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# Revealing The Motifs, Properties, and Phylogeny of Lupeol Synthase Using Bioinformatics Approach

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## ABSTRACT

Lupane-type triterpenoid saponins are potent plants' secondary metabolites for drug development as they showed various activities such as anticancer, Sarcoplasmic Reticulum $Ca^{2+}$-ATPase (SERCA) activator which is very important for neurons, and antileishmanial. The triterpenoid saponin backbone is produced by cyclization and rearrangement of the 2,3-oxidosqualene precursor by the oxidosqualene cyclase. The type of oxidosqualene cyclase involved determines the type of saponins so it is referred to as a key enzyme. Lupane-type saponins are produced by 2,3-oxidosqualene cyclization through the chair-chair-chair conformation and the formation of various cation intermediates. This study aimed to analyze lupeol synthase, the key enzyme which determines the conversion of 2,3-oxidosqualene into lupane-type saponins. This in silico project was done using bioinformatics programs including Multiple Em for Motif Elicitation (MEME), ProtParam, and Molecular Evolutionary Genetic Analysis (MEGA-X) for relationship analysis. The amino acid sequences analysis using the MEME program showed that lupeol synthase has QW, DCTAE, and CYCR conserved motifs in the oxidosqualene cyclase family even though some evolutions were also present. Analysis of chemical and physical parameters with ProtParam indicated that lupeol synthase had lower stability than lanosterol synthase from *Saccharomyces cerevisiae*. The phylogenetic tree showed that lupeol synthase was closely related to other plant oxidosqualene cyclases. The results of this study are expected to support the modification strategy determination to increase the production of lupane-type saponins using a biotechnological approach in the pharmaceutical industry.

**Keywords**: bioinformatics, lupeol synthase, lupane, motifs, saponins

## ABSTRAK

Saponin triterpenoid tipe lupan merupakan metabolit sekunder tumbuhan yang sangat potensial untuk dikembangkan sebagai obat karena memiliki berbagai aktivitas seperti antikanker, aktivator Sarcoplasmic Reticulum $Ca^{2+}$-ATPase (SERCA) yang sangat penting untuk neuron, dan *antileishmanial*. Kerangka dasar triterpenoid saponin dihasilkan melalui siklisasi dan penataulangan prekursor 2,3-oksidoskualen oleh enzim oksidoskualen siklase. Jenis enzim oksidoskualen siklase yang terlibat menentukan tipe produk akhir saponin sehingga disebut sebagai enzim kunci. Saponin tipe lupan dihasilkan dari siklisasi oksidoskualen melewati konformasi *chair-chair-chair* dan pembentukan berbagai intermediet kation. Tujuan dari penelitian ini adalah untuk menganalisis enzim kunci lupeol sintase yang menentukan perubahan prekursor 2,3-oksidoskualen menjadi saponin tipe lupan. Studi *in silico* ini dilakukan menggunakan program bioinformatika yang meliputi *Multiple Em for Motif Elicitation* (MEME), ProtParam dan *Molecular Evolutionary Genetic Analysis* (MEGA- X) untuk analisis kekerabatan. Analisis menggunakan program MEME menunjukkan bahwa urutan asam amino dari lupeol sintase memiliki motif QW, DCTAE, dan CYCR yang lestari dalam famili oksidoskualen siklase meskipun kemungkinan

terjadi evolusi. Analisis parameter kimiawi dan fisik dengan ProtParam menunjukkan bahwa lupeol sintase memiliki stabilitas yang lebih rendah dibandingkan lanosterol sintase dari *Saccharomyces cerevisiae*. Pohon filogenetik menunjukkan bahwa lupeol sintase memiliki hubungan yang sangat erat dengan kelompok enzim oksidosqualen siklase tanaman lain. Hasil penelitian ini diharapkan dapat mendukung penentuan strategi modifikasi untuk meningkatkan produksi saponin tipe lupan dengan menggunakan pendekatan bioteknologi pada industri farmasi.

