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Metabolite Profiling of 96% Ethanol Extract from Marsilea crenata Presl. Leaves Using UPLC-QToF-MS/MS and Anti-Neuroinflammatory Predicition Activity with Molecular Docking

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ABSTRACT

Phytoestrogen is a group of compounds that can replace the estrogen function in the body. One of its roles was as anti-neuroinflammatory by inhibiting the microglia M_1 polarity activation. *Marsilea crenata* Presl. is a plant that suspected to contain phytoestrogens. The aim of this research was to determine the metabolite profile of 96% ethanol extract of *M. crenata* using UPLC-QToF-MS/MS, and prediction the anti-neuroinflammatory activity of compounds with molecular docking. The 100 ppm of 96% ethanol extract in DCM and methanol were injected 5 µl each into the UPLC-QToF-MS/MS, and then analyzed by Masslynx 4.1 software to determine the compounds. The result of metabolite profiling shows a total 59 compounds in both DCM and methanol. Molecular docking was done with Autodock 4.2.6. After being analyzed, there are 3 compounds that are predicted to have activities similar to 17β-estradiol, they are prochlorperazine, 12-Aminododecanoic acid, and 1-methyl-2-[(4-methylpiperazin-1-yl)methyl]benzimidaol-5-amine hydrochloride. The results showed that the three compounds were predicted to be phytoestrogen compounds from *M. crenata* leaves, which have potential as anti-neuroinflammatory.

Keywords: Phytoestrogens, *M. crenata*, metabolite profiling, anti-neuroinflammatory, in silico

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INTRODUCTION

Marsilea. crenata Presl. is an aquatic plant that widely used as an ingredient for traditional food in Surabaya, Indonesia [1, 2]. Some of the research that had been done showed that 96% ethanol extract, *n*-hexane extract, and ethyl acetate extract of *M. crenata* leaves can inhibit osteoporosis in female mouse (*mus musculus*) with mechanism of bone formation improvement [3-6]. Other studies were also showed that *n*-hexane extract of *M. crenata* leaves can increase the alkaline phosphatase production in MC3T3-E1 preosteoblast cell differentiation process, which indirectly also play a role in bone formation improvement [3, 7].

This activity appears to be suspected because of the phytoestrogens content in *M. crenata*. Phytoestrogens are a group of compounds contained in plants which have estrogen-like structures or can replace the function of estrogen, either in association with estrogen receptors (ER-dependent) or not (ER-independent) [8-10]. The high phytoestrogen content in *M. crenata* raises allegations of its activity as a neuroprotective agent with anti-neuroinflammatory mechanism. This mechanism can occur due to inhibition of microglia cells M_1 polarity activation, where phytoestrogens bind to ER-β in microglia cells and prevent their activity to induce neuron apoptosis [11, 12].

Although it has great potential as a medicinal plants, the phytochemical properties of *M. crenata* has not been completely confirmed yet. This research was done to identify the metabolite profile of 96% ethanol extract of *M. crenata* using UPLC-QToF-MS/MS, which is a powerful technique used for metabolite profiling which has improved in performance of chromatographic resolution, speed and sensitivity analysis, saves time, also reduces solvent consumption [13].

The in silico approach was used to develop new drugs from *M. crenata* leaves as anti-neuroinflammatory, as a preliminary test with the principle of tethering compounds from metabolite profiling analysis to a certain macromolecules such as ER-β, and determine the physicochemical properties of compounds against it [14]. Through the in silico approach, researchers can make the initial hypothesis and make simple predictions related to the potential activity of compounds, by correlating the structure and physicochemical properties using software analysis [15].

METHODS

Material

M. crenata were collected in Benowo, Surabaya, Indonesia at November 2017, and identified in UPT Materia Medica, Batu, Indonesia at December 2017 with specimen number 1a-17b-18a-1. The leaves were prepared to get dry powder of *M. crenata*.

