromatogram using toluene-ethyl-acetate; 99:1 as eluent; (i) *XNT* reference, (ii) C-7 isolate.

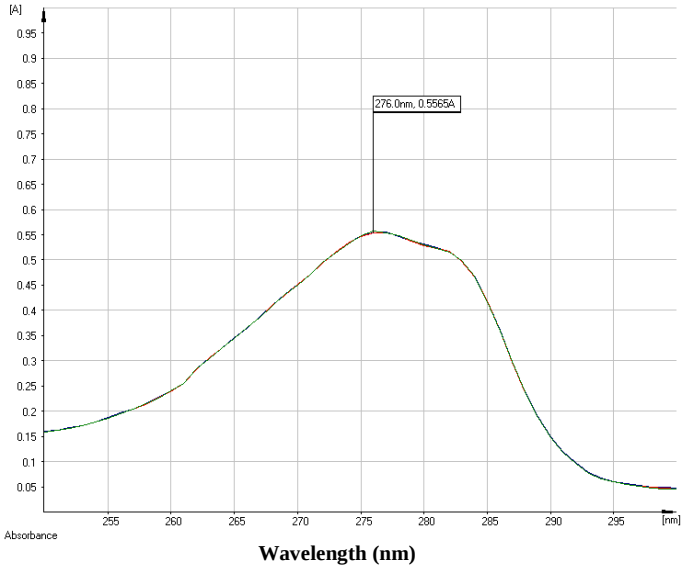


Fig. 2: C-7 isolate ultraviolet Spectrum.

In the IR spectrum (Fig. 3), it showed the absorption characteristic at 3681 **cm**$^{-1}$ (-OH in alcohol and phenol), 2966 **cm**$^{-1}$ (-CH_3 and -CH_2 aliphatic), 2873 **cm**$^{-1}$ (-CH_3 and -CH_2 aliphatic), 1725 **cm**$^{-1}$ (substituted benzene ring), 1515 **cm**$^{-1}$ (benzene ring in aromatic compounds) wave number. The finger print of isolate C-7 at wave number less than 1500.. This shows that the C-7 isolate contains those functional groups.

Cytotoxic Test

The cytotoxic test is based on the surviving pasted SRB pigmented cell protein after the sample was given (Vichai and Kirtikara, 2006). The surviving cells were pigmented with 0.4% SRB diluted in acetic acid 1%. The pigmented cells were then further diluted in Tris base to determine the optical density using ELISA plate reader with 515 nm wavelength. The reading of the ELISA plate reader showing absorption values for each sample and cisplatin as the positive control can be seen in Table 2.