**Kata Kunci:** bioinformatika, lupeol sintase, lupan, motif, saponin

## INTRODUCTION

Lupane is a triterpenoid saponin group that has various biological activities, so it is very potential to be developed in the pharmaceutical industry. The derivative compound of lupane saponin that was chemically reacted to add triphenylphosphonium has been shown to inhibit cancer cell proliferation and migration in zebrafish model organisms through mechanisms that targeted the mitochondria (Ye et al., 2017). The lupane-type saponin schekwanglupaside C isolated from the Chinese herbal plant *Schefflera kwangsiensis* using ethanol solvent can act as an activator for Sarcoplasmic Reticulum $Ca^{2+}$-ATPase (SERCA) which regulates Spontaneous $Ca^{2+}$ Oscillation (SCO) in neuronal cell cultures. This activator is potential to improve memory in sufferers of neuron damage disease (Yang et al., 2019). The survival index of *Leishmania (L.) amazonensis* which causes leishmaniasis in tropical and sub-tropical regions can be reduced by administering lupane-type saponin extract isolated from *Combretum leprosum* (Teles et al., 2015).

The triterpenoid saponin backbone is produced by cyclization and rearrangement of 2,3-oxidosqualene precursors by the oxidosqualene cyclase enzyme group. These enzymes act as an acid for the initiation of the 2,3-oxidosqualene protonation, provide an active site for a series of cyclization reactions, and protect the reactive intermediates from side reactions occurrence. The type of oxidosqualene cyclase enzyme involved determines the types of saponins end product thus referred to as a key enzyme. In addition, to form the basic structure of triterpenoid saponins, there are oxidosqualene cyclase enzymes that catalyze the sterol precursor production namely lanosterol synthase and cycloartenol synthase. These two enzymes convert 2,3-oxidosqualene into the backbone of sterol through the formation of protosteryl cations intermediate. Several enzymes forming the backbone of triterpenoid saponins that have been identified include tirucalladienol synthase which forms tirucallane-type saponins, lupeol synthase forms lupane-type, β-amyrin synthase forms oleanane-type, dammarenediol synthase forms dammarane-type, and α-amyrin synthase forms ursane-type saponins. The lupane-type saponins are produced by oxidosqualene cyclization through the chair-chair-chair conformation and the formation of dammarenyl, baccharenyl, and lupanyl cation intermediates (Augustin et al., 2011).

The lupeol synthases have been isolated from various organisms such as *Ricinus communis* (Julian et al., 2006), *Kalanchoe daigremontiana* (Wang et al., 2010), *Bruguiera gymnorhiza* (Basyuni et al., 2007), *Glycyrrhiza glabra* (Hayashi et al., 2004), *Lotus japonicus* (Sawai et al., 2006), *Morella rubra* (Jia et al., 2019) and other plants. Various modifications through the biotechnological approach using this enzyme have been successfully conducted to increase the production of lupane-type saponins to meet the pharmaceutical industry's needs. Improvement of lupane-type saponins production has been successfully carried out by engineering the metabolic pathway of *Saccharomyces cerevisiae* through the insertion of the lupeol synthase gene from *Olea europaea*. In addition, the squalene synthase from *Thermosynechococcus elongates* and squalene epoxidase gene from *Rattus norvegicus* were introduced to increase the precursor compounds (Qiao et al., 2019).

Bioinformatics studies have been carried out to examine the coding DNA sequences and amino acid constituents of oxydosqulane cyclase from mangrove plants. The results provided information regarding the prediction of secondary structure, protein localization in cells, and the relationship through phylogenetic tree construction (M. Basyuni & Wati, 2017). Besides, the three-dimensional structural modeling of the oxidosqualene cyclase protein was performed using the Phyre2 and Swiss-model programs (M. Basyuni et al., 2018). In this study, lupeol synthase analysis was conducted using the bioinformatics approach to examine the motifs, physical and chemical parameters, and relationship studies. The information obtained is very essential because lupeol synthase is a key enzyme that determines the direction of the 2,3-oxidosqualene precursor into the lupane-type saponins.

## METHODS

### Searching for the amino acid database sequence

The thirty-three amino acid sequences of lupeol synthase from various plant species were obtained from the National Center for Biotechnology Information (NCBI) which can be accessed at http://www.ncbi.nlm.nih.gov. Furthermore, other amino acid sequences of oxidosqualene cyclases were collected, including cycloartenol synthase, β-amyrin synthase, dammarenediol synthase, and α-amyrin synthase.

### Analysis of Conserved Motifs

The search for conserved motifs on 34 lupeol synthase protein sequences was performed using the Multiple Em for Motif Elicitation (MEME) program version 5.3.2 (T. L. Bailey & Elkan, 1994) that can be accessed at https://meme-suite.org/meme/. The parameters that applied in the analysis were classical mode, motif sites distribution was of occurrence per sequence (OOPS), the number of motifs should MEME find was 10, the minimum motifs number was 4 and the maximum motifs number was 10.