All chemicals and reagent were analytical grade and used as received. 96% ethanol as solvent were purchased from Pharmacy Department, Faculty of Medical and Health Science, Maulana Malik Ibrahim State Islamic University. Dichloromethane, metanol, acetonitrile, and formic acid as solvent and mobile phase on UPLC-QToF-MS/MS were purchased from Central Forensic Laboratory Badan Reserse Kriminal Kepolisian Negara Republik Indonesia.

Extraction

Dry powder of *M. crenata* leaves were extracted with 96% ethanol using ultrasonic assisted extraction methods (Sonica 5300EP S3). This process was repeated, collecting all the supernatants, which were finally evaporated in a rotary evaporator (Heidolph) to get 96% ethanol extract.

Figure 1. *Marsilea crenata* Presl.

Metabolite Profiling by UPLC-QToF-MS/MS

A simple, rapid, reliable and precise reversed phase UPLC-QToF-MS/MS method has been developed and validated according to the regulator guidelines. The 96% ethanol extract preparation was done using solid phase extraction, 100 ppm of ethyl acetate extract in DCM and methanol then injected 5 µl each into the an ACQUITY UPLC® H-Class System (Waters, USA) coupled to an MS detector Xevo G2-S QToF (Waters, USA). Sample were separated on an ACQUITY BEH C18 (1.7 µm 2.1×50 mm) with acetonitril + 0.05 % formic acid and water + 0.05 % formic acid as mobile phase, with flowrate 0.2 ml/min. The results of UPLC-MS analysis was processed using the Masslynx 4.1 software, to obtain the data of peak and m/z spectra of each detected peak. The compound content can then be predicted using the chemspider website.

Molecular Docking Simulation

Receptor structure of ER-β used in this research was obtained from Protein Data Bank (http://www.rcsb.org) with code 3OLS [16]. Initial preparation was done to separate internal ligand (17β-estradiol) from the protein using Biovia Discovery Studio Visualizer 2016. The secondary metabolite of *M. crenata* was prepared with SwissADME Web Tool [17] to predict its physicochemicals properties. The compounds that were predicted to penetrate the brain by using BOILED-Egg method [18] will be used for the next step as test compounds. Internal ligand and test compounds was prepared with Avogrado 1.90.0 for energy optimization by using MMF94s method. Molecular docking was done using Pyrx 0.8 [19] with Autodock Vina [20] for molecular docking simulation. The receptor-ligand complexes from docking simulation were visualized using Biovia Discovery Studio Visualizer 2016.

RESULTS AND DISCUSSION

Metabolite Profiling by UPLC-QToF-MS/MS

A total of 440 g dry powder of *M. crenata* leaves were extracted with 96% ethanol to produce 7 g extract. The 96% ethanol extract of *M. crenata* were analysed by UPLC-QToF-MS/MS to better interpret the diversity of available phytochemicals. **Table 1** and **Table 2** summarise all the compounds characterized in 96% ethanol extract of *M. crenata* leaves, including retention times, % area, measured m/z, molecular formula, putative compounds, and its activity based on references. In total there were 42 peak of compounds identified in the methanol solvent, and 33 peak in the DCM solvent. The use of two types of solvent aimed to elute the 96% ethanol extract optimally.

From all the peaks, only 59 peaks can be identified, while the rest are unknown compounds. Unknown compounds may be identified as impure compounds which are still detected by the instrument, or they may be a new compounds, which is undetectable in chemspider database, especially unknown compounds with high concentrations.

Molecular Docking Simulation

The 59 compounds from the metabolite profiling analysis were screened by using SwissADME Web Tool [17] to predict the ability of compounds to penetrate BBB. The method used was the Boiled-EGG method [18], where the compounds that have topology polar surface area (TPSA) value 0-79 $Å^2$ [21] and a logP value 0.4-6.0 possess the ability to penetrate the blood brain barrier (BBB). From the results of the analysis, there are 19 compounds that are predicted to be able to penetrate the BBB.

