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Butenedioic Acid of Soursop Leaves (*Annona muricata*) Water Extract as Dipeptidyl-peptidase 4 (DPP4) Inhibitor

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Abstract. *The aim of the study was to prove the potential of Soursop Leaves Water Extract (SLWE) content as DPP4 inhibitor an in silico method. The extraction process of soursop leaves uses water solvent with the infusion method. Withdrawal of the active compounds using acetone solvent. Identification using the GCMS method. Molecular docking of SLWE active compounds, Linagliptin as a control and DPP4 protein target using Autodoc Vina application. 2D visualization using LigPlot. Validation of affinity of active compounds of Annona muricata Leaves using the measurement results of free binding energy, the number similarity of bond position to active site ot the protein target, and the number of hydrogen bond compared to controls. To determine the potential effect as a drug and toxicity using the 5 rules of Lipinski and ADME. The results of molecular docking found that the active SLWE compounds that have affinity close to control is butenedioic acid. It has the ΔG of -7 kcal / mol, binds 53% of amino acid residues of DPP4 and has one hydrogen bond. Based on 5th rules of Lipinski and ADMET, Buetenoic acid has the potential to be developed as DPP4 inhibitor drugs which is administered orally and had a non-toxic effect. The the Conclusion Butenedioic acid, the active compound found from SLWE is potential as DPP4 inhibitors*.

Insulin resistance is a condition of decreasing the sensitivity of cells in the tissue to normal insulin levels. This condition is characterized by impaired glucose uptake and oxidation processes, decreased glycogen synthesis, increased gluconeogenesis and reduced ability to inhibit lipid oxidation. Insulin resistance is the most common complication of obesity and increases the risk of developing type 2 diabetes (De Tata, 2014).

Dipeptidyl Peptidase -4 (DPP4) is a protein that plays a role in glucose metabolism. DPP4 is widely expressed in vascular endothelium, liver, pancreas, intestinal epithelium and renal tubule, and immune cells (CD26) (Deacon, 2019). DPP4 cleaves other peptide amino acid residues at the position of proline, valine, gly-

cine, alanine, and serine (Santana, Godoy-matos and Kraemer-aguiar, 2015; Deacon, 2019). Conditions of obesity and type 2 diabetes cause an increase in DPP4 production and activity (Santana, Godoy-matos and Kraemer-aguiar, 2015). The effects of increasing DPP4 include causing insulin resistance in fat tissue and liver due to decreased expression of GLUT4 (Spellman, 2011), activating anorixogenic Y2 receptor (Gautier-Stein and Mithieux, 2013), catalyzing GLP-1, GIP and oxintomodulin (Santana, Godoy-matos and Kraemer-aguiar, 2015), inducing the formation of inflammatory mediators (Zhong, Kankanala and Rajagopalan, 2016), stimulating cell α pancreas to activate proglucagon gene synthesizing and secreting GLP-1 (Deacon, 2019), and inhibiting Stromal Derived Factors (SDF-1) (Haddad et al., 2020).

Incretin based therapy consisting of GLP-1 agonist class drugs and DPP4 inhibitor class drugs successfully reduce blood glucose levels, reduce HbA1c, prevent weight gain, and reduce the incidence of side effects in the form of hypoglycemia in type 2 DM patients who experience failure with metformin or glibenclamit treatment (Spellman, 2011; Gallwitz, 2019). The DPP4 inhibitor class drug that most potently inhibits DPP4 is Linagliptin. Linagliptin can reduce blood glucose levels, Hb A1c, lose weight, and prevent hypoglycemia in type 2 DM patients who are tolerant of metformin and sulfonyl urea groups. Linagliptin is safe for use in patients with renal failure, hepatic dysfunction, the elderly and obese (Guedes et al., 2013). In addition to the benefits of using incretin-based therapy, several researchers mentioned the side effects of using this drug in the long term because it increases the risk of acute pancreatitis and medullary carcinoma of the thyroid gland (Zhong, Kankanala and Rajagopalan, 2016).

