Elucidation of the Mechanism of Indonesian Traditional Medicine (*Jamu*) Based on Case Studies of Type 2 Diabetes Networks

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ABSTRACT

Plant medicine is a kind of plant that can be used to solve various problems in the human body either due to illness or other disorders. *Jamu* is an Indonesian traditional medicine. It is essentially herbal medicine that made from natural materials taken from several parts of medicinal plants which contain some substances and compounds that important and beneficial for the body. So far, the efficacy for some type of *jamu* has been proven empirically. In this paper, to fill this gap, we intend to elucidate the mechanism of *jamu* using computational base approach. This research focus to a *jamu* for Type 2 Diabetes (T2D) which prescription consist of four plants: *Ginger (Zingiber officinale)*, *Bratawali (Tinospora crispa)*, *Sembung (Blumea balsamifera)*, and *Bitter Melon (Momordica charantia)*. The framework of analysis starts with generating the network with 3 components: active compounds, proteins target and gene ontology. After that, will implement clustering to those components using concept of tri-partite graphs fuzzy clustering. The main ingredients of 15 active compounds have high score probability which divided in different cluster by the pair of active compounds that have high synergic effect. T2D is not solely caused by protein abnormalities from insulin secretion (isoform insulin-degrading enzyme 1), but also caused by other proteins that involved in the inhibition of insulin in the pancreas. These proteins are Alpha-2C adrenergic receptors, beta-1 adrenergic receptor, and peroxisome proliferator-activated receptor delta, which have high probability in the same group.

Keywords — Fuzzy Clustering, Graph Tri-Partite, *Jamu*, Network, Type 2 Diabetes

I. INTRODUCTION

Disease is an abnormal condition in the body that can cause damage to those it affects. There are some alternative treatments that can be done, such as chemical treatment and treatment-based plants. Chemical drugs, usually involves only one chemical compound single with one target (one-drug-one target), is different from medicinal plants involves many components of chemical compounds that works on one or several targets (multicomponent-network target) [9]. Many of the diseases that are common in humans. Degenerative metabolic disease is a disease caused by metabolic disorders due to consumption of various types of instant foods that are not controlled by the degeneration process. There are some types of degenerative metabolic diseases like type 2 diabetes, stroke, hepatitis, cardiovascular, and dyslipidemia. In 2012, the number of people with diabetes is estimated more than 371 billion and 90% of them with type 2 diabetics. Type II diabetes is a disease with characterized by high blood glucose levels. It is due to insulin in the body is weak and therefore can not control the circulation of glucose properly, made glucose levels in the body increase. It can happen due to a less regulated lifestyle, age, pregnancy and overweight (obesity) [2].

Base on research herbs for type 2 diabetes, which is being developed at the Center for Biopharmaceutical Research in Bogor. In the research tested on zebrafish as an animal model given special treatment, there are four plants: bitter melon (*Momordica charantia*), sembung (*Blumea balsamifera*), Bratawali (*Tinospora crispa*) and ginger (*Zingiber officinale*) is predicted to treat type II diabetes. Therefore, research will be conducted to formulate herbal medicine to treat type 2 diabetes in humans. Herbal medicine, which involves the active compounds of plants, through the computing-based statistical approach can be used to find compounds which were actively treating a specific disease based on the target protein. In previous research [7], using the NIMS method (Network targets-based Identification of Multicomponent Sinergic) to see the synergistic effect between the combination of active ingredients (a score of synergies). It was shown on the score of the synergies that have relatively high. NIMS method is an effort to determine the mechanism of action of a medicinal herb. Couple several active ingredients that have a relatively high score and significant synergies are couple some of the active ingredients of plants ginger and bitter melon, Bratawali plant and ginger, and sembung and Bratawali plant, as well as crop sembung and Ginger. Analysis of the data network pharmacology (drugCIPHER method) is performed to find the target proteins that have greatest correlation with each compound. DrugCIPHER method capable of changing the closeness between the protein and the chemical structure of the compound into numerical values that can be analyzed and statistical

computation. There are two of three compounds Bratawali and 11 of 44 compounds ginger as a potential antidiabetic drug. Otherwise, sembung and Pare have a potential as an antidiabetic plant.