The compounds then analyzed with molecular docking using the PyRx 0.8 program and AutoDock Vina [20] as a docking simulator. From the redocking test, the RMSD value was < 2 Å, so that the docking protocol can be used to docking the 19 compounds (Figure 2). From the results of analysis with Discovery Studio Visualizer 2016, it was found that there were 3 compounds predicted as ER-β agonists such as DCM10, MTL7, and MTL17 (**Table 3**), 6 compounds predicted as ER-β antagonist such as DCM4, DCM11, DCM14, DCM24, and MTL6 (**Table 4**), and 10 compounds do not have activity in ER-β. To be able to act as an ER-β agonist, the compound must interact with His475 amino acid residues and interact either with Glu305 or Arg346. The absence of interaction between the compound and His475 residue will cause the compound to act as an ER-β antagonist.

Table 1 : Predicted compounds of 96% ethanol extract of *M. crenata* from Benowo District, Surabaya in methanol solvent

No	Rt (min)	% Area	Measured m/z	Molecular Formula	Proposed Metabolite	Activity
1	0.201	0.0228	124.9790	CH_3NO_6	Unknown	-
2	1.535	2.4313	235.1423	$C_{10}H_{21}NO_5$	4-(3-Hydroxypropyl)-4-nitro-1,7-heptanediol	-
3	2.232	0.1510	179.1315	$C_{11}H_{21}NO_7$	2[(tertButoxycarbonyl)amino-2-deoxy-D glucopyranose	-
4	2.518	1.5144	293.1479	$C_{12}H_{23}NO_7$	Methyl 6-deoxy-6-({[(2-methyl-2-propanyl)oxy]carbonyl}amino)-β-D-glucopyranoside	-
5	3.799	1.4856	327.1314	$C_{15}H_{21}NO_7$	Methyl (3,4,5-triethoxy-2-nitrophenyl)acetate	-
6	4.427	1.4055	187.0642	$C_5H_{15}N3C_{l2}$	4-Hydrazinopiperidine dihydrochloride	-
7	4.610	0.3629	162.0321	$C_9H_6O_3$	3-Hydroxy-2H-chromen-2-one (3 hydroxycoumarin)	Competitive inhibition of human recombinant DAAO [23].
8	4.896	0.1836	373.1375	$C_{20}H_{24}N_3SCl$	Prochlorperazine	Analgesics [24], antiemetics [25]
9	5.228	0.9215	359.0997	$C_{13}H_{18}N_5O_5Cl$	Ethyl 4-[3-(4-chloro-3-nitro-1H-pyrazol-1-yl)propanoyl]-1-piperazinecarboxylate	-
10	5.445	0.0257	475.2990	$C_{33}H_{37}N_3$	4-{Bis[4-(1-pyrrolidinyl)phenyl]methyl}-N,N-dimethyl-1-naphthalenamine	-
11	5.628	0.9906	343.1051	$C_{10}H_{21}N_3O_8S$	1-Azido-1-deoxy-2,3-bis-O-methoxymethyl)-5-O-(methylsulfonyl)-D-ribitol	-
12	5.845	0.6908	550.0951	$C_{24}H_{22}O_{15}$	Quercetin-3-(6"-malonyl)-Glucoside	Antioxidant and antiatherogenic protective [26]
13	6.177	1.0895	498.1166	$C_{25}H_{22}O_{11}$	4-(1,3-Benzodioxol-5-yl)-6-hydroxy-1-oxo 1,3-dihydronaphtho [2,3-c]furan-5-yl hexopyranoside	-
14	6.577	0.3205	534.1013	$C_{24}H_{22}O_{14}$	2(3,4Dihydroxyphenyl)-5-hydroxy-4-oxo-4H chromen-7-yl 6-O (carboxyacetyl)-β-Dglucopyranoside "luteolin 7-O-(6-O-malonyl-β-D-glucoside"	-
15	6.908	0.2713	219.1625	$C_{14}H_{21}NO$	1-[1-(4-Methoxyphenyl)cyclohexyl]methanamine	-
16	7.206	2.0878	196.1105	$C_{11}H_{16}O_3$	1-carboxy-3-hydroxyadamantane	-