The use of herbs to prevent disease or treat disease is common among Indonesians, especially in rural communities (Kemenkes RI, 2018). The belief that herbal plants can cure various diseases, are safe and at low prices is the main reason for the use of traditional health services (Ekor, 2014). Indonesian people usually consume herbal medicine in the form of decoction (Elfahmi Woerdenbag and Kayser, 2014). One of the herbs that are widely consumed by people to reduce blood glucose levels is soursop leaves (Moghadamtousi et al., 2015). Soursop leaves (*Annona muricata*) contain active compounds, especially the acetogenin group, followed by other groups such as alkaloids, terpenoids, coumarin flavonoids, steroids, fatty acids, phlobatin, phenolic compounds, tannins, and saponins (Bhardwaj et al., 2019). In vivo experimental animals soursop leaves have been shown to lower blood cholesterol levels (Adeyemi et al., 2008), increase insulin secretion (Adeyemi et al., 2009), reduce blood glucose levels and body weight through anti-inflammatory mechanisms (Ishola et al., 2014), antioxidants (Florence, Benoit and Jonas, 2014), and reduce glucose absorption (Yunivita, Lubis and Arifin, 2019). However, so far there has been no research discussing the potential of soursop leaf water extract as an inhibitor of DPP4.

In silico is a computer-based modeling method that is applied in the process of new drug discovery (Wadood et al., 2013). Molecular docking is an in silico method that uses the principle of structure based drug design or ligand based drug design (Wadood et al., 2013; Aamir et al., 2018). The use of this computer-based modeling method serves to predict the affinity of an active compound for a protein to cause biological effects. The advantage of using this computational method is also to reduce the time and cost of researching new drug discoveries (Wadood et al., 2013). To understand the pharmacodynamic processes of a drug, it is very important to know the physicochemical, pharmacokinetic and toxic properties of the drug. PkcSM was used to predict physicochemical properties, ADME and toxicity of natural active compounds (Lagorce et al., 2017).

Based on the description above, the researchers are interested in researching the mechanism of SLWE as a DPP4 inhibitor using in silico method.

METHOD

Tools and Materials Used

The tools used in the study were a laptop with hardware specifications: Intel® Pentium® Core i5 @ 1.86Ghz, 4GB RAM, Windows 8 64-bit

Operating System connected to an internet connection, rotary evaporator R-300 (Buchi, Switzerland), oven UN-30 (Memmert, Germany), GCMS (Shimadzu QP 2010, Japan). The materials used were 0.05N ammonia solution, FeCl3 solution, 2N HCl solution, 2N sulfuric acid (H2SO4), acetic acid anhydride, Ac2O, Meyer reagent, soursop leaf powder, fresh soursop leaves, aquadest, Hexana Solution (Merck), Solutions acetone (Merck).

Procedure For Making Soursop Leaf Water Extract (SLWE)

Soursop leaves were selected good leaves, not too old or too young (about 3-4 from the base) soursop leaves that had been selected, then washed thoroughly with running water and drained. Soursop leaves were roasted at a temperature of 50 C, until soursop leaves were obtained with a weight of about 10 % of the wet weight. Dried soursop leaves in a blender to get dry soursop leaf powder. 250g of soursop leaf dry powder was taken and put in an infusion pan and added distilled water with a ratio of 1:10. Extraction using the infusion method. Heating was carried out for 15 minutes which counts from when the temperature in the infusion pan reaches 90^{o}C. Filtering using Whatmann paper no 1. The extract was evaporated using a rotatory evaporator to evaporate water. The evaporation process is carried out at a temperature of 600C, until it reaches a volume of 1/3 of the initial volume (Vongsak et al., 2013). This procedure had been conducted in Biochemistry Laboratory of the Faculty of Medicine, University of Islam Malang. The thick liquid extract was freeze dryed to remove the water component and obtained dry extracts of 35-36g with a yield of 14% (Shukla and Road, 2011). The freeze drying process was carried out in the central laboratory of Ma Chung University, Malang.

SLWE active compound identification procedure using GCMS

GCMS is used for qualitative identification and quantitative measurement of the individual components in volatile complex mixtures (Sri Widyawati et al., 2014). Identification of active compounds from aqueous extracts that are volatile cannot be done using the GCMS method because GCMS colums are not compatible with water components (Wonorahardjo et al., 2015). In order for identification, the water extract must be treated to remove the water component. SLWE was carried out freeze drying to remove aqueous components (Shukla and Road, 2011; Bhatta, Janezic and Ratti, 2020), then dissolved with acetone solvent in a ratio of 1 (g): 1 (ml). The purpose of using acetone solvent is to attract the active compounds in SLWE which are polar and semi-polar (Chiou, Kobayashi and Adachi, 2013). The interpretation of the results from GC-MS was converted to the data base contained in Wiley8.Lib based on the mass spectrum. The Mass Spectrum of active compounds from GC-MS results will be compared with the Mass Spectrum of compounds contained in the Wiley8.Lib data base (Ezhilan and Neelamegam, 2012). The higher the percentage of similarity, it is assumed that the active ingredients identified are closer to the truth. Identification of active compounds using the GCMS method was carried out at the Advanced Materials Laboratory, State University of Malang.