### Analysis of Chemical and Physical Parameters

The chemical and physical parameters analysis of protein was conducted using ProtParam that can be accessed https://web.expasy.org/protparam/ (Gasteiger et al., 2005). The properties analyzed comprised molecular weight, theoretical isoelectric point, amino acid composition, instability index, aliphatic index, and Grand Average of Hydropathicity (GRAVY).

### Phylogenetic Analysis of Lupeol synthase

The phylogenetic tree was constructed using *Molecular Evolutionary Genetics Analysis* (MEGA-X) (Kumar et al., 2018). The thirty-three amino acid sequences of lupeol synthase from various plant species and other oxidosqualene cyclases, including cycloartenol synthase, β-amyrin synthase, dammarenediol synthase, and α-amyrin synthase were aligned using the MUSCLE program. In addition, the amino acid sequence of lanosterol synthase from *Saccharomyces cerevisiae* was added as an outgroup. The maximum likelihood method and bootstrapping evaluation with 1000 replications were performed in the phylogenetic tree construction

## RESULTS AND DISCUSSION

The thirty-three amino acid sequences of lupeol synthase from the Euphorbiaceae, Crassulaceae, Rhizophoraceae, Betulaceae, Fabaceae, Brassicaceae, Solanaceae, Asteraceae, Oleaceae, Cleomaceae, Araliaceae, Cannabaceae, Myricaceae, Malvaceae, Fagaceae, Chenopodiaceae, Juglandaceae, Vitaceae, and Malvaceae plant family were used in this study. Those sequences were obtained from the National Center for Biotechnology Information (NCBI) database with the accession number listed in Table 1. In addition, other plant-based oxidosqualene cyclases and lanosterol synthase from *Saccharomyces cerevisiae* were used as comparisons.

Table 1. The list of the accession numbers and sources of protein organisms used in this study. LUS indicated lupeol synthase, CS indicated cycloartenol synthase, DS indicated dammarenediol synthase, bAS indicated β-amyrin synthase, aAS indicated α-amyrin synthase, and LS indicated lanosterol synthase.

| Accession number | Organism Source | Length (aa) | Abbreviation | Family |
|---|---|---|---|---|
| Q2XPU7.1 | *Ricinus communis* | 769 | *Rc*-LUS | Euphorbiaceae |
| E2IUA9.1 | *Bryophyllum daigremontianum* | 765 | *Bd*-LUS | Crassulaceae |
| A8CDT3.1 | *Bruguiera gymnorhiza* | 761 | *Bg*-LUS | Rhizophoraceae |
| Q8W3Z2.1 | *Betula platyphylla* | 755 | *Bp*-LUS | Betulaceae |
| Q764T8.1 | *Glycyrrhiza glabra* | 758 | *Gg*-LUS | Fabaceae |
| AAD05032.1 | *Arabidopsis thaliana* | 757 | *At*-LUS | Brassicaceae |
| AGA17939.1 | *Withania somnifera* | 755 | *Ws*-LUS | Solanaceae |
| BAA86932.1 | *Taraxacum officinale* | 758 | *To*-LUS | Asteraceae |
| BAA86930.1 | *Olea europaea* | 758 | *Oe*-LUS | Oleaceae |
| AQS23741.1 | *Cleome arabica* | 762 | *Ca*-LUS | Cleomaceae |
| AFM82492.1 | *Eleutherococcus trifoliatus* | 763 | *Et*-LUS | Araliaceae |
| XP_030499884.1 | *Cannabis sativa* | 759 | *Cs*-LUS | Cannabaceae |
| KAB1212044.1 | *Morella rubra* | 758 | *Mr*-LUS | Myricaceae |
| KAE8735495.1 | *Hibiscus syriacus* | 767 | *Hs*-LUS | Malvaceae |
| XP_030945069.1 | *Quercus lobata* | 755 | *Ql*-LUS | Fagaceae |
| BAE53430.1 | *Lotus japonicus* | 755 | *Lj*-LUS | Fabaceae |
| TKY44487.1 | *Spatholobus suberectus* | 755 | *Ss*-LUS | Fabaceae |
| QBO24615.1 | *Taraxacum coreanum* | 757 | *Tco*-LUS | Asteraceae |
| XP_028220611.1 | *Glycine soja* | 755 | *Gs*-LUS | Fabaceae |
| XP_006606309.1 | *Glycine max* | 755 | *Gm*-LUS | Fabaceae |
| XP_013451810.1 | *Medicago truncatula* | 763 | *Mt*-LUS | Fabaceae |
| XP_015966342.1 | *Arachis duranensis* | 756 | *Ad*-LUS | Fabaceae |
| XP_010669609.1 | *Beta vulgaris* | 762 | *Bv*-LUS | Chenopodiaceae |
| AJE29379.1 | *Artemisia annua* | 757 | *Aa*-LUS | Asteraceae |
| XP_009786280.1 | *Nicotiana sylvestris* | 755 | *Ns*-LUS | Solanaceae |
| BAL41371.1 | *Glycyrrhiza uralensis* | 758 | *Gu*-LUS | Fabaceae |
| XP_035547196.1 | *Juglans regia* | 755 | *Jr*-LUS | Juglandaceae |
| QBZ67649.1 | *Euphorbia tirucalli* | 761 | *Eti*-LUS | Euphorbiaceae |
| KAF3620028.1 | *Capsicum annuum* | 758 | *Can*-LUS | Solanaceae |
| QIB85404.1 | *Lactuca sativa* | 760 | *Ls*-LUS | Asteraceae |
| RVW59286.1 | *Vitis vinifera* | 753 | *Vv*-LUS | Vitaceae |
| AXU93517.1 | *Taraxacum kok-saghyz* | 758 | *Tk*-LUS | Asteraceae |
| XP_023893584.1 | *Quercus suber* | 755 | *Qs*-LUS | Fagaceae |
| XP_017984003.1 | *Theobroma cacao* | 755 | *Tca*-LUS | Malvaceae |
| Q2XPU6.1 | *Ricinus communis* | 759 | *Rc*-CS | Euphorbiaceae |
| P38605.2 | *Arabidopsis thaliana* | 759 | *At*-CS | Brassicaceae |
| AEO27862.1 | *Panax ginseng* | 769 | *Pg*-DS | Araliaceae |
| AGI15962.1 | *Panax quinquefolius* | 769 | *Pq*-DS | Araliaceae |
| B6EXY6.2 | *Arabidopsis thaliana* | 759 | *At*-bAS | Brassicaceae |
| Q9MB42.1 | *Glycyrrhiza glabra* | 765 | *Gg*-bAS | Fabaceae |
| QBZ67648.1 | *Euphorbia tirucalli* | 764 | *Eti*-aAS | Euphorbiaceae |
| AAA16975.1 | *Saccharomyces cerevisiae* | 731 | *Sc*-LS | Saccharomycetaceae |