17	7.423	0.8892	261.1731	$C_{16}H_{23}NO_2$	1-(7-Ethyl-1-benzofuran-2-yl)-2-[(2-methyl-2-propanyl)amino]ethanol	-
18	7.903	0.3096	295.1565	$C_{14}H_{22}N5Cl$	1-methyl-2-[(4-methylpiperazin-1-yl)methyl]benzimidaol-5-amine hydrochloride	-
19	8.406	1.4141	550.3668	$C_{36}H_{46}N_4O$	"Manzamine J"	-
20	8.886	0.0560	393.1997	$C_{17}H_{31}NO_9$	Ethyl (4S,5R,6R,7S,8R)-4,6,7,8,9pentahydroxy-2-methylene-5-({[(2 methyl-2 propanyl) oxy]carbonyl}amino)nonanoate	-
21	9.321	0.1071	289.2038	$C_{18}H_{27}NO_2$	1-(4-Butoxyphenyl)-3-(1-piperidinyl)-1-propanone (dyclonine)	Inhibitor aldehyde dehydrogenase 1 [27], antimicroba [28]
22	9.584	0.1649	323.1878	$C_{13}H_{29}N_3O_4S$	(3R,4R)-3-{[(2-Hydroxyethyl)(methyl)amino]methyl}-4-(hydroxymethyl)-N-isopropyl-N-methyl-1-pyrrolidinesulfonamide	-
23	10.601	0.6568	191.1315	$C_{12}H_{18}NO$	N,N,N-Trimethyl-3-oxo-3-phenyl-1-propanaminium	-
24	10.830	0.3341	827.4180	$C_{34}H_{57}N_{11}O_{13}$	L-α-Aspartyl-L-threonyl-3-(4H-imidazol-4-yl)-L-alanyl-L-lysyl-L-seryl-L-α-glutamyl-L-isoleucinamide	-
25	11.082	0.4582	287.2817	Unknown	Unknown	-
26	11.379	0.8714	665.3629	$C_{36}H_{51}N_5O_5S$	(3R)-Tetrahydro-3-furanyl [(2S,3S,5S)-3-hydroxy-5-{[(2S)-2-{2-[(2-isopropyl-1,3-thiazol-4-yl)methyl]-2-methylhydrazino}-3-methylbutanoyl]amino}-1,6-diphenyl-2-hexanyl]carbamate	-
27	11.562	1.7782	310.1208	$C_{14}H_{19}N_4O_2Cl$	Lintopride	-
28	11.928	0.4325	591.3256	C28H49NO12	2-Methyl-2-propanyl 2-cyano-3-[(4S,5R)-5-{(5S,6R)-6-[(4R)-2,2-dimethyl-1,3dioxolan-4-yl]-2,4,7,9-tetraoxadecan-5-yl}-2,2-dimethyl-1,3-dioxolan-4-yl]-2-(1-ethoxyethoxy)propanoate	-
29	12.179	0.3815	467.3253	$C_{27}H_{49}NOS_2$	2-[(Bis{2-[(2-methyl-2propanyl)sulfanyl]ethyl}amino)methyl]-4,6-bis(2-methyl-2-propanyl)phenol	-
30	12.397	1.5741	503.3094	$C_{25}H_{45}NO_9$	Pederin	Anticancer [29]
31	12.614	1.9858	693.3938	$C_{46}H_{51}N_3O_3$	2-{(2R)-2-[Benzoyl(methyl)amino]-2-phenylethyl}-6-{(2S)-2-[benzoyl(methyl)amino]-2-phenylethyl}-N,N-diisopropyl-4-methylbenzamide	-
32	12.797	2.5108	517.3168	$C_{29}H_{39}N_7O_2$	1-(2-Methylalanyl-5-phenyl-D-norvalyl)-4-{2-[2-(2H-tetrazol-5-yl)ethyl]phenyl}piperidine	-
33	13.208	0.9465	619.3571	$C_{30}H_{53}NO_{12}$	(3S)-16-{[(1S)-1-Carboxyethyl]amino}-2-methyl-16-oxo-3-hexadecanyl 6-O-(3-carboxypropanoyl)-β-D-glucopyranoside	-
34	13.460	2.6423	495.3579	$C_{29}H_{45}N_5O_2$	8-(Benzylamino)-7-hexadecyl-3-methyl-3,7-dihydro-1H-purine-2,6-dione	-
35	13.677	2.4722	519.3339	$C_{28}H_{46}N_5O_2Cl$	N4-(5-Chloro-2,4-dimethoxyphenyl)-N6-hexadecyl-4,5,6-pyrimidinetriamine	-
36	14.409	10.3549	495.3331	$C_{25}H_{50}NO_6Cl$	Unknown	-
37	15.106	25.3622	746.3726	$C_{22}H_{48}N_9Cl$	N2-[3-({12-[(3-Aminopropyl)amino]dodecyl}amino)propyl]-N4-methyl-1,3,5-triazine-2,4,6-triamine hydrochloride (1:1)	-
38	15.404	4.7166	499.3879	$C_{24}H_{50}N_9Cl$	Unknown	-
39	16.718	5.9510	701.2070	$C_{36}H_{36}N_5O_6SCl$	4-[(N-{2-[(6-Chloro2-methyl-4-quinolinyl) amino]ethyl}-N-[(4-methoxyphenyl)sulfonyl]-β-alanyl)amino]-3-methoxy-N-phenylbenzamide	-
40	17.004	1,3681	553.1696	$C_7H_{24}N_{19}O_9Cl$	Unknown	-
41	17.999	46577	849.2435	$C_{46}H_{48}N_5OS_4Cl$	Unknown	-
42	18.330	13.6488	698.5885	$C_{36}H_{70}N_{14}$	Unknown	-

