BAREKENG: Journal of Mathematics and Its Applications
December 2024 Volume 18 Issue 4 Page 2459–2470
P-ISSN: 1978-7227 E-ISSN: 2615-3017
https://doi.org/10.30598/barekengvol18iss4pp2459-2470

AVERAGE LINKAGE CLUSTERING METHOD AND MOLECULAR DOCKING STUDY ON DATE PALM (PHOENIX DACTYLIFERA L.) AS POTENTIAL ANTI-ANEMIA AGENT

Siswanto Siswanto[1*], Herlina Rasyid[2], Nur Aliah Ramadhani[3], Nadia Nazwadiah Caesar[4], Nurtiti Sunusi[5], Zaraswati Dwyana Zainuddin[6]

[1,3,4,5]Department of Statistics, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin
Jln. Perintis Kemerdekaan Km 10 Tamalanrea Indah, Makassar, 90245, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin
Jln. Perintis Kemerdekaan Km 10 Tamalanrea Indah, Makassar, 90245, Indonesia
[6]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin
Jln. Perintis Kemerdekaan Km 10 Tamalanrea Indah, Makassar, 90245, Indonesia
*Corresponding author's e-mail: *siswanto@unhas.ac.id*

Article History:

Received: 5th, April 2024
Revised: 3rd, June 2024
Accepted: 30th, July 2024
Published:14th, October 2024

Keywords:

Anemia;
Average Linkage Clustering;
Dates;
Molecular Docking.

ABSTRACT

Anemia, characterized by blood hemoglobin (Hb) levels below the World Health Organization's (WHO) normal limit, remains a significant health concern. Date fruit (Phoenix dactylifera L.) stands out as an herbal plant boasting the highest iron content at 13.7 mg, suggesting its potential as an anti-anemia agent. This study aimed to explore the anti-anemia potential of active compounds in date fruit using average linkage clustering and validated using molecular docking. Compounds from dates were gathered via GC-MS analysis and online databases, totaling 145 compounds—50 from GC-MS and 95 from Knapsack and Dr. Duke databases. Additionally, 5 lead compounds served as positive controls for comparison. SwissADME online servers assessed the compounds' properties, serving as materials for the clustering method. The average linkage clustering method was employed, yielding an excellent dendrogram with a cophenetic correlation of 0.711. Notably, a total of 17 date fruit compounds are in the same cluster as the lead compounds. Molecular docking revealed 4 date palm fruit-derived compounds as potential PHD enzyme inhibitors, promising for anemia treatment. In conclusion, the average linkage clustering method and validation using molecular docking approaches highlight date fruit's potential as an alternative anemia treatment, showcasing the significance of interdisciplinary methodologies in drug discovery.

How to cite this article:

Siswanto, H. Rasyid, N. A. Ramadhani, N. N. Caesar, N. Sunusi, and Z. D. Zainuddin. "AVERAGE LINKAGE CLUSTERING METHOD AND MOLECULAR DOCKING STUDY ON DATE PALM (PHOENIX DACTYLIFERA L.) AS POTENTIAL ANTI-ANEMIA AGENT", *BAREKENG: J. Math. & App.*, vol. 18, iss. 4, pp. 2459-2470, December, 2024.

Journal homepage: *https://ojs3.unpatti.ac.id/index.php/barekeng/*
Journal e-mail: *barekeng.math@yahoo.com; barekeng.journal@mail.unpatti.ac.id*
Research Article · Open Access

1. INTRODUCTION

Anemia is still a serious issue in global health, especially in developing countries like Indonesia. Anemia is characterized by blood hemoglobin (Hb) levels below the normal limit (less than 11 g/dL in pregnant women and less than 12 g/dL in nonpregnant women) set by the World Health Organization (WHO). Anemia can lead to decreased immunity, growth problems, stunted mental development, and decreased physical fitness [1]. Globally, based on estimates made by WHO in 2019, there are almost 270 million children aged 6-59 months and over 500 million women aged 15-49 years old affected by anemia [2]. Meanwhile, In Indonesia the incidence of anemia is quite high, according to Basic Health Research data in 2018, anemia occurred at 32% among adolescents aged 15-24 years [3]. Adolescent girls with anemia are at risk of developing this condition during pregnancy, which can affect fetal growth, such as stunting. Therefore, anemia is one of the focuses of the Sustainable Development Goals (SDGs) [4].

The 2nd SDG, which focuses on "Zero Hunger", makes reducing the prevalence of anemia in adolescent girls one of the crucial targets that need to be done to accelerate progress towards achieving the 2nd SDG. The most common deficiency leading to anemia is iron deficiency, which is referred to as Iron Deficiency Anemia (ADB) [4]. The estimation of anemia made by WHO in 2019 shows that almost 60% of the anemia cases were caused by dietary iron deficiency [5]. In developed countries, ADB is an important public health issue [6]. Pregnant women with ADB have a higher rate of low birth weight (<2500 gr) which causes stunting in infants [7]. One of the strategies to treat anemia is using Blood Addition Tablets as an iron supplement. Unfortunately, research conducted by Moretti and the team shows that more iron supplementation taken would likely produce poorer results because only a relatively small amount of oral iron is absorbed by the body [8]. Furthermore, based on research conducted by Tolkien and the team, it is shown that oral supplements taken on an empty stomach can cause epigastric pain and nausea in some women [9]. Therefore, alternatives such as herbal plants are needed that have less effect [10]. Date fruit *Phoenix dactylifera* L. is an herbal plant that has a lot of vitamins, minerals, folic acid, and zinc. Research conducted by Husnah and team shows a significant increase in Hb levels in participants after the administration of dates [11].