## Analysis of Conserved Motifs

Analysis of patterns that occur repeatedly in groups of related protein sequences was carried out using the Multiple Em for Motif Elicitation (MEME) program version 5.3.2. MEME found the novel repeated motifs and have a fixed length of a protein sequence with a statistical approach. The motif is conceived as a probability matrix of each possible letter at each position in the pattern (T. L. Bailey & Elkan, 1994). The conserved motifs of proteins during the evolutionary process may be posses as an active side of enzymes or a protein folding guide which is very essential to produce active structures (Timothy L. Bailey et al., 2015). From the result of the motif analysis, it was found that there were 10 conserved motifs in all the lupeol synthase protein sequences. The sequence of the ten motifs can be observed in Figure 1, while the positions of the ten motifs in the amino acid sequence can be found in Figure 2.

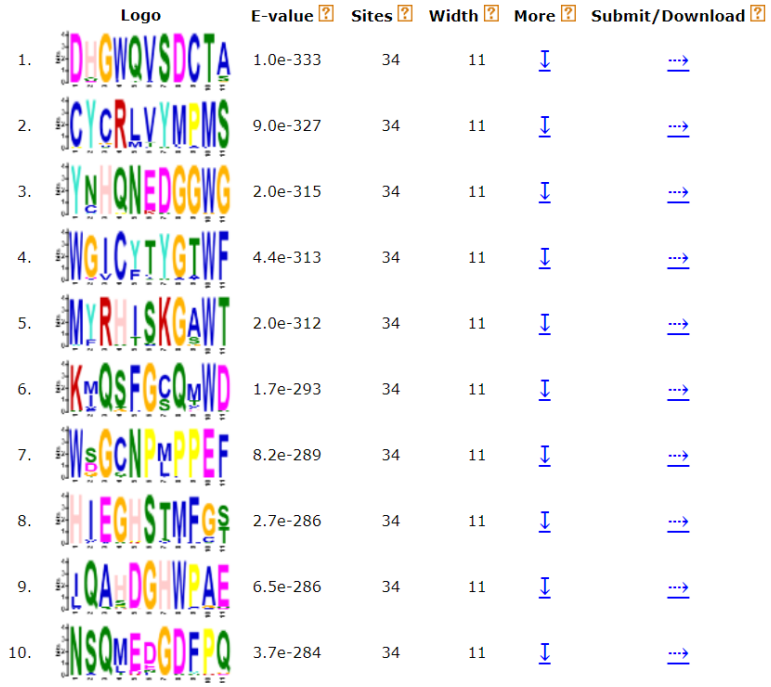



Figure 1. Motifs that were found in the 34 lupeol synthase protein sequences using the MEME program