Table 2 : Predicted compounds of 96% ethanol extract of *M. crenata* from Benowo District, Surabaya in DCM solvent

No	Rt (min)	% Area	Measurec m/z	Molecular Formula	Proposed Metabolite	Activity
1	0.289	0.0032	278.1510	$C_{11}H_{23}N_4O_2Cl$	2-Methyl-2-propanyl 4-carbamimidamido 1-piperidinecarboxylate hydrochloride (1:1)	-
2	0.540	0.0278	278.1518	$C_{16}H_{22}O_4$	Dibutyl phthalate	Antibacterial [30], glycosidase inhibitors [31], estrogenic [32]
3	0.906	0.0049	278.1526	$C_9H_{22}N_6O_2S$	Unknown	-
4	1.969	0.0041	278.1503	$C_{10}H_{23}N_4O_3P$	2[(Diisopropylphosphoryl)methyl]malonohydrazidePropanedioic acid,2[[bis(1methylethyl)phosphinyl]methyl]-, dihydrazide	-
5	2.084	0.1072	201.1730	$C_{11}H_{23}NO_2$	11-Aminoundecanoic Acid	
6	4.427	0.0282	301.1885	$C_{15}H_{27}NO_5$	Megalanthonine	Atifeedant and antifungal [33]
7	4.930	0.0127	299.1935	$C_9H_{21}N_{11}O$	Unknown	-
8	5.342	0.2477	149.1205	$C_{10}H_{15}N$	4-Butylaniline	-
9	5.479	0.0731	431.2734	$C_{18}H_{41}NO_{10}$	Unknown	-
10	5.662	0.0912	210.1261	$C_{12}H_{18}O_3$	(2,4,6Trimethylbenzene1,3,5triyl)trimethanol	-
11	5.925	0.0405	519.3254	$C_{35}H_{41}N_3O$	N-Benzyl 2cycloheptyl-2-{4-[(4-methyl-5,6,7,8-tetrahydro-9H-pyrido[2,3-b]indol-9-yl)methyl]phenyl}acetamide	-
12	6.211	0.0164	563.3512	$C_{37}H_{45}N_3O_2$	2,2'-{[2-Phenyl-2-(2pyridinyl)1,1ethenediyl]bis(4,1phenyleneoxy)}bis(N,Ndiethylethanamine)	-
13	6.474	0.0109	607.3767	$C_{23}H_{49}N_{11}O_8$	Unknown	-
14	6.840	0.0041	122.0837	Unknown	Unknown	-