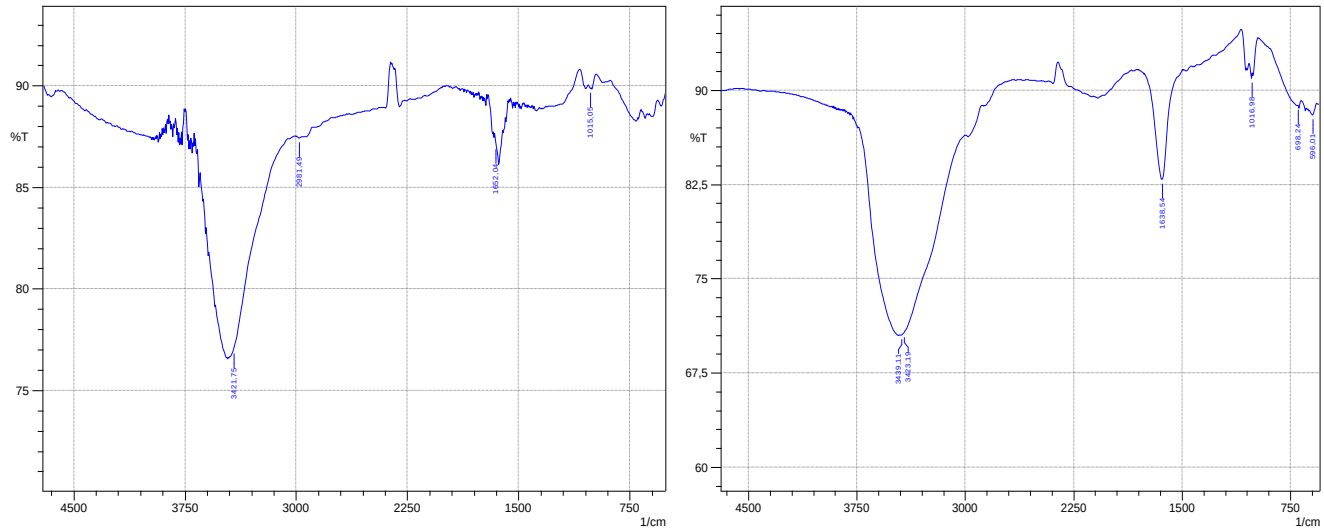


Fig. 3: Infra Red Spectrum between C-7 isolate (left) from temulawak and xanthorrizol reference (right).

Table. 2: The Average Absorption Values of Cisplatin, Essential Oil, Isolate, and XNT.

Concentration (µg/mL)	Average Absorption Values ± SD			
	Cisplatin	Essential oil	Isolate	XNT Reference
100.000	0.167 ± 0.011	0.121 ± 0.025	0.276 ± 0.003	0.093 ± 0.016
50.000	0.238 ± 0.005	0.271 ± 0.044	0.316 ± 0.025	0.246 ± 0.060
25.000	0.262 ± 0.001	0.277 ± 0.030	0.309 ± 0.008	0.310 ± 0.003
12.500	0.319 ± 0.025	0.338 ± 0.067	0.307 ± 0.031	0.362 ± 0.015
6.250	0.352 ± 0.027	0.368 ± 0.062	0.303 ± 0.019	0.371 ± 0.003
3.125	0.334 ± 0.028	0.363 ± 0.010	0.319 ± 0.011	0.363 ± 0.016

The results showed that increasing the concentration of the samples showed lower absorbance due to the smaller number of survival cells. The absorption values were taken from the number of cells remains after the test sample and cisplatin as the positive control. The greater the absorption value is, the greater the number of the living and SRB pigmented cells is; moreover, it can also be stated that the cytotoxic value is smaller if the absorption value is bigger. The absorption values, it can be determined the value of survival percentage which shows the cytotoxic effect.

Table. 3: Survival Percentage of XNT against T47D.

Concentration (µg/mL)	Survival Percentage			
	Cisplatin	Essential oil	Isolate	XNT ref
100.000	21.186	-1.418	69.143	-13.897
50.000	56.516	66.648	87.294	55.530
25.000	68.128	69.597	84.118	84.572
12.500	96.541	97.277	82.983	108.168
6.250	112.847	110.664	81.395	112.252
3.125	103.706	108.395	88.429	108.622

The survival percentage result can be seen in Table 3, and the graph shows the survival percentage can be shown in Fig. 4. The results showed that increasing the concentration of the samples showed higher anticancer activity of cancer cells, as indicated by the survival percentage were lower. Best activity provided by xanthorrhizol reference, essential oil and xanthorrhizol isolated. According to the data of survival percentage, the smaller the survival percentage value is, the better the cytotoxic value will be. The increase of cisplatin, essential oil, isolate, and standard XNT concentration results in the increase of cytotoxic effect against T47D breast cancer cells. Furthermore, the IC_{50} value for essential oil, isolate, and XNT reference were 55.503 µg/mL, 100 µg/mL, and 52.469 µg/mL, respectively according of the data. The small IC_{50} values indicate that the three tested substances possess strong toxicity against T47D breast cancer cells.