To identify active compounds, 10g of dry extract is dissolved in acetone solution, then analyzed by GCMS with GCMS optimization conditions as follows:

Table 1 Optimization conditions for the GCMS tool

Collum Oven Temperature	*80°C*	
Injection Temperature	*250 ° C*	
Injection mode	*Split*	
Flow control mode	*Pressure*	
Total Flow	*588.8 mL/min*	
Collum flow	*1.46 mL/min*	
Linier velocity	*45.5 cm/sec*	
Purge flow	*3.0 mL/min*	
Split ratio	*400*	
Oven temperature program		
Ratte (min)	*Temperature*	*Hold time*
-	*80.0*	*1.00*
10.00	*250.0*	*1.00*
Equilibrium time	*3.0 min*	

In silico Study Procedure

The 3D structure of the active compound SLWE and the control of the DPP4 inhibitor was downloaded from the Pub Chem server (https:// pubchem.ncbi.nml.nih.gov) with the CID recorded. Docking is done using Autodock Vina on the PyRx 9.5 program. The target protein used is DPP4 (PDB 2RGU Chain A). Meanwhile, the ligands used are the active compounds from EADS that have been identified. The ligand control used was Linagliptin. Visualization of docking results using LigPlot 2.1. Docking that is done is specific docking by mimicking the binding binding between the target protein and the control drug. The docking grid box is as follows: Vina Search Space for DPP4. Center X: 50.66, Y: 49,547, Z: 35,987, dimensions (Angstrom) X: 18,957, Y: 11,848, Z: 13,595. The validation of the affinity of SLWE active compounds for the DPP4 protein was assessed based on the value of free energy binding, the number of bond of ligan to protein DPP4 amino acid residues and the number of hydrogen bonds compared to controls.

RESULT AND DISCUSSION

Identification of active compounds using the GCMS method

The results of identification of SLWE active compounds using the GCMS method confirmed by Willey Lib.8 are listed in the table below.

Table 2 Results of Identification of The Active Compound SLWE with Acetone Solvent

Peak	RT	SI (%)	MW	Area (%)	Active compound
1	5.045	94	134	19.27	Benzene, 1,2,3,4-tetramethyl
2	6.44	98	228	3.94	2-Oxazolidinone, 3,3'-ethylidenebis[5-methyl,
3	6.44	98	126	3.94	Ethane, 1-bromo-2-fluoro-, 2
4	6.44	98	172	3.94	Butenedioic acid (e)-, diethyl ester
5	10.051	100	395	4.42	2-(((carbobenzyloxy) amino) methyl)-4-benzyl-5-((carbomethoxy)-amino) oxazole
6	10.051	100%	530	4.42%)	Ditridecyl Ester Phthalic Acid.

RT: retention time, SI: similary index, MW: molecular weight . Benzene 1,2,3,4 tetrametyl is the active compound of SLWE with the largest area.

The results of the identification of the active SLWE compound obtained Benzene 1, 2, 3, 4-tetramethyl (an aromatic hydrocarbon compound with a non-polar benzene and 4 methyl groups) # 2-Oxazolidinone 3,3'-ethylidenebis [5-methyl (a polar compound group alkaloid) # Ethane, 1-bromo-2-fluoro-, 2 (polar alkaloid group compound) # Butenedioic acid (e) -, diethyl ester (polar dicarboxylate compound) # 2 - (carbobenzyloxy) amino) methyl) -4 -benzyl-5 - ((carbomethoxy) -amino) oxazole (a polar alkaloid group) and Ditridecyl Ester Phthalic Acid (a polar aromatic dicarboxylate compound) (Pubchem). Of the seven active compounds, Benzene 1,2,3,4-tetramethyl (19%) is the compound with the largest amount. The purpose of using acetone solution as a solvent in this study was attract was to attract polar and semi polar SLWE active compounds (Chiou, Kobayashi and Adachi, 2013). The existence of non-polar active compounds in this research is already due to the effect of heating done during the extraction process.

Potential of SLWE Compounds Inhibit DPP4 In Silico

Molecular Docking method was used to predict the affinity of SLWE active compounds against the DPP4 target protein, with rigid docking approaches and specific docking. The results of molecular docking between SLWE active compounds against the DPP4 target protein can be seen in the table below.