15	7.640	0.0242	215.1887	$C_{12}H_{25}NO_2$	12-Aminododecanoic acid	-
16	8.006	0.1302	271.1935	$C_{18}H_{25}NO$	Cyclazocine	Hipnotic sedativa [34]
17	9.504	0.0908	301.2406	$C_{20}H_{31}NO$	Trihexyphenidyl	Antiparkinson, antikolinergic [35], antioxidant [36]
18	10.967	0.5387	191.1318	Unknown	Unknown	-
19	11.448	2.3323	241.2775	$C_{16}H_{35}N$	Hexadecylamine	Antibacteri, adjuvant for diphtheria, tetanus toxoid, and influenza [37]
20	11.630	0.3879	287.2827	$C_{17}H_{37}NO_2$	2-Amino-2-tetradecyl-1,3-propanediol	-
21	12.111	0.0640	310.1781	$C_{17}H_{26}O_5$	Portentol	Anticancer [38]
22	12.248	0.0123	227.2618	$C_{15}H_{33}N$	Pentadecylamine	-
23	12.396	0.0027	315.3143	$C_{19}H_{41}NO_2$	3-(Hexadecylamino)-1,2-propanediol	-
24	12.694	0.7068	310.1202	$C_{19}H_{18}O_4$	Benzylbutylphthalat	Estrogenic [32]
25	12.842	0.9778	303.2928	$C_{21}H_{37}N$	4-Pentadecylaniline	-
26	13.894	0.9962	331.3237	$C_{23}H_{41}N$	N-Benzyl-N-octyl-1-octanamine	-
27	15.072	23.3154	627.1879	$C_{12}H_{21}N_{25}O_5S$	Unknown	-
28	15.987	26.3455	775.2257	$C_{38}H_{38}N_5O_{11}Cl$	(1R,13S,16S,17R,28)-28-Amino-20-chloro-17,25dihydroxy5,8,10,24tetramethoxyNmethyl-15,29,31trioxo-22oxa14,30,32triazahexacyclo[14.14.2.218,21.12,6.123,27.07,12]hexatriaconta-2(36),3,5,7,9,11,18,20,23(33),24,26,34-dodecaene-13-carboxamide	-
29	17.050	0.4132	923.2661	$C_{41}H_{35}N_{21}O_2C_{l2}$	Unknown	-
30	17.599	1.9907	592.2688	$C_{35}H_{36}N_4O_5$	Pheophorbide A	Anticancer [39]
31	18.433	37.6384	701.2070	$C_{36}H_{36}N_5O_6SCl$	4[(N{2[(6Chloro2methyl4quinolinyl)amino]ethyl}N[(4methoxyphenyl)sulfonyl]βalanyl)amino]-3-methoxy-N-phenylbenzamide	-
32	21.658	0.0399	156.9953	$C_{12}N$	Unknown	-
33	22.572	2.8467	278.1517	$C_7H_{10}N_2$	2-Pyridylethylamine	Histamine agonist [40]