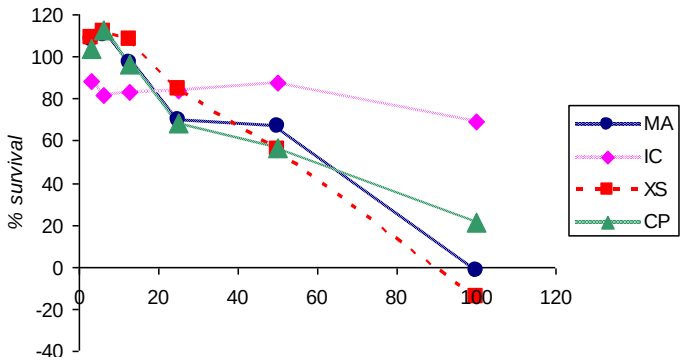


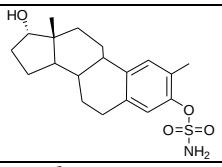
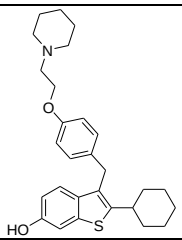
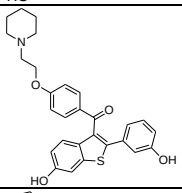
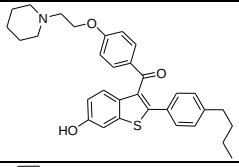
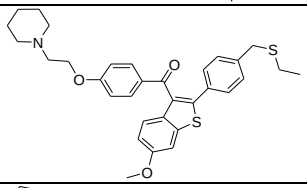
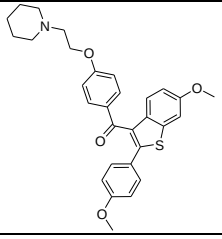
Fig. 4: Graph of survival percentage of essential oil, C-7 isolate, reference of XNT, and Cisplatin. (MA ; essential oils, IC ; isolate compounds, XS : xanthorrizol reference, CP; cisplatin.

Other author (Cheah *et al.*, 2006) reported that XNT inhibits the proliferation of the same human breast cancer cell line in this research, with an EC50 value of 1.71 µg/ml. In addition, xanthorrhizol also play a role as antiproliperative against the others human breast cancer lines MDA-MB-231 with IC50 8.67 µg/mL (Cheah *et al.*, 2009, Cheah *et al.*, 2008). However, the mechanism of xanthorrhizol as antiproliperative is not published yet.

Table 4 Binding Interaction of xanthorrhizol (XNT), tamoxifen (OHT) and E2 (Estrogen) into ERα.

Compounds	FEB (kcal/mol) (2HU4)	Hydrogen Bond		Distance (Å)	Hydrophobic Interaction	
		Donor	Acceptor		Aa residue of ERα	Xanthorrhizol
(XNT)	-8.32				Leu387	CH_3-2, Benzene
		XAN:OH-1	Glu353:O	3.701	Ala350, Phe404,	Benzene
		XAN:OH-1	Arg394:N	3.110	Glu387, Leu349	CH_3-6', C-4'
		XAN:OH-1	Phe404:O	3.446	Leu346	
(OHT)	-11.46	OHT:O4	GLU353:O-2	2.427	Phe404	C-9
		ARG394:NH2	OHT:O4	3.025	Ala350	Ring A, B
		OHT:O4	GLU353:O2	2.475	Met343,	Ring C
		Leu387	OHT:O4	3.919	Glu419, Glu420,	C-9
		Asp351	OHT:N24	3.818	Leu525, Gly521	
		Thr347	OHT:O20	3.963	Leu346	
E2	-8.86	E3:O3	GLU353:O2	2.427	Phe404	Ring A
		ARG394:NH2	E2:O3	2.802	Leu347	C-12
		E2:O4	GLU353:O2	2.944	Leu387	Ring A
		His524	E2:O17	3.615	Gly521, Met348	Ring D
		E2:O3	Leu387	3.157	Leu384	Ring C

Table 5. Training Set for HipHop1.

No.	Structure	IC_{50} (nm)	Principal	MaxOmitFeat
1		0.06	2	0
2		2	2	0
3		3.2	2	0
4		10	2	1
5		100	1	2
6		300	1	2

To know the mechanism, we tried to predict by using two methods of computational modeling; molecular docking simulation and pharmacophore modeling. The binding interaction of xanthorrhizol with human estrogen receptor-α can be explained through the following molecular docking simulation, while pharmacophore modelling could predict active chemical feature of xanthorrhizol that responsible for its biological activity.