Testing the potential of date fruit as an anti-anemia can be done in vivo. However, in-vivo tests generally require a large number of test animals, time, and labor. Therefore, the development of alternative methods, such as molecular docking, an in-silico test method that is more efficient in terms of time, cost, and energy [12]. The method has been used by Arthi and team in 2023 to identify novel anti-HCV protease inhibitors [13]. Although the results are accurate, this method still requires many tools and stages. Therefore, a more efficient method or approach is needed regarding cost, time, tools, and stages. One possible approach is a statistical approach, such as clustering. The clustering method is used for grouping similar objects based on their characteristics [14]. Clustering methods are divided into two types, namely hierarchical and non-hierarchical. A study conducted by Lu and team in 2016 used an algorithm in the non-hierarchical method, namely K-means, to identify compounds that have potential as alternative lung cancer drugs based on the nature of the compounds. However, this algorithm has not provided sufficiently accurate clustering results because the selection of the optimal number of clusters is based on the subjectivity of the researcher [15].

As an alternative method, the hierarchical clustering method is a more objective choice because the optimal number of clusters does not depend on the subjectivity of the researcher, but is obtained from the dendrogram of the data formed [16]. This method was carried out by Yusniyanti and team in 2021 in mapping provinces in Indonesia based on the welfare of its people and obtained the result that the average linkage method is more effective than the K-means method [17]. This is characterized by a smaller variance ratio, which is 0.08. There are three types of linkage clustering methods: single linkage, complete linkage, and average linkage. In 2021 Reinaldi and team were researching to map community welfare in East Java using three linkage methods: single linkage, complete linkage, and average linkage. The results showed that the average linkage method was also the best method with a silhouette index value of 0.61 [18]. Among the single linkage, complete linkage, and average linkage methods only the average linkage method is used for most applications because both the single and complete linkage methods results can be greatly affected by noise.

To validate the results, in-silico methods known as molecular docking will be employed. In the molecular docking method, the aim is to determine how strongly a drug binds to its target, such as a target protein in the human body. A higher binding energy indicates a stronger bond between the drug and its target, which is desired in drug development to ensure optimal therapeutic effectiveness. In this study, if the binding energy of a drug compound and a date compound that are in the same cluster are similar, then the clustering

result can be assumed to be valid. The identification of active compounds in date fruit will be conducted through the maceration extraction process and Gas Chromatography-Mass Spectrometer (GC-MS) analysis [19].

2. RESEARCH METHODS

2.1 Materials

The materials used were dates fruit (*Phoenix dactylifera* L.), distilled water, and 96% ethanol.

2.2 Tools

The tools used are beakers, erlenmeyer, test tubes, stirring rods, funnels, hot plates, Gas Chromatography-Mass Spectrometer (GC-MS) grinders, ovens, Google Colab, Autodock4, Autodock Tools, Chimera, and Discovery Studio Visualizer.

2.3 Research Variables

The variables used for compound clustering in this research are structural and physicochemical properties of date fruit compounds and compared with synthetic chemical drugs for anemia, especially iron deficiency anemia (IDA), obtained through the SwissADME database at http://www.swissadme.ch/. The properties of compounds used are structural, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry. The obtained active compounds will then be verified using the Simplified Molecular Input Line Entry System (SMILES) to describe their chemical structure in code format. SMILES will facilitate the retrieval of compound properties on the SwissADME website [20].

2.4 Extraction and Identification of Date Fruit

The extraction process was carried out by separating 1 kg of date fruit flesh from the seeds. Furthermore, the date fruit was chopped and dried using an oven at 50°C to form dry simplisia. The date fruit simplisia obtained was then grounded and resulted in 150 grams of date fruit powder. The maceration method was conducted using 96% ethanol solvent for 3 × 24 hours with occasional stirring. After that, all the macerates were combined and then evaporated until a thick extract was obtained. Therefore, the extract was analyzed using a GC-MS instrument to identify the compounds contained in date fruit. Identification of date fruit compounds was also obtained using Knapsack (http://www.knapsackfamily.com/) and Dr. Duke (https://phytochem.nal.usda.gov/) online databases. All compounds collected have their respective SMILES and are collected using the PubChem online database at https://pubchem.ncbi.nlm.nih.gov/. The SMILES code is used to gain research variables in the form of structural and physicochemical properties of compounds in the SwissADME online database.