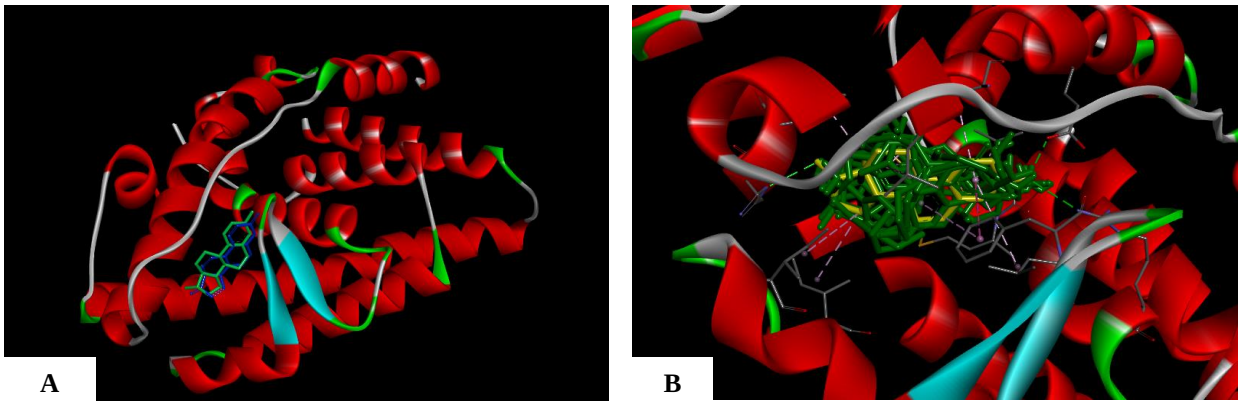

Figure 2. Docking Result : (A) Ligand position after docking (green) in redocking process, (B) interaction between predicted compound (green) and original ligand (yellow) with amino acids in ER-β

Table 3. Compounds from 96% ethanol extract of *M. crenata* leaves which act as ER-β agonist

No.	Compound Code	Compounds	Binding Affinity	TPSA	% Area	Amino Acid Interaction
1	MTL7	Prochlorperazine	-4.9	35.02	0.1836	Glu305, His475
2	MTL17	1-methyl-2-[(4-methylpiperazin-1-yl)methyl]benzimidaol-5-amine hydrochloride	-5.8	50.32	0.3096	Glu305, His475
3	DCM10	12-Aminododecanoic acid	-5.6	63.32	0.0246	Glu305, His475
Control	-	17β-estradiol	- 10.5	-	-	Glu305, His475, Arg 46

Table 3. Compounds from 96% ethanol extract of *M. crenata* leaves which act as ER-β antagonis

No.	Compound Code	Compounds	Binding Affinity	TPSA	% Area	Amino Acid Interaction
1	DCM24	2-Pyridylethylamine	-4.5	38.91	2.8467	Glu305
2	DCM4	11-Aminoundecanoic acid	-6.6	63.32	0.1072	Glu305
3	MTL6	3-Hydroxy-2H-chromen-2-one	-6.5	50.44	0.3629	Glu305
4	DCM11	Cyclazocine	-7.5	23.47	0.1302	Glu305
5	DCM14	2-Amino-2-tetradecyl-1,3-propanediol	-6.1	66.48	0.3879	Glu305
6	DCM16	Pentadecylamine	-6	26.02	0.0123	Glu305