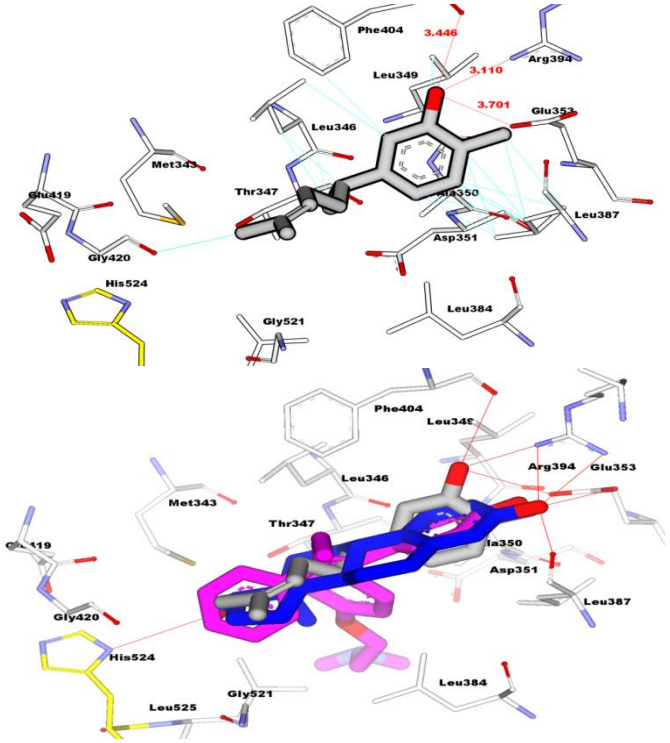


Fig 5 Interaction between XNT on the active side of HER-α (top) and overlay binding interaction between xanthorrizol (grey carbon), tamoxifen (pink carbon), and E2 (white carbon) on the active side of HER-α (bottom). Red line: hydrogen bond interaction, blue line: hydrophobic interaction.

Interaction Study Result of XNT against HER-α by Computational Modeling

Molecular docking simulation was employed to predict interaction of xanthorrhizol into hERα. Protein data bank of estrogen alpha (3ERT) was used with tamoxifen as crystal ligand. We docked xanthorrhizol, tamoxifen and E2 to clarify biding interaction into the site pocket of hERα as shown in Table 4 and Fig. 5. The value of free energy binding (ΔG) and Ki of XNT were obtained -8.32 kcal/mol and 7.93 x 10^{-7}, respectively. The molecular docking simulation shows that XNT has less activity compared than tamoxifen . As shown Fig. 5, Hydroxyl groups of xanthorrhizol strongly formed hydrogen bond with Arg394 and Glu353 as mention E2 and tamoxifen that formed same interaction with same residue as shown in table 4.

Such as tamoxifen, XNT has formed a strong hydrophobic interaction with hERα. Phe404, Le346, and Leu387 predominantly produced hydrophobic interaction with xanthorrhizol as shown in Table 4. It might explain that 1-OH and alkyl chain of xanthorrhizol that the hydroxyl group has played an important role as chemical features responsible for their activity. XNT has hydrogen bond with Glu353 and also forms hydrogen bond with Arg394, but unfortunately, XNT loss interactions with residues of His524. Glu353, Arg394, and His524 are important residue since E2 formed four hydrogen bonds between hydroxyl groups of E2 (Table 4) and residues of side Glu353, Arg394, His524. His524 is important in binding of copep (coprotein peptida) into H12, because H524 shows a conserved hydrogen bond from E2(O17) to His524(N) during MD simulation (Celik *et al.*, 2007). This residue is also role in building zipper network H3 and H11 with the others residues (K531, E419, and E339) (Gangloff *et al.*, 2001).

The presence of a network zipping H3 and H11 stabilized conformation of agonist. This zipper network confirms that H12 is placed in the "mouse trap", as consequence it can not reach the antagonist position on the hER LBD surface when H11 is not allowed to relax (Celik *et al.*, 2007). However, when antagonist (4-OHT) is placed, this network zipper is lost. In this study, the presence XNT in binding pocket also has eliminated this network zipper as shown Fig. 6.