Based on the result to get a potion an-diabetic, will explore Gene Ontology biological process proteins targeted by the compounds contained in the four plants and processes associated therein. Gene Ontology (GO) biological process is a process of biological proteins which involves a lot of genes in it. The mechanism of biological processes is described by a computational approach based on the active compounds, protein target, and biological processes. The four of plants that are predicted to treat type II diabetes found as many as 416 unique proteins derived from 58 active ingredients that target proteins. To determine the mechanism of biological processes, statistical methods can be applied with a network using graph Tri-partite fuzzy clustering. Then, the information obtained compounds that lead to specific groups of proteins with biological processes owned efficacious as a diabetes drug.

II. METHODOLOGY

This research uses the data of the active compound, target protein, and GO-BP from herbs Bratawali (*Tinospora crispa*), Ginger (*Zingiber officinale*), bitter melon (*Momordica charantia*) and sembung (*Blumea balsamivera*)) that are being developed in the Center for Biopharmaceutical Research in Bogor, predicted to treat type 2 diabetes. The working mechanism analysis of *jamu* use Graph Tri-Partite Fuzzy Clustering. The illustration can be seen in Figure 1 and Table I.
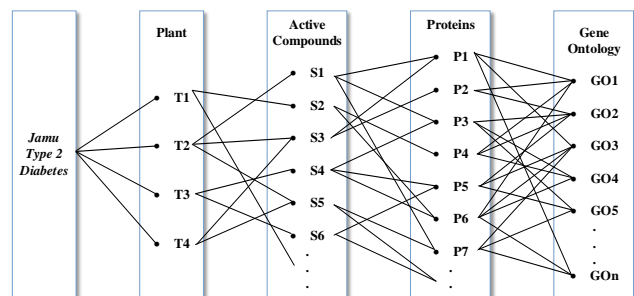

Figure 1: Illustration Structure of Data Research

Table I
Number of Active Compound and Target Protein

Plant	Compound	Source	Protein	Source
Ginger	47	KNApSAcK	2394	Pubchem
Bitter Melon	4	KNApSAcK	47	Pubchem
Sembung	4	KNApSAcK	548	Pubchem
Bratawali	3	KNApSAcK	70	Pubchem
Sum	**58**		**3059**	

III. GRAPH TRI-PARTITE

Graph G = (V, E), with V = {S1, S2, …, S58, P1, P2, …, P413, GO-BP1, GO-BP2, …, GO-BP3104}, and connection E = S1, S2, …, S58, P1, P2, …, P413, GO-BP 1, GO-BP 2, …, GO-BPn.

1. Form the matrix A which as active compound, target protein, and GO-BP.
2. Initialization of matrix B and C randomly in the form of non-negative matrix to perform the next iteration.

$$A^{(ij)} = C^{(i)} B^{(ij)} \left(C^{(j)} \right)^T$$

3. Conducting iteration to obtain a matrix B, the matrix B is a matrix whose elements consist of the results element clustering the proximity matrix A.
4. Conducting iteration to obtain a C matrix, the matrix C is the matrix in the form of opportunities element each node on a particular group.
5. Iteration matrix B and C carried out to obtain convergent results.
6. Decomposition matrix B and C using graph fuzzy clustering, thus obtained graph H = (V, E) has a more sample.
7. Elucidation target protein biological processes that take place through the group formed.

IV. CONCLUSION

Network of pharmacology is a special approach in an effort to find a new herb medicine [9]. *Jamu* for T2D which prescription consist of four plants: Bratawali, ginger, bitter melon, and sembung have as many as 58 compounds and target proteins as many as 416 unique proteins. The number of connections between the compound and there as many as 3059 proteins (Figure 2).

Networking in Figure 2 is a network of 58 active ingredients originating of four plants Bratawali many as 3 active compounds (B013, B015, B018), bitter melon as many as four active ingredients (P044, P183, P185, P195), sembung as many 4 active ingredients (S001, S002, S030, S031), and the remaining 47 are the active ingredients of ginger plants. Bratawali plants have a connection to the target protein as many as 70 connections. Bitter melon plant has a connection to the target protein as many as 47 connections. Sembung plants have a connection to the target protein as much as 544 connections. Ginger plant has a connection to the target protein as much as 2383 connections.