2.5 Lead Compound Identification

There are five compounds used as lead compounds from synthetic drugs that have previously been tested on living organisms. The lead compound data and research variables are listed in supplementary files. The five lead compounds are:

1. Ascorbic acid [21]
2. Ferric cation [22]
3. Ferric derisomaltose [23]
4. Ferrous sulfate anhydrous [24]
5. Ferumoxytol [25]

All SMILES code of lead compounds was collected from the Drugbank database, and the evaluation of structural properties was obtained from the SwissADME online database.

2.6 Data Analysis

Data analysis in this study used the clustering method to group date fruit compounds that have the potential as an alternative to anemia treatment. The clustering method used is the average linkage method. The following are the steps of data analysis carried out in this study:

a. Standardize the scaler on numeric type data.
b. Decoding categorical type data.
c. Using PCA to reduce the dimensions or research variables.
d. Using the average linkage algorithm to group compounds based on the number of clusters and research variables obtained in the previous stages.

2.7 Molecular Docking Validation

The 3D structure of the test compound was downloaded to the PubChem database and optimized using the AM1 method in the Chimera software. The enzyme used was Prolyl hydroxylase domain (PHD) enzyme with PDB code 3HQU which was prepared by 'dockprep' menu in Chimera [26]. Molecular docking analysis was performed using Autodock4 and Autodock Tools software [27] with a grid box size of 40 × 40 × 40 Å and spacing of 0.375. The algorithm used was the Lamarckian Algorithm [28] and set up to produce 10 conformations. Selection of conformations due to the lowest energy. Visualization of the interaction was done using Discovery Studio Visualizer software.

3. RESULTS AND DISCUSSION

3.1 GC-MS Analysis

Based on the results of analysis using GC-MS, it is shown that there are 50 compounds in the ethanol extract of date palm fruit flesh. 5 compounds with the highest percent area are shown in Table 1. The compounds obtained will be searched for SMILES and structural properties of compounds through PubChem and SwissADME online databases.

Table 1. Date Fruit Compounds Result of GC-MS Analysis

No	Retention Time	Area	Area%	Name
1	8.561	12771115	12.02	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	8.963	11801142	11.10	5-Hydroxymethylfurfural
3	15.858	6977327	6.56	3-Deoxy-d-mannonic acid
4	15.367	6709249	6.31	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)-
5	3.443	6091932	5.73	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl-

SMILES search of date fruit compounds from GC-MS analysis was carried out through the PubChem database which was then used to search for structural properties of compounds in the SwissADME database. In supplementary files, the structural properties of compounds are attached which then become research variables from GC-MS date palm compounds.

3.2 Online Database of Date Fruit Compounds

Based on the results of collecting data on the names of date fruit compounds through the Knapsack and Dr.Duke online databases and searching for SMILES and structural properties of compounds through the PubChem and SwissADME online databases, a list of a collection of 95 date fruit compounds is obtained which is attached in supplementary files for the Dr.Duke online database and supplementary files for the Knapsack online database.

3.3 Principal Component Analysis

Principal Component Analysis (PCA) is used to decompose a large matrix into orthogonal vectors using eigenvectors and eigenvalues. The research variables that have been obtained will be reduced to k new variables called principal components using the Principal Component Analysis algorithm and the resulting 46 principal components are formed. The Cumulative Variance (CV) value is used to determine how much the principal component can explain the total diversity. Visualization for the CV value on the number of principal components can be seen in **Figure 1**.

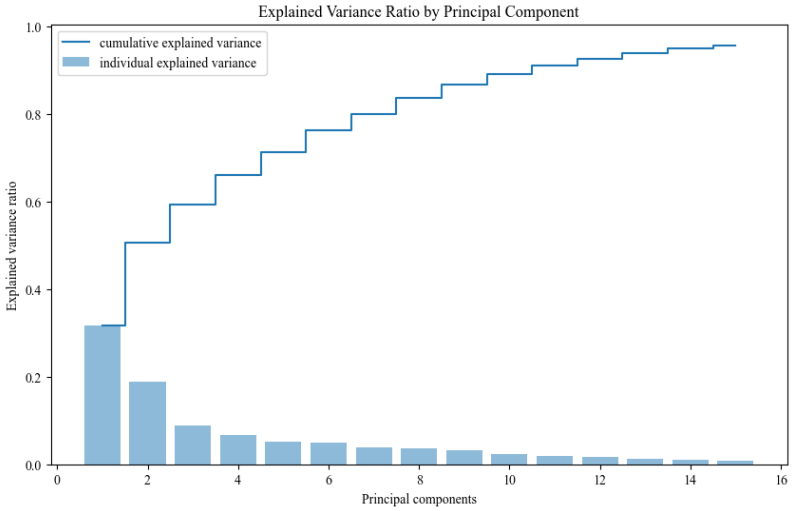

Figure 1. Plot between the Number of Principal Components and CV value

The CV value used is $> 95\%$, so 15 principal components will be used for the Average Linkage Clustering method shown in **Table 2**.