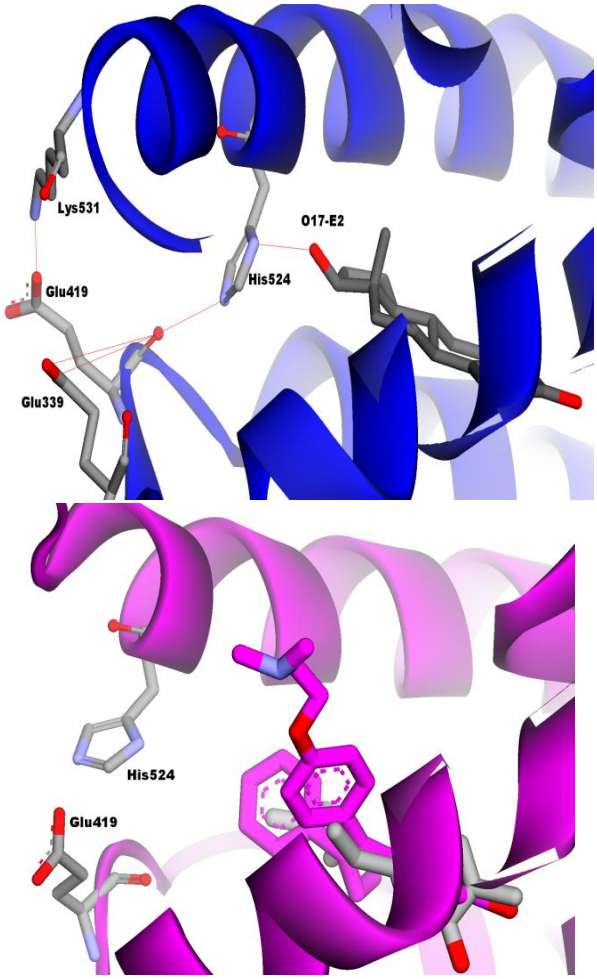
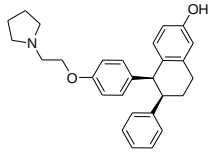
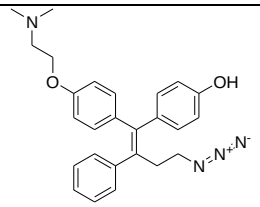
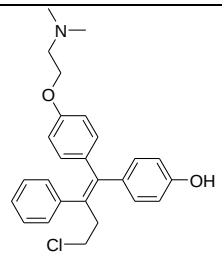
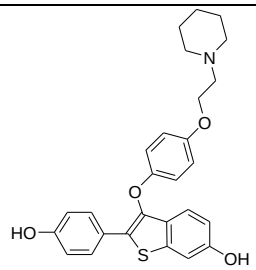


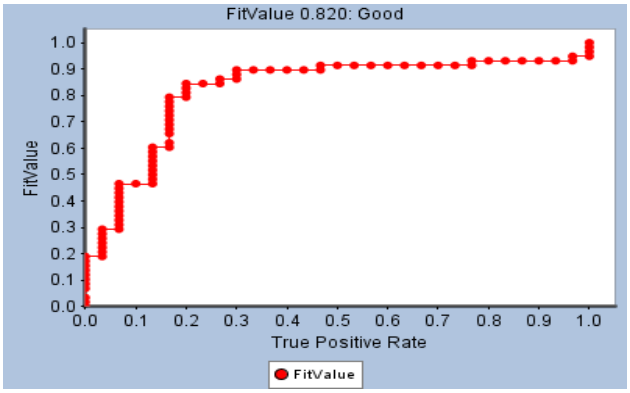
Fig 6. Zipper network were built by His524, Lys531, Glu419, and Glu339 on agonist (E2) repositioned of hERα (left blue), while zipper network go away from hERα on antagonist repositioned (right pink), inside in site active; XNT (grey carbon) and OHT (pink carbon).

Table 6. Training set for HipHop2.