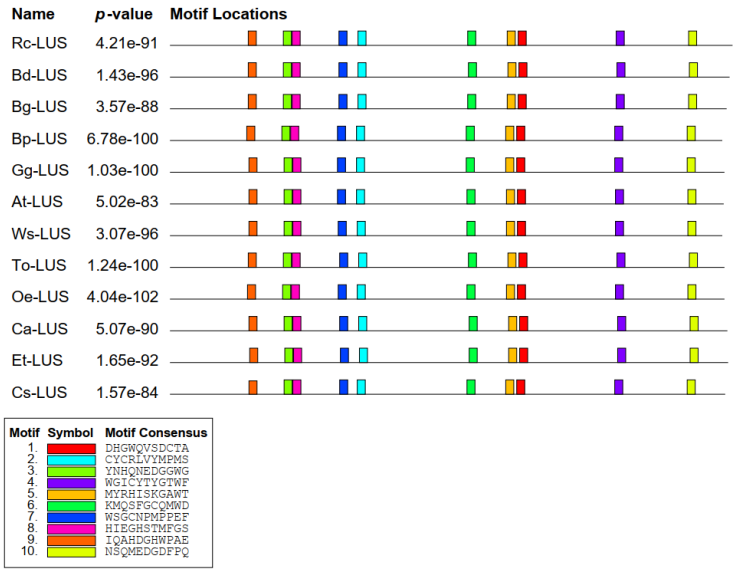
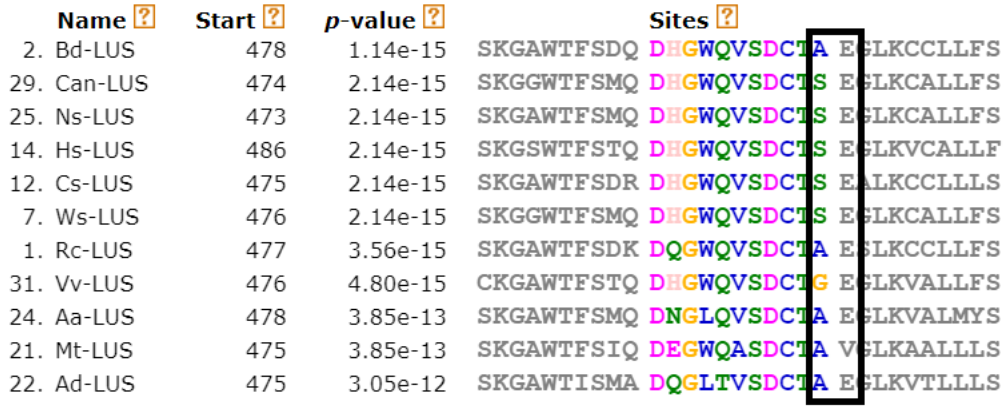



Figure 2. The position of the ten conserves motifs on the amino acid sequence

The analysis indicated that DCTAE, CYCR, and QW motifs sequences were conserved in the oxidosqualene cyclases group. The QW motif is present in the amino acid sequence of oxidosqualene cyclases and squalene cyclase. In the analyzed protein sequence, it was found that there were amino acid alterations in the motif from DCTAE to DCT (A/S/G)(E/V) as depicted in Figure 3. The QW motif was found 3 times (3, 6, and 9 motifs). This sequence was considered prominent in supporting protein structure and stability as well as enzyme catalytic activity previously. The negative charge of aromatic amino acids in the QW motif will interact with the cation intermediates formed in the cyclization reaction (Haralampidis et al., 2002). However, further research showed that this motif only acts as the structural support because it is located on the surface of the oxidosqualene cyclase (Kushiro & Ebizuka, 2010).