Table 2. Principal Components of PCA Analysis Results

Components	Eigenvalues	Variance
PC 1	13.1410	0.3172
PC 2	7.8088	0.1885
PC 3	3.6307	0.0876
PC 4	2.7876	0.0673
PC 5	2.1433	0.0517
PC 6	2.0386	0.0492
PC 7	1.5961	0.0385
PC 8	1.4717	0.0355
PC 9	1.3308	0.0321
PC 10	0.9361	0.0226
PC 11	0.7928	0.0191
PC 12	0.6834	0.0165
PC 13	0.5130	0.0123
PC 14	0.4253	0.0102
PC 15	0.3221	0.0077

3.4 Average Linkage Clustering

The clustering outcomes utilizing the Average Linkage method exhibit commendable performance, evidenced by a Cophenetic Correlation Coefficient of 0.9435. This value is notably high, considering the

standard range of Cophenetic Correlation Coefficients falling between 0 and 1. The dendrogram of the clustering results is depicted in **Figure 2**.

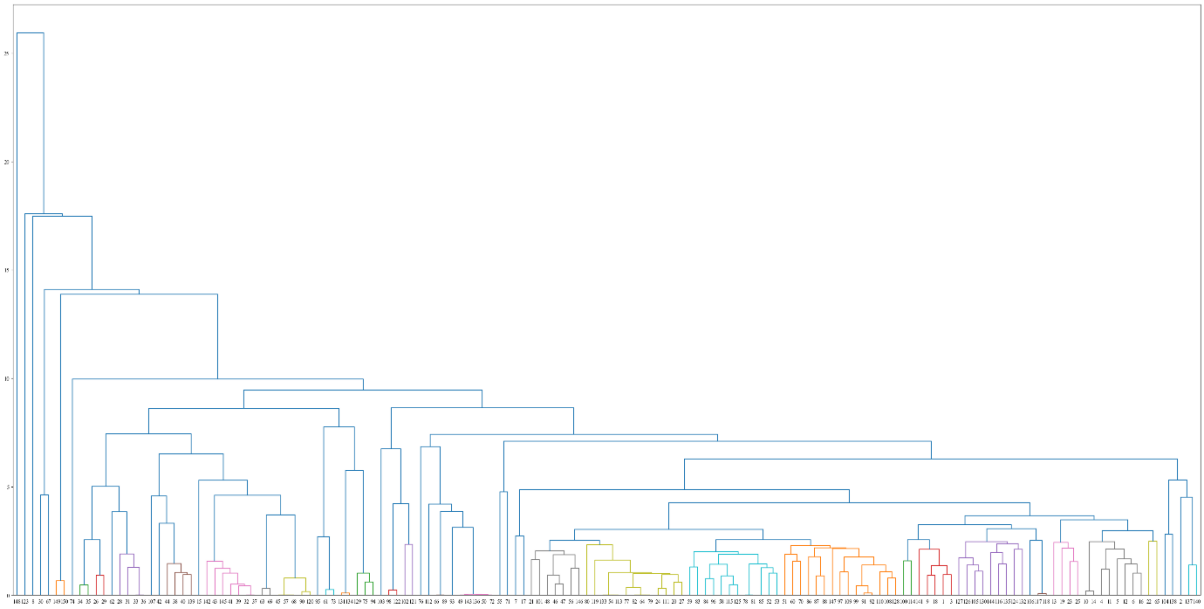

Figure 2. Dendrogram of Average Linkage Clustering

The clustering results indicate 2 optimal clusters with Silhouette Scores of 0.7027 where cluster 1 contains the Ferric derisomaltose compound, while cluster 2 contains all compounds in the data except the Ferric derisomaltose compound. The clustering is repeated to obtain more accurate results until a cluster that shows maximum proximity between lead compounds and date fruit compounds is obtained. Clustering is performed up to 10 times, as shown in **Table 3**.

Table 3. Each Clustering Results

i	N cluster	Silhouette Score	Deleted Compounds Code
1	2	0.7027	147
2	2	0.5664	122
3	2	0.5826	8
4	2	0.4744	30, 67
5	2	0.5043	149, 150
6	8	0.4317	26, 28, 29, 31, 33, 34, 35, 36, 62, 15, 32, 37, 38, 39, 40, 41, 42, 43, 44, 45, 57, 63, 68, 69, 90, 107, 120, 139, 142, 145, 61, 73, 95, 75, 94, 129, 131, 134, 98, 102, 103, 121, 122, 49, 50, 66, 72, 76, 89, 93, 112, 136, 143, 74
7	2	0.4479	55, 71
8	2	0.3969	2, 104, 137, 138, 140
9	3	0.3056	7, 17, 1, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 16, 18, 19, 22, 23, 25, 65, 100, 105, 106, 114, 116, 117, 118, 124, 126, 127, 130, 132, 135, 141, 144
10	6	0.3155	20, 24, 27, 54, 64, 77, 79, 82, 111, 113, 119, 133, 80, 52, 53, 58, 59, 78, 81, 83, 84, 85, 96, 115, 125, 51, 60, 70

The number of clusters in **Table 3** is obtained based on the highest Silhouette Score. The list of deleted compounds consists of clusters with no lead compounds. In the 10^{th} clustering, there are only 2 clusters containing lead compounds, as seen in the dendrogram in **Figure 3**.