Table 3 Molecular Docking Results of All SLWE Active Compounds (PDB 2RGU Chain A)

Pubchem ID	Active compound	Free Energy Binding (kcal/mol)
7269	Benzene, 1,2,3,4-tetramethyl	-5.5
8379	Ditridecyl ester phthalic acid	-5.9
73949	2-Oxazolidinone,3,3'-ethylidenebis[5-methyl,	-3.7
12982	Ethane, 1-bromo-2-fluoro-, 2	-2.8
588882	Butenedioic acid (e)-, diethyl ester	-7.0
Control	**Linagliptin**	**-10.4**

Table 4 Intermolecular Interactions Between Linagliptin, SWLE Active Compounds and DPP4 Protein (PDB 2RGU Chain A).

Active compound	Hydrogen bond	Hydrophobic bond	The number of DPP4 amino acid residues bound by ligands (%)
Linagliptin	Tyr631, Glu205, Glu 206	Tyr662,Tyr666, Trp629,Val656, Phe357, Tyr752, Ser630,His740, Tyr547	69%
Ditridecyl Ester Phthalic acid	Ser630,Arg125	His740,Tyr631, Val546, Trp629, Tyr547,Tyr662, Val656, Glu206, Phe 357	61%
Benzene 1.2.3.4 tetrametyl	-	Arg358,Arg669 ,Phe357,Glu20 6,Tyr547,Tyr66 6	23%
Butenedioic acid (e)-,	*Tyr547*	*Tyr662*,V*al656* Trp659,*Ser630 Phe357*,*Glu206* Tyr666 , *Tyr631*	53%

Docking is done using Autodock Vina on the PyRx 9.5 program. The target protein used is DPP4 (PDB 2RGU Chain A). The active side of DPP4 consists of amino acids Arg 125, Glu 205, Glu206, Phe357, Tyr 547, Ser 630, Tyr631, Val656, Tyr662, Tyr667, ASN710, Val 711 and His740 (Gallwitz, 2019). The drug used as a control was Linagliptin.

The results of in silico study conducted by researchers showed that Linagliptin had the highest affinity among other DPP4 inhibitors based on the value of nilaiG (-10.9) and the number of bonds in the active side amino acid residue of DPP4 as much as 69%, and had 3 hydrogen bonds. The active compound of SWLE, namely butenedioic acid, has an affinity for DPP4. Butenedioic acid has Δ G of -7 kcal / mol, binds to the amino acid residue of DPP4 by 58%, and 1 hydrogen bond.

The In silico method is a computational method to predict the potential of a compound against its target protein. This method has been developed in order to find new drugs. Molecular docking is an in silico method that uses the principles of structure based drug design or ligand based drug design [20]. In order to have an effect, a drug/ligand will bind to the receptor or drug/receptor ligand interaction. The principle of the drug/receptor ligand interaction is that the ligands will bind to specific receptors causing the conformation of the receptors, then inducing a biological effect. The ability of a drug or ligand to bind to a receptor is called the affinity of a drug or ligand (Noblet, 2008).

The affinity of a drug with a receptor depends on the similarity in structure and shape of the ligand to the receptor, the size of the ligand and the electrical energy generated by a bond (Noblet, 2008; Aamir et al., 2018). The similarity of structure is determined from the RMSD value. The smaller the distance between the drug molecule and the target protein, the more likely it is that the drug has a structure similar to its receptor (Aamir et al., 2018). The size of the molecules that are less than 900 are called small molecules. The smaller the size of a molecule, the easier it is to bind to the receptor (Ghersi and Roberto Sanchez, 2009).

Affinity is also determined by the type of chemical bond. Chemical bonds can result from electrostatic attraction between oppositely charged ions such as in ionic bonds or by sharing electrons such as in covalent bonds. The strength of a chemical bond depends on the transfer of electrons to 2 atoms. The electrostatic bond type is the type of bond most often used to determine the strength of the bond between the drug and the receptor, namely the hydrogen bond (Kojić-Prodić and Molčanov, 2008) (Batista et al., 2016). This is because hydrogen bonds are weak bonds, and form drug and receptor bonds that are reversible. In addition, hydrogen bonds are very important to determine the structure and characteristics of a molecule. The greater the number of hydrogen bonds between the drug and the receptor, the stronger the drug affinity for the receptor (Chen et al., 2016). In addition to hydrogen bonds, hydrophobic bonds also play a role in the strength of a compound's affinity for its target. The more hydrophobic bonds, the stronger the affinity of a compound for its receptor (Berg, Tymoczko and Stryer, 2002).