| | Name | Start | *p*-value | Sites |
|---|---|---|---|---|
| 2. | Bd-LUS | 478 | 1.14e-15 | SKGAWTFSDQ DHGWQVSDCTA EGLKCCLLFS |
| 29. | Can-LUS | 474 | 2.14e-15 | SKGGWTFSMQ DHGWQVSDCTS EGLKCALLFS |
| 25. | Ns-LUS | 473 | 2.14e-15 | SKGAWTFSMQ DHGWQVSDCTS EGLKCALLFS |
| 14. | Hs-LUS | 486 | 2.14e-15 | SKGSWTFSTQ DHGWQVSDCTS EGLKVCALLF |
| 12. | Cs-LUS | 475 | 2.14e-15 | SKGAWTFSDR DHGWQVSDCTS EALKCCLLLS |
| 7. | Ws-LUS | 476 | 2.14e-15 | SKGGWTFSMQ DHGWQVSDCTS EGLKCALLFS |
| 1. | Rc-LUS | 477 | 3.56e-15 | SKGAWTFSDK DQGWQVSDCTA EGLKCCLLFS |
| 31. | Vv-LUS | 476 | 4.80e-15 | CKGAWTFSTQ DHGWQVSDCTG EGLKVALLFS |
| 24. | Aa-LUS | 478 | 3.85e-13 | SKGAWTFSMQ DNGLQVSDCTA EGLKVALMYS |
| 21. | Mt-LUS | 475 | 3.85e-13 | SKGAWTFSIQ DEGWQASDCTA VGLKAALLLS |
| 22. | Ad-LUS | 475 | 3.05e-12 | SKGAWTISMA DQGLTVSDCTA EGLKVTLLLS |

Figure 3. DCTAE motif that may have changed during evolution

## Analysis of Chemical and Physical Parameters

Chemical and physical parameter analysis on the primary protein structure using ProtParam are depicted in Table 2. Information regarding isoelectric points is prominently in determining the design of protein purification methods. The instability index estimates the stability of the protein when existing outside the cell. Proteins that have an instability index less than 40 are predicted to be stable, while those with a value above 40 are likely to be unstable (Gasteiger et al., 2005). From the analysis, it is indicated the most stable enzyme is lanosterol synthase from *Saccharomyces cerevisiae* with a value of 39.76. The aliphatic index of a protein is defined as the relative volume occupied by the aliphatic side chains (Alanine, Valine, Isoleucine, and Leucine) and is a thermostability-enhancing factor for globular proteins. While the GRAVY is defined as the sum of the hydropathic values of all amino acids, divided by the number of residues in a sequence (Gasteiger et al., 2005). The hydropathic index posses the hydrophobic and hydrophilic properties of the side chains. A negative GRAVY value indicates that the protein is hydrophilic. Analysis of chemical and physical parameters of protein was performed using ProtParam which can be accessed at https://web.expasy.org/protparam/ (Gasteiger et al., 2005). The properties analyzed comprised molecular weight, theoretical isoelectric point, amino acid composition, instability index, aliphatic index, and Grand Average of Hydropathicity (GRAVY).

## Phylogenetic Analysis of Lupeol synthase

Phylogenetic tree construction was conducted to determine the relationship between lupeol synthase and other enzymes of the oxidosqualene cyclase. In this study, a phylogenetic tree was built using MEGA-X software with the maximum likelihood method. This character-based approach selects the best tree with the highest probability to reproduce the observed data. This

method produces a tree that most likely reflects the actual evolutionary process (Xiong, 2006). A bootstrapping technique with 1000 replications was applied to evaluate the phylogenetic tree. From the phylogenetic tree constructed (Figure 4), it is shown that lupeol synthase is closely related to other enzymes of the oxidosqualene cyclase group such as cycloartenol synthase, dammarenediol synthase, β-amyrin synthase, and α-amyrin synthase derived from other plants and lanosterol synthase from *Saccharomyces cerevisiae*. This result indicated that the oxidosqualene cyclase catalyzing the formation of sterols in fungi (*Saccharomyces cerevisiae*) and triterpenoid saponins in plants probably originated from a common ancestor (Haralampidis et al., 2002). Local tandem gene duplication contributes to the expansion of the oxidosqualene cyclase-coding gene family. This process occurs due to biotic and abiotic stress so that plants must synthesize certain compounds as a defense mechanism against these stresses (Xue et al., 2012).