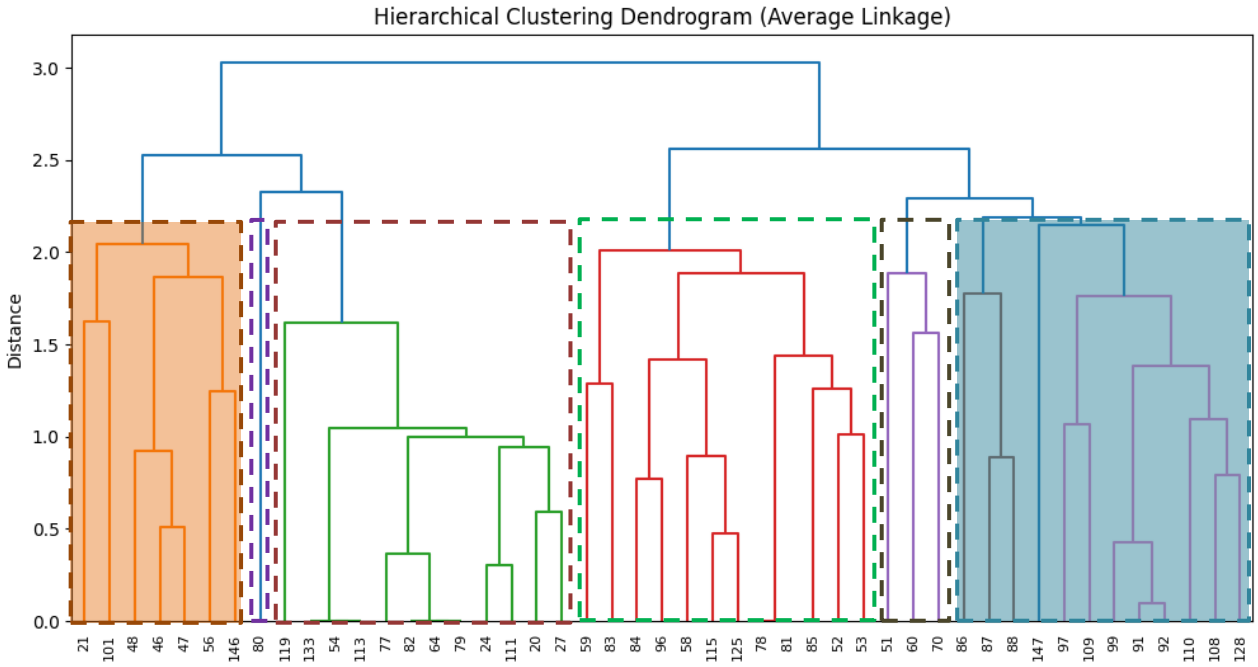

Figure 3. Dendrogram of the 10th Average Linkage Clustering

The dendrogram in **Figure 3** has a Cophenetic Correlation Coefficient of 0.711 which indicates the high dendrogram quality. With the best Silhouette Scores value of 0.3155, The 10th cluster contains 6 clusters where the clusters with lead compounds are clusters 1 and 6 which are marked with orange and blue colors. The list of compounds in those 2 clusters can be seen in the following **Table 4**.

Table 4. Clustering Results

No	Date Fruit Compounds	Lead Compounds
1	2,4-Methylene-D-epirhamnitol	Ascorbic acid
2	PANTOTHENIC-ACID	
3	2-O-METHYLXYLOSE*	
4	2,3,4-TRI-O-METHYLXYLOSE	
5	2,3-DI-O-METHYLXYLOSE	
6	ASCORBIC-ACID*	
7	HISTIDINE*	Ferric cation
8	IODINE	
9	IRON	
10	MAGNESIUM*	
11	POTASSIUM	
12	METHIONINE	
13	ISOLEUCINE	
14	LEUCINE	
15	PROLINE	
16	PIPECOLIC-ACID	
17	VALINE	

These compounds will then be further examined for their proximity by conducting Molecular Docking. Based on the hierarchical proximity, 6 compounds are selected, consisting of 2 lead compounds and 4 date fruit compounds. Among them are 2-O-methylxylose and ascorbic-acid, which are clustered with lead compounds Ascorbic acid, and compound magnesium and histidine, which are clustered with lead compound Ferric cation.

3.5 Molecular Docking

Molecular docking analysis was conducted to validate the clustering analysis. There were four compounds docked which have the highest similarity with the lead compounds based on average linkage clustering results, namely 2-O-methylxylose, ascorbic-acid, magnesium, and histidine. These four compounds were obtained based on GC-MS data for the compound 2-O-methylxylose, and the remaining compounds (ascorbic-acid, magnesium, and histidine) resulted from Dr. Duke's database. The molecular docking results of the four compounds were compared with two lead compounds that represent each cluster, L-ascorbic acid and L-ferric cation (**Table 5**). Docking was done by inserting the tested compounds on the active side of Prolyl Hydroxylase domain enzymes (PHDs).