Table 5 Physicochemestry of SLWE Active Compound

Properties	Butenedioic Acid	Ditridecyl Ester Phthalic Acid	Benzene 1.2.3.4 tetrametyl
Molecular Weight	116.07	530.8	134.22
AlogP	-0.29	10.62	2.92
H-Bond Acceptor	2	4	0
H-Bond Donor	2	0	0
Rotatable Bonds	2	26	0
Lipinski rules	Yes	No	Yes

Active compounds that have free energy binding greater than -5 kcal/mol are not listed. Prediction of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and Rule's of 5 of Lipinski using PKCSM on line tool.

Table 6 Prediction Farmacokinetics of Active Compounds of Nimba Leaves

Properties	Butenedioic Acid	Ditridecyl Ester Phthalic Acid	Benzene 1.2.3.4 Tetrametyl
Human Intestinal Absorbtion (%)	78	87	95%
Blood Brain Barier (logBB)	-0.13	-0.65	0.54
Metabolism	No active /inhibitor CYP450	CYP3A4 substrate	CYP1A2inhibitor
Excretion (log ml/mnt/kg)	0.89	1.92	0.29
Carsinogenic	No	No	No
Hepatotoxic	No	No	No
Water solubility (log mol/L)	-0.64	-5.27	-3.22

Based on prediction physicochemestry and ADMET, Butenedioic acid is a small molecule, water soluble, good intestinal absorption, non toxic to CNS, no activation or inhibition of CYP450 enzime, good excretion, non toxic and non carcinogenic

Pharmacokinetic indicators based on the process of absorption, distribution, metabolism and excretion. Absorption is assessed by the ability of the drug to be absorbed in the intestine. The value of absorption in the intestine is said to be high if the value is more than 60%. The distribution of the drug is assessed based on the amount of free drug in the circulation. The distribution process is assessed from the ability of the drug to penetrate the blood brain barrier. The higher the BBB value, the drug is estimated to have the ability to penetrate the blood brain barrier so that it affects neuron cells in the brain. The process of drug metabolism that occurs in the liver aims to change drugs or xenobiotics to become inactive, and to change drugs/xenobiotics that are lipophilic to hydrophilic so that they are easily excreted through the liver, biliary system, and kidneys. Excessive CPY activity will cause an increase in the production of free radicals which can cause hepatotoxic effects, but the inhibition of P450 (CYP) activity also causes the drug not to be metabolized so that the risk of drug intoxication increases. The process of excretion shows how many drug metabolites are excreted by the kidneys, liver and biliary system per unit time. The more excreted, the lower the possibility of toxicity due to drug accumulation in the blood. The toxicity of a drug is seen from the possibility of inducing hepatotoxicity and carcinogenicity when given in the long term (Lagorce et al., 2017; Sultan et al., 2020).

Soursop leaves are often consumed by people to reduce blood glucose levels and lose weight (Moghadamtousi et al., 2015). In vivo study showd that soursop leaves are known to have the ability to reduce blood glucose levels (Adeyemi et al., 2009), improve pancreatic β cell function and reduce body weight through mechanisms as antioxidants (George et al., 2015), increase insulin secretion, reduce TNF-α levels, increase Leptin levels, and prevent excessive pancreatic beta cell proliferation (Damayanti, Kusuma and Soeatmadji, 2019).

The active compound SWLE, Butenedioic acid has the ability to inhibit DPP4 based on the bond-free energy value of -7kcal / mol, the number of DPP4 amino acid residues that are bound is 53%, and 1 number of hydrogen bonds compared to controls. Butenedioic acid meets Lipinski criteria and is well absorbed in the intestine, cannot penetrate the blood brain barrier, not actived or inhibit CYPP450 enzime, good excretion, non carcinogenic or hepatotoxic. Phthalic acid has the ability to bind to the

active site of DPP4 protein better than butenedioic acid, but phthalic acid has higher free energy binding and lower solubility. Thus, phthalic acid is predicted to have a lower affinity to DPP4 protein than butenedioic acid.

CONCLUSION

The conclusion that can be drawn from this study is that EADS contains the active compound Butenedioic acid with affinity for DPP4 and has the potential to be used as a drug that can be administered orally as a DPP4 inhibitor. In the development of drug discovery, in silico research is an initial step to predict the mechanism of action of an active herbal compound to cause pharmacological effects. Further research is needed to prove the potential of soursop leaf water extract as an inhibitor of DPP4 both in vitro and in vivo in animal models of obesity or type 2 diabetes.

Conflict Of Interest

The researcher stated that there was no conflict of interest in this study. This research is a part of the dissertation that has never been published.

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