Each of protein targeted by the compound has the mechanism of action associated with biological processes called Gene Ontology (GO) biological processes. From the 416 targets protein, is owned by 58 active compounds searched GO-BP significantly associated with biological processes on DAVID database by Entrez Gene-ID as the ID

of each protein. GO-BP selected in the DAVID database is the most relevant GO-BP associated with a list of specific genes. Criteria GO-BP DAVID Database significant (p-value ≤ 0.01 and EASE Score = 0.06), obtained as many as 3104 GO-BP of David database, the connection between the protein and the GO-BP as much as 17626 (Figure 3).
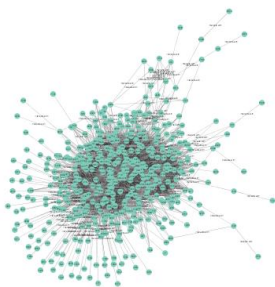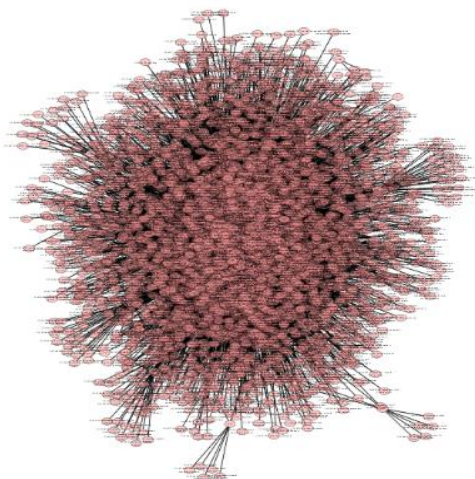

Figure 2: Connect of Compound and Protein

Figure 3: Connect of Protein and GO-BP

Tri-Partite Graph

Graph used in this research consists of 3 components there are active compound, target protein and GO (Figure 1). According to [5] Tri-partite graph that is established between the active compound and target protein, target protein and GO biological process in the form of binary data (0 and 1). On further analysis Graph Tri-partite formed called matrix A. Matrix A in the graph is worth 1 score if the connection between the compounds with protein, or a protein with GO biological process. And, it's 0 score if not have a connection between a compound with protein, or a protein with GO biological process. Moreover, it will be 0 score when the connection between the compounds and compounds, proteins and protein, GO and GO, as well as compounds and GO biological process.

Fuzzy Clustering

In this research method used to determine what are the chances of an object included in a particular cluster is fuzzy clustering method. Fuzzy clustering is a method of non-hierarchical clustering. Fundamental algorithms in this clustering method is determining the amount clump and calculate the degree of membership held on each object. In this research the degree of membership that is used is a tri-partite graph formed above. The basic principle of fuzzy clustering is based on clustering opportunity, or in other words the amount of chances each object to be a particular clump of between 0 and 1 [6].

Compound Fuzzy Clustering

Clustering in the 58 compound that is owned by four of medicinal plants that are predicted to treat type 2 diabetes will be clustered as much as six clusters.

Based on research using the simultaneous clustering method, from the PCA plots and network similarity there are 15 active compounds were predicted as the main composition an-diabetic herbs. Here is presented proteins predicted as the main composition of T2D.

Table II
The Predicted Protein Main Ingredients Medicinal Herb T2D

ID-S	Name
B018	N-Formylanonaine
J036	Gingerols; (6)-Gingerol
J270	Hexahydrocurcumin
J010	Gingerdiols; (6)-Gingerdiol, 3-Epimer
J044	Gingerols; (6)-Gingerol, Demethoxy
J135	(3R,5R)-form, 3,5-Diketone, 3',3''-di-Me ether
J153	4-(3,4-Dihydroxyphenyl)-2-butanone; 3-Me ether
B013	(-)-Secoisolariciresinol
J091	Shogaols; (8)-Shogaol
J127	(3S,5S)-form, 3',3''-Di-Me ether
J205	Safrole
J206	(6)-Shogaol
J249	10-Shogaol
S030	3,4-Dihydroxybenzoic acid
S031	Xanthoxylin

On the research [7], using the NIMS method of used as an early initiation to estimate the synergic effects between partners active compound based on the phenotype of the disease, acquired several pairs of the active compound with a relatively high score of synergy between different plants. However, by using fuzzy clustering tri-partite graph is only partly a couple of active ingredients that have a relatively high synergy score, a big chance to be in the same cluster (Table III). That 15 of the active compounds is predicted as main ingredients an-diabetic herbs have different activities, but has a target to treat the same disease (T2D).

