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Network Pharmacology Analysis of Secondary Metabolites from *Alstonia spectabilis* for Antimalaria Activity Prediction

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Abstract

Malaria, which is prevalent in 85 countries worldwide, poses a significant threat to global mortality rates, particularly in regions like East Nusa Tenggara, Indonesia. Conventional anti-malarial drugs like chloroquine and artemisinin face diminishing effectiveness due to *Plasmodium sp.* resistance. Historically, among the Tetun people in East Nusa Tenggara, *Alstonia spectabilis* served as an anti-malarial remedy. The purpose of this research was to predict *A. spectabilis's* potential as an antimalaria treatment through network pharmacology.By analyzing SMILES metabolite codes from UPLC-QToF-MS/MS and GC-MS via BindingDB, TargetNet, Ensemble Similarity Approach, and SwissTargetPrediction, potential protein targets implicated in malaria pathogenesis were identified. Leveraging databases like GeneCards®, The Human Gene Database, DrugBank Online Database, and OMIM, numerous protein targets associated with malaria and *Plasmodium sp.*were revealed. Interactions between active compounds and protein targets were forecasted using GeneCard, Drugbank, OMIM, and DisGeNET. 2,707 genes from pharmacological activity databases and 6,802 therapy targets for malaria were identified. The Venn diagram analysis refined the selection to 657 target genes. Protein-protein interaction networks were constructed using the STRING database and Cytoscape software, with Cluster 6 spotlighted for its association with malaria pathogenesis. Top-ranking genes, including ITGB2, ITGB1, ITGAL, ITGA4, and ITGB3, were identified based on degree parameters. While ITGB2 remains in the preliminary stage, its potential correlation with malaria is hypothesized, given its association with immune responses like inflammation and adaptive immunity. Finally, *A. spectabilis* shows promise as a potential antimalaria drug because it changes the immune system by increasing ITGB2 expression. This research sheds light on novel avenues for combating malaria.

Keywords: *Alstonia spectabilis*; Antimalaria; ITGB2; Network Pharmacology

1. Introduction

Malaria, an ancient disease, continues to affect around 85 countries, with 241 million new cases in 2022 causing 627,000 deaths, two-thirds of which are children under five years old [1]. In Indonesia, malaria is a major health issue in the eastern regions such as Nusa Tenggara, Maluku, and Papua, where it remains hyperendemic [2]. The 2013 Indonesian Health Profile reported that East Nusa Tenggara has one of the highest malaria endemic rates, with an Annual Parasite Incidence (API) of 16.37%, significantly higher than the national average of 1.38% [3].

Current antimalarial drugs