Prolyl hydroxylases domain (PHDs) are enzymes crucial for regulating the stability of hypoxia-inducible factor (HIF) proteins through hydroxylation reactions. HIF serves as a transcription factor vital in the cellular response to hypoxia, orchestrating various adaptive mechanisms. Inhibition of prolyl hydroxylases can lead to the stabilization of HIF, which can increase the production of erythropoietin (EPO), a hormone that stimulates red blood cell production. EPO is essential for red blood cell production, and its deficiency can lead to anemia. Inhibition of prolyl hydroxylases has been shown to increase EPO production and improve red blood cell production in patients with renal failure **[29].**

Table 5. Results of Molecular Docking of Test Compounds on the Active Side of PHD Enzyme

Compounds	Binding Energy (kcal/mol)	Ki (mM)
L-Ascorbic Acid (Lead 1)	-3.89	1.41
Ascorbic-Acid	-5.65	0.07
2-O-Methylxylose	-3.64	2.15
L-Ferric Cation (Lead 2)	+0.04	-
Magnesium	-1.07	164.40
Histidine	-4.25	0.77

The molecular docking results of tested compounds showed better binding energy values than the lead compound, except for 2-O-methylxylose that have binding energy bigger than the lead compound. However, the binding energy from the 2-O-methylxylose compound is similar to the lead compound's binding energy (L-ascorbic acid). The binding energy generated from each test compound is the result of the contributions of each energy interaction that appears in the protein complex and the test compound. **Figure 4** shows that many molecular interactions that arise between test compounds and enzymes such as conventional hydrogen bonds, carbon-hydrogen bonds, and phi-sigma.

All of these interactions have energy which is then summed up to produce an intermolecular energy value. The more interactions that appear, the more negative the intermolecular energy value will be, and the more interactions that appear, the better the strength of protein and ligand interactions in inhibiting enzyme activity. The 2-dimensional interaction between the test compounds and the enzyme is shown in **Figure 4** which shows that Ascorbic-Acid has more molecular interactions with the PHD enzyme, causing its binding energy to be smaller than the other compounds. Therefore, the molecular docking results indicate the potential of the four compounds derived from date palm fruit as PHD enzyme inhibitors that can be used as anemia drugs.