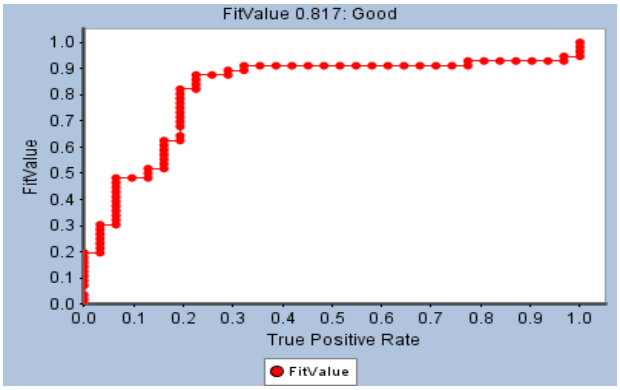
No.	Structure	IC_{50} (nm)	Principal	MaxOmitFeat
1		4	1	0
2		1	2	0
3		4	1	0
4		7.8	1	1

The unleashing of zipper network in antagonist interaction is speculated as a consequence of the rotation of His524, hence Glu339 and Lys531 are positioned too far away from each other to interact. As shown in Table 4, hydrogen bond distance between OHT and important residues (Arg394 and Glu353) was smaller than hydrogen bond distance between XNT and residues. In addition, hydrophobic interaction of OHT-hERα LBD was more strongly than XNT- hERα LBD. It was reason why XNT was less active than OHT. Based on molecular docking results, we postulated that 1-OH and alkyl chain of xanthorrhizol has been responsible into the activity. To test of our postulates, we created pharmacopohore modelling using HipHop running. HipHop running would identify important chemical features based on most active compounds against MCF7. We created two HipHop with different training set consisted by using catalyst embedded Discovery Studio 2.5. HipHop1 model was built by training set (Table 5) that consisted of six compounds as shown in Fig. 7. Four compounds were set as active and two compounds were set as moderate active using Principal and MaxOmitFeat of HipHop parameters (Barnum *et al.*, 1996). 2-ethylE2-3-O-sulfamate (Newman *et al.*, 2004) (**1**), raloxifen derivate (Grese *et al.*, 1996) (**2**), and two arilbenzothiophene (Grese *et al.*, 1997) (**3** and **4**) derivates were set as most active compounds.

Model HipHop2 (Figure 8) was created by four compounds of tamoxifen derivatives (Chao *et al.*, 2006) compounds (Table 6). Feature of best HipHop models were used to provide the pharamacophoric features of xanthorrhizol. Best HipHop models successful were selected based on ROC analysis. The both of models consisted the same features Hydrogen Bond Donor (HBD), Hydrogen Bond Acceptor (HBA), Hydrophobic (HY), and Ring Aromatic (RA), however different position, angle, and torsion. HipHop1 models (was shown in Fig. 8a that has ROC value 0.82 (Fig. 7a), while HipHop2 has ROC value 0.817 (Fig 7b). Xanthorrhizol missed two features (RA and HBD) of HipHop1 as shown in Figure 8.



(A)



(B)

Fig 7. Received operator curves (ROC) conducted for pharmacophore models: (**A**) HipHop1 and (**B**) HipHop2 (numbers of active are 35 of 60 molecules). As predicted of molecular docking results, only 1-OH and tail of 1'-alkyl were mapped by HBA and HY, respectively.

Figure 8 ilustrated that HBA, RA, and HY of HipHop2 successfully mapped 1-OH, benzene ring, and 1'-alkyl of xanthorrhizol, respectively. However, xanthorrhizol missed HBD features of HipHop2. On the other hand, in molecular docking results might show that RA only play role as hydrophobic interaction. The molecular docking results were in accordance with pharmacophore mapping results.

The both methods generated the postulate that xanthorrhizol might has almost mechanism as partial antagonist hERα, such as tamoxifen. Based on *in silico* results, 1-OH and