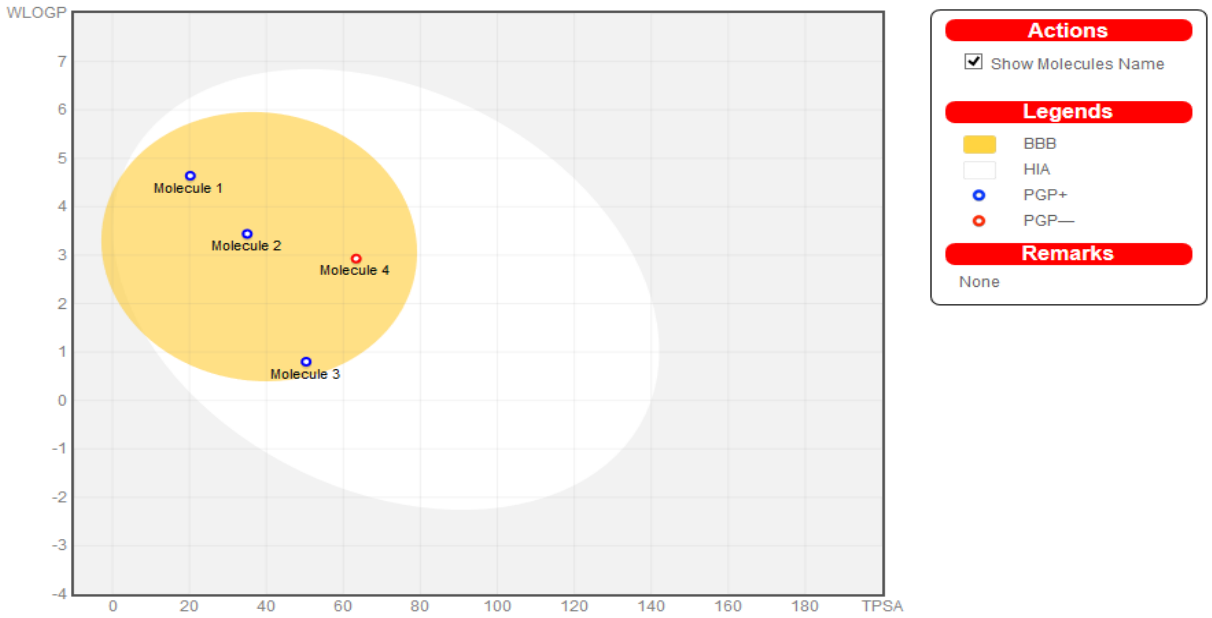

Figure 3. Boiled-Egg diagram of three compounds from 96% ethanol extract of *M. crenata* leaves which act as ER-β agonist : Molecule 1 : 17β estradiol; Molecule 2 : Prochlorperazine; Molecule 3 : 1-methyl-2-[(4-methylpiperazin-1-yl) methyl] benzimidaol-5-amine

From the Boiled-Egg diagram in Figure 3, it can be observed that the three compounds which act as ER-β agonist and 17β-estradiol as positif control are in the yellow region, which indicates that these compounds can penetrate the BBB and can work on ER-β. In the diagram, all compounds except DCM10 (12-aminododecanoic acid) has a blue colour indicating that it can bind to the P-gp substrate in the brain, which causes the compound to have a short duration of action and easily excreted from the body [17]. While the DCM10 did not show any bond with the P-gp substrate, so it could work with longer duration in the brain [40].

ER-β is present in several types of cells in the brain, one of them is microglia cells. Microglia cells are responsible for the neuroinflamation process which is one of the main neurodegenerative causes [11]. In neuroinflamation, an increase in the immune system response characterized by an increase in the number of microglia cells activated in M_1 polarity pathway due to estrogen deficiency, causes an increase in inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and nitric oxide (NO) in the brain [41]. This event will reduce synaptic function and neuronal plasticity, and induce neuronal cell

apoptosis [11, 41]. Even so, further research is still needed to ascertain the activity of compounds in 96% ethanol extract of *M. crenata* leaves as anti-neuroinflammatory.

CONCLUSIONS

From UPLC-QToF-MS/MS analysis, There were 75 compounds contained in 96% ethanol extract of M. crenata leaves, either detected compounds (59 compounds), or unknown compounds. The molecular docking simulation shows there are 3 compounds that are predicted to have activities similar to 17β-estradiol as anti-neuroinflammatory, they are prochlorperazine, 12-Aminododecanoic acid, and 1-methyl-2-[(4-methylpiperazin-1-yl)methyl]benzimidaol-5-amine hydrochloride.

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