Table 2. The result of primary structure analysis using ProtParam. LUS indicated lupeol synthase, bAS indicated β-amyrin synthase, LS indicated lanosterol synthase, Gm indicated *Glycine max*, Ns indicated *Nicotiana sylvestris*, Aa indicated *Artemisia annua*, At indicated *Arabidopsis thaliana*, and Sc indicated *Saccharomyces cerevisiae*.

| **Parameter** | **Enzymes** | | | | |
|---|---|---|---|---|---|
| | *Gm*-LUS | *Ns*-LUS | *Aa*-LUS | *At*-bAS | *Sc*-LS |
| Number of amino acids (aa) | 755 | 755 | 757 | 759 | 731 |
| Molecular weight (MW) | 86410,89 | 86774,09 | 86563,57 | 86953,30 | 83437,38 |
| Theoretical isoelectric point (pI) | 6,33 | 6,44 | 6,08 | 6,26 | 6,13 |
| Total number of negatively charged residues (Asp+Glu) | 84 | 86 | 86 | 86 | 81 |
| Total number of positively charged residues (Arg+Lys) | 76 | 80 | 74 | 76 | 72 |
| Instability index | 43,93 | 46,47 | 42,02 | 47,10 | 39,76 |
| Aliphatic index | 81,27 | 76,78 | 81,33 | 79,08 | 81,23 |
| Grand average of hydropathicity (GRAVY) | -0,293 | -0,387 | -0,324 | -0,284 | -0,209 |

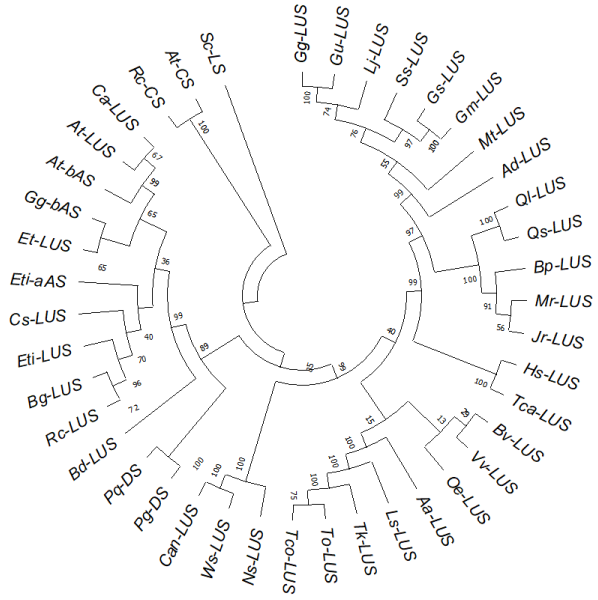



Figure 4. Phylogenetic tree constructed using MEGA-X

## CONCLUSIONS

Analysis using MEME indicated that the amino acid sequence of lupeol synthase comprised DCTAE, CYCR, and QW motifs that conserve in the oxidosqualene cyclase group. Chemical and physical parameters analysis with ProtParam showed that lupeol synthase had lower stability than lanosterol synthase produced by *Saccharomyces cerevisiae*. Lupeol synthase was closely related to other plant oxidosqualene cyclase groups and might have a common ancestor with lanosterol synthase from *Saccharomyces cerevisiae*.

## REFERENCES

Augustin, J. M., Kuzina, V., Andersen, S. B., & Bak, S. (2011). Molecular activities, biosynthesis and evolution of triterpenoid saponins. In Phytochemistry. https://doi.org/10.1016/j.phytochem.2011.01.015

Bailey, T. L., & Elkan, C. (1994). Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proceedings / ... International Conference on Intelligent Systems for Molecular Biology ; ISMB. International Conference on Intelligent Systems for Molecular Biology.

Bailey, Timothy L., Johnson, J., Grant, C. E., & Noble, W. S. (2015). The MEME Suite. Nucleic Acids Research. https://doi.org/10.1093/nar/gkv416

Basyuni, M., & Wati, R. (2017). Bioinformatics analysis of the oxidosqualene cyclase gene and the amino acid sequence in mangrove plants. Journal of Physics: Conference Series. https://doi.org/10.1088/1742-6596/801/1/012011

Basyuni, M., Wati, R., Sulistiyono, N., Hayati, R., Sumardi, Oku, H., Baba, S., & Sagami, H. (2018). Protein modelling of triterpene synthase genes from mangrove plants using Phyre2 and Swiss-model. Journal of Physics: Conference Series. https://doi.org/10.1088/1742-6596/978/1/012095

Basyuni, Mohammad, Oku, H., Tsujimoto, E., Kinjo, K., Baba, S., & Takara, K. (2007). Triterpene synthases from the Okinawan mangrove tribe, Rhizophoraceae. FEBS Journal. https://doi.org/10.1111/j.1742-4658.2007.06025.x