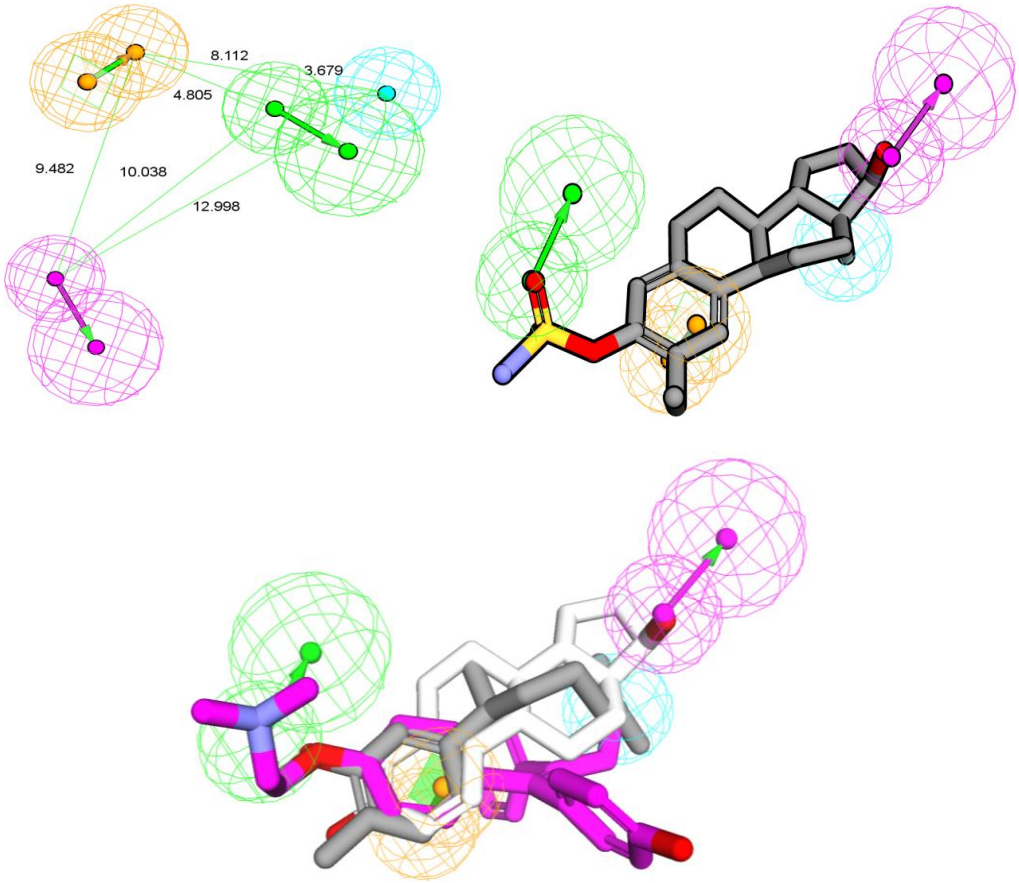


Fig 8. (**A**) HipHop2 model generated by HipHop module of Catalyst in DS 2.5 Package with view distance inter-features **(B)** HipHop2 aligned on 2Ete2MATE (4-hydroxy-tamoxifen) **(7),** the most-active compound (IC_{50}: 2 nM), (**C**) xanthorrizol (grey carbon), tamoxifen (purple carbon), and E2 (white carbon) were mapped into HipHop1model. Pharmacophore features are color coded; magenta - hydrogen bond donor (HBD), green - hydrogen bond acceptor (HBA), blue – hydrophobic feature (Hy), orange – ring aromatic (RA).

alkyl chain of xanthorrhizol were two chemical features that have an important role in biological activity as partial antagonist hERα. Ring aromatic of xanthorrhizol also contributed in molecular interaction with hERα. To improve activity of xanthorrhizol, we have suggestion that modified 6-methyl and attachment some HBD groups in 1' and 2' of alkyl chain may allow increased activity against MCF7 human breast cancer lines.

CONCLUSIONS

Based on these studies, it is known that essential oil, temulawak's rhizome isolate and pure xanthorrhizol possess cytotoxic effects on T47D breast cancer cells with IC50 concentration, 55.503 µg/mL, 100 µg/mL and 52.469 µg/mL, respectively. The increased essential oil, isolate and xanthorrhizol concentration of temulawak's rhizome can result in cytotoxic increase. The xanthorrhizol mechanism as a medicine for breast cancer in that xanthorrhizol is hypothesized as partial estrogen antagonist is justifiable based on its competitive characteristic versus tamoxifen (OHT-200) which was located on the active side of HER-α.

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