Table III
The Synergistic Similarity Score

Active Compound Main	Active Compound Candidates
B015	J091
B015	J249
B015	J036
P044	P195
P044	P183
P044	S002
P183	P195
P183	S002
P183	S031
P195	S002
P195	S031

Protein Fuzzy Clustering

Number of Clustering on 416 unique proteins targeted by 58 active compounds is 6 cluster. On the [3] pancreas functions as an endocrine and exocrine organ. Its function as an endocrine organ supported by the islands of Langerhans (Islets of Langerhans) which consists of three types of cells including, cell alpha (α) produce glucagon, beta cells (β) produce insulin and the type of pancreatic cell at most, cell deltha (D) produces somatostatin. The main control on insulin secretion is the direct negative feedback system between β cells that produce insulin in pancreas with the concentration of glucose in the blood. Increased blood glucose levels, a case that occurs after ingestion of food will directly stimulate the synthesis and secretion of insulin by pancreatic β cells. With the increased levels of insulin, it will lower blood glucose to normal levels due to the increased usage and storage of glucose.

The results of statistical clustering, proteins obtained there some proteins of which are involved in cell metabolism α, cell β and D cells in the production of insulin in pancreas are Alpha-2C adrenergic receptor potentially amounting to 0.99 are in cluster 1, Beta-1 adrenergic receptor opportunity for 0.96 is the first cluster, and peroxisome proliferator-activated receptor delta with a chance of 0.95 is at cluster 1. Whereas, for insulin-degrading enzyme isoform 1 is a protein that plays an important role in insulin production is likely to 1.00 at 4 with a cluster of proteins advocates like phosphocholine phosphatase isoform 1, alpha-galactosidase and other proteins that play a role in the production of insulin.

GO Fuzzy Clustering

GO biological process significantly gained on Data Base DAVID gained as 17626 GO is connected with the 416 targets protein. The 17626 Connect of GO owned by as many as 3104 protein unique. On the [8] there are two biological processes involved in T2D. First, the biological processes associated with downregulated, both upregulated of T2D. Gene involved in biological processes in decline regulated T2D namely: cell activation, macromolecule biosynthetic process, hydrogen peroxide biosynthetic process, immune response and regulation of glycolysis. On the results of fuzzy dendrogram clustering GO biological process, clump 1 and 3 the majority of gene-gene is a gene involved in biological processes down-regulation of T2D. Meanwhile, the gene is involved in the upregulated of T2D, namely: aromatic compound biosynthetic process, nitric oxide biosynthetic process, and regulation of glycolysis. The overall biological processes that occur in the 416 proteins a targeted by the NII 4 plants, there are four biological processes involved in the upregulated of the T2D that is: GO: 0006809 ~ nitric oxide biosynthetic process, GO: 0045019 ~ negative regulation of nitric oxide biosynthetic process, GO:0045428 ~ regulation of nitric oxide biosynthetic process, and GO: 0045429 ~ positive regulation of nitric oxide biosynthetic process. From 4 to biological processes that have a big opportunity to be on a clump of 1 and 3.

There are 3 connection weights are quite large at first, by 0.7716 held between GO-BP in cluster 4 and protein on the cluster 2. Secondly, by 0407 the weight of the GO-BP in cluster 2 and proteins on the clusters 2. Third, amounting to 0.2499 weight which is owned by GO-BP in clusters 3 and protein in all 3 clusters. However, of the three connection weights high enough that if it is associated with the results of clustering on proteins that are in clusters 1 is a biological process involved in the metabolic processes of cells α, cell β and D cells in insulin production on pancreas although many biological processes other involved. In addition, the weight of connection you have between clusters of GO-BP with a protein on the 4th cluster should have a great connection weights, since insulin-degrading enzyme isoform 1 is a protein that plays an important role in insulin production is likely to 1.00 in four clusters.

V. CONCLUSION

The results of clustering compounds, protein, and GO-BP were conducted using the Fuzzy Clustering Tri-partite graph method through formed by a few conclusions:

1. The compound predictable as the main composition an-diabetic herbs each has a different activity in tackling the disease, but has the same disease target (T2D).
2. Cluster on the protein a targeted by the compound 58 has a big opportunity to be a particular cluster associated with protein metabolism inhibitor of insulin, leading to T2D. Protein in cluster 1, namely

Alpha-2C adrenergic receptors, beta-1 adrenergic receptor, and peroxisome proliferator-activated receptor delta is involved with inhibition of metabolism of insulin in the pancreas. Meanwhile, the biological processes associated with the production of insulin is insulin-degrading enzyme protein isoform 1 is located on the 4th cluster with proteins such as phosphocholine supporting phosphatase isoform 1, alpha-galactosidase and other proteins that play a role in the production of insulin.

3. The results using Fuzzy Clustering method on GO be obtained cluster 1 and 3 is a biological process involved in increasing and decreasing regulation of T2D. However, the results are more dominant showing gene-gene involved in biological processes down-regulation of T2D.

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