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. In The Proteomics Protocols Handbook. https://doi.org/10.1385/1-59259-890-0:571

Guhling, O., Hobl, B., Yeats, T., & Jetter, R. (2006). Cloning and characterization of a lupeol sintase involved in the synthesis of epicuticular wax crystals on stem and hypocotyl surfaces of Ricinus communis. Archives of Biochemistry and Biophysics. https://doi.org/10.1016/j.abb.2005.12.013

Haralampidis, K., Trojanowska, M., & Osbourn, A. E. (2002). Biosynthesis of triterpenoid saponins in plants. In Advances in biochemical engineering/biotechnology. https://doi.org/10.1007/3-540-44604-4_2

Hayashi, H., Huang, P., Takada, S., Obinata, M., Inoue, K., Shibuya, M., & Ebizuka, Y. (2004). Differential expression of three oxidosqualene cyclase mRNAs in Glycyrrhiza glabra. Biological and Pharmaceutical Bulletin. https://doi.org/10.1248/bpb.27.1086

Jia, H. M., Jia, H. J., Cai, Q. Le, Wang, Y., Zhao, H. B., Yang, W. F., Wang, G. Y., Li, Y. H., Zhan, D. L., Shen, Y. T., Niu, Q. F., Chang, L., Qiu, J., Zhao, L., Xie, H. B., Fu, W. Y., Jin, J., Li, X. W., Jiao, Y., … Gao, Z. S. (2019). The red bayberry genome and genetic basis of sex determination. Plant Biotechnology Journal. https://doi.org/10.1111/pbi.12985

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. https://doi.org/10.1093/molbev/msy096

Kushiro, T., & Ebizuka, Y. (2010). Triterpenes. In Comprehensive Natural Products II: Chemistry and Biology. https://doi.org/10.1016/b978-008045382-8.00007-1

Qiao, W., Zhou, Z., Liang, Q., Mosongo, I., Li, C., & Zhang, Y. (2019). Improving lupeol production in yeast by recruiting pathway genes from different organisms. Scientific Reports. https://doi.org/10.1038/s41598-019-39497-4

Sawai, S., Shindo, T., Sato, S., Kaneko, T., Tabata, S., Ayabe, S. I., & Aoki, T. (2006). Functional and structural analysis of genes encoding oxidosqualene cyclases of Lotus japonicus. Plant Science. https://doi.org/10.1016/j.plantsci.2005.08.027

Teles, C. B. G., Moreira-Dill, L. S., Silva, A. de A., Facundo, V. A., de Azevedo, W. F., da Silva, L. H. P., Motta, M. C. M., Stábeli, R. G., & Silva-Jardim, I. (2015). A lupane-triterpene isolated from Combretum leprosum Mart. fruit extracts that interferes with the intracellular development of Leishmania (L.) amazonensis in vitro. BMC Complementary and Alternative Medicine. https://doi.org/10.1186/s12906-015-0681-9

Wang, Z., Yeats, T., Han, H., & Jetter, R. (2010). Cloning and characterization of oxidosqualene cyclases from Kalanchoe daigremontiana: Enzymes catalyzing up to 10 rearrangement steps yielding friedelin and other triterpenoids. Journal of Biological Chemistry. https://doi.org/10.1074/jbc.M109.098871

Xiong, J. (2006). Essential bioinformatics. In Essential Bioinformatics. https://doi.org/10.1017/CBO9780511806087

Xue, Z., Duan, L., Liu, D., Guo, J., Ge, S., Dicks, J., Ómáille, P., Osbourn, A., & Qi, X. (2012). Divergent evolution of oxidosqualene cyclases in plants. New Phytologist. https://doi.org/10.1111/j.1469-8137.2011.03997.x

Yang, G., Wang, Y., Yu, Y., Zheng, J., Chen, J., Li, S., Chen, R., Zhang, C., Naman, C. B., Yu, D., & Cao, Z. (2019). Schekwanglupaside C, a new lupane saponin from Schefflera kwangsiensis, is a potent activator of sarcoplasmic reticulum Ca2+-ATPase. Fitoterapia. https://doi.org/10.1016/j.fitote.2019.04.005

Ye, Y., Zhang, T., Yuan, H., Li, D., Lou, H., & Fan, P. (2017). Mitochondria-Targeted Lupane Triterpenoid Derivatives and Their Selective Apoptosis-Inducing Anticancer Mechanisms. Journal of Medicinal Chemistry. https://doi.org/10.1021/acs.jmedchem.7b00679