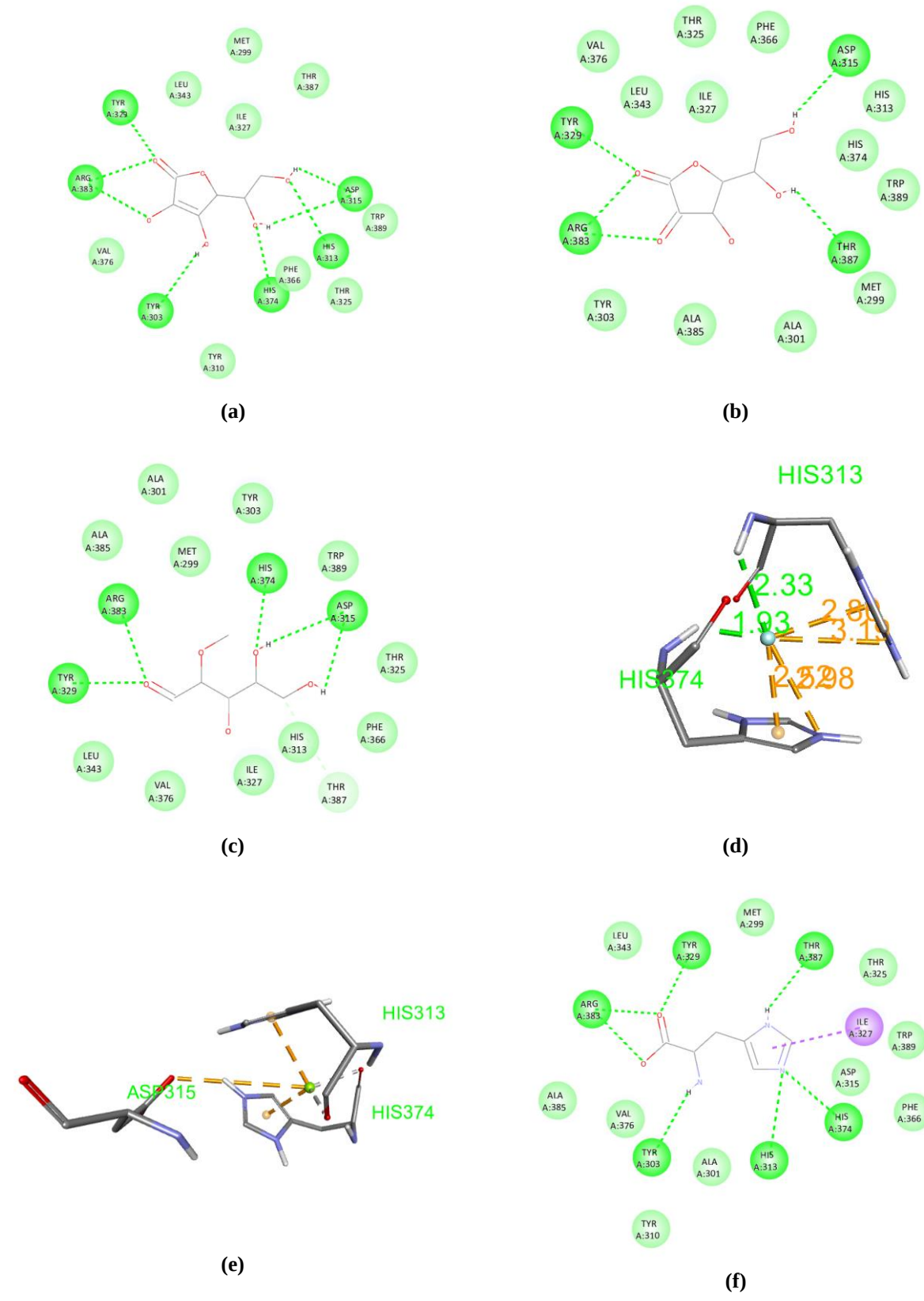

Figure 4. 2-Dimensional interaction of (a) L-Ascorbic-Acid, (b) Ascorbic-Acid, (c) 2-O-Methylxylose, (d) L-Ferric Cation, (e) Magnesium, (f) Histidine on the active side of PHD enzyme

4. CONCLUSIONS

Based on the clustering results obtained, it is known that:

1. Based on the average linkage methods result, the compounds from synthetic chemical drugs are only found in two out of six final clusters. 17 compounds of date fruit have structural properties similar to compounds in synthetic chemical drugs, which concludes that date fruit can be used as an alternative prevention and treatment for anemia.
2. Validation carried out using molecular docking also shows similar results namely the results from molecular docking of compounds ascorbic-acid, magnesium, and histidine show better binding energy values than lead compounds, and 2-O-methylxylose shows similar binding energy with lead compound. So, the molecular docking results show the potential of the four compounds derived from date palm fruit as PHD enzyme inhibitors that can be used as anemia drugs.
3. Therefore, it can be concluded that date fruit can be an alternative treatment for anemia, and the average linkage clustering method can be used in future cases or fields similar to this research.

ACKNOWLEDGMENT

The authors thank Hasanuddin University for the funds from Hasanuddin University's revenue funds with contract number 00310/UN4.22/PT.01.03/2024 and the support provided for this research.

REFERENCES

[1] R. J. Derman and A. Patted, "Overview of Iron Deficiency and Iron Deficiency Anemia in Women and Girls of Reproductive Age," *International Journal of Gynecology and Obstetrics*, vol. 162, no. S2, pp. 78–82, Aug. 2023, doi: 10.1002/ijgo.14950.
[2] S. Y. Hess, A. Owais, M. E. D. Jefferds, M. F. Young, A. Cahill, and L. M. Rogers, "Accelerating Action to Reduce Anemia: Review of Causes and Risk Factors and Related Data Needs," *Annals of the New York Academy of Sciences*, vol. 1523, no. 1. John Wiley and Sons Inc, pp. 11–23, May 01, 2023. doi: 10.1111/nyas.14985.
[3] R. I. Kemenkes, "Laporan hasil riset kesehatan dasar (Riskesdas) Indonesia tahun 2018," *Riset Kesehatan Dasar*, vol. 2018, pp. 182–183, 2018.
[4] C. M. Chaparro and P. S. Suchdev, "Anemia Epidemiology, Pathophysiology, and Etiology in Low-and Middle-Income Countries," *Ann N Y Acad Sci*, vol. 1450, no. 1, pp. 15–31, 2019.
[5] Institute for Health Metrics and Evaluation, "The Global Burden of Disease."
[6] T. W. Wu and F. P. Tsai, "Comparison of the Therapeutic Effects and Side Effects of Oral Iron Supplements in Iron Deficiency Anemia," *Drug Res*, vol. 66, no. 5, pp. 257–261, May 2016, doi: 10.1055/s-0035-1569326.
[7] K. J. Kumar, N. Asha, D. S. Murthy, M. S. Sujatha, and V. G. Manjunath, "Maternal Anemia in Various Trimesters and Its Effect on Newborn Weight and Maturity: An Observational Study," *Int J Prev Med*, vol. 4, no. 2, p. 193, 2013.
[8] D. Moretti *et al.*, "Oral Iron Supplements Increase Hepcidin and Decrease Iron Absorption from Daily or Twice-Daily Doses in Iron-Depleted Young Women," *The Journal of the American Society of Hematology Blood*, vol. 126, no. 17, pp. 1981–1989, 2015.
[9] Z. Tolkien, L. Stecher, A. P. Mander, D. I. A. Pereira, and J. J. Powell, "Ferrous Sulfate Supplementation Causes Significant Gastrointestinal Side-Effects in Adults: A Systematic Review and Meta-Analysis," *PLoS One*, vol. 10, no. 2, p. 117383, 2015.
[10] N. U. Stoffel, H. K. von Siebenthal, D. Moretti, and M. B. Zimmermann, "Oral Iron Supplementation in Iron-Deficient Women: How Much and How Often?," *Mol Aspects Med*, vol. 75, Oct. 2020, doi: 10.1016/j.mam.2020.100865.
[11] R. Husnah, S. As'ad, W. Sinrang, N. Usman, M. Ahmad, and H. Hidayanty, "Addition Of Sukkari Dates (Phoenix Dactylifera L) And Fe Supplementation In Increasing Ferritin Levels To Young Women With Anemia," *Annals of R.S.C.B.*, vol. 25, no. 4, pp. 17377–17389, 2021, [Online]. Available: http://annalsofrscb.ro
[12] F. D. Prieto-Martínez, M. Arciniega, and J. L. Medina-Franco, "Molecular docking: current advances and challenges," *TIP. Revista especializada en ciencias químico-biológicas*, vol. 21, 2018.
[13] A. Venkatesan, M. Rambabu, S. Jayanthi, and J. Febin Prabhu Dass, "Pharmacophore Feature Prediction and Molecular Docking Approach to Identify Novel Anti-HCV Protease Inhibitors," *J Cell Biochem*, vol. 119, no. 1, pp. 960–966, Jan. 2018, doi: 10.1002/jcb.26262.
[14] P. Govender and V. Sivakumar, "Application of K-Means and Hierarchical Clustering Techniques for Analysis of Air Pollution: A Review (1980–2019)," *Atmospheric Pollution Research*, vol. 11, no. 1. Elsevier B.V., pp. 40–56, Jan. 01, 2020. doi: 10.1016/j.apr.2019.09.009.
[15] J. Lu *et al.*, "Identification of new candidate drugs for lung cancer using chemical–chemical interactions, chemical–protein interactions and a K-means clustering algorithm," *J Biomol Struct Dyn*, vol. 34, no. 4, pp. 906–917, 2016.

[16] C. Anneke and C. Andreas, "Application of The Hierarchical Cluster Method in Analyzing The Characteristics of Parenting Patterns for Early Childhood in Indonesia," *International Journal of Academic and Applied Research (IJAAR)* , vol. 6, no. 12, pp. 180–186, 2022.
[17] A. L. Yusniyanti, F. Virgantari, and Y. E. Faridhan, "Comparison of Average Linkage and K-Means Methods in Clustering Indonesia's Provinces Based on Welfare Indicators," in *Journal of Physics: Conference Series*, IOP Publishing, 2021, p. 012071.
[18] Y. Reinaldi, N. Ulinnuha, and M. Hafiyusholeh, "Comparison of Single Linkage, Complete Linkage, and Average Linkage Methods on Community Welfare Analysis in Cities and Regencies in East Java," *Jurnal Matematika, Statistika dan Komputasi*, vol. 18, no. 1, pp. 130–140, 2021.
[19] R. Jakhar, M. Dangi, A. Khichi, and A. K. Chhillar, "Relevance of Molecular Docking Studies in Drug Designing," *Curr Bioinform*, vol. 15, no. 4, pp. 270–278, 2020.
[20] I. M. Fitriani, D. E. Ratnawati, and S. Anam, "Klasifikasi Senyawa Kimia dengan Notasi Simplified Molecular Input Line Entry System (SMILES) menggunakan Metode Extreme Learning Machine (ELM)," *Jurnal Pengembangan Teknologi Informasi dan Ilmu Komputer*, vol. 3, no. 5, pp. 4516–4524, 2019.
[21] J.-K. Lee *et al.*, "Alleviation of Ascorbic Acid-Induced Gastric High Acidity by Calcium Ascorbate In Vitro and In Vivo," *The Korean Journal of Physiology & Pharmacology*, vol. 22, no. 1, pp. 35–42, 2018.
[22] N. Abbaspour, R. Hurrell, and R. Kelishadi, "Review on Iron and Its Importance For Human Health," *J Res Med Sci*, vol. 19, no. 2, p. 164, 2014.
[23] A. I. Khan *et al.*, "Efficacy and Safety of Ferric Derisomaltose for Treatment of Anemia in Chronic Kidney Disease Patients: A Systematic Review," *Blood*, p. 138, 2021.
[24] L. P. Węgier *et al.*, "Ferrous Sulfate Oral Solution in Young Children with Iron Deficiency Anemia: An Open-Label Trial of Efficacy, Safety, and Acceptability," *Pediatrics International*, vol. 62, no. 7, pp. 820–827, 2020.
[25] D. C. Ford *et al.*, "Ferumoxytol Versus Placebo in Iron Deficiency Anemia: Efficacy, Safety, And Quality Of Life in Patients with Gastrointestinal Disorders," *Clin Exp Gastroenterol*, vol. 9, pp. 151–162, 2016.
[26] E. F. Pettersen *et al.*, "UCSF Chimera—A visualization system for exploratory research and analysis," *J Comput Chem*, vol. 25, no. 13, pp. 1605–1612, Oct. 2004, doi: 10.1002/jcc.20084.
[27] G. M. Morris *et al.*, "AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility," *J Comput Chem*, vol. 30, no. 16, pp. 2785–2791, Dec. 2009, doi: 10.1002/jcc.21256.
[28] G. M. Morris *et al.*, "Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function," *J Comput Chem*, vol. 19, no. 14, 1998.
[29] M. J. Koury, "Can Prolyl Hydroxylase Inhibition Treat EPO-Deficient Anemia of Renal Failure With Fewer Vascular Complications Than EPO Itself?," *The Hematologist*, vol. 12, no. 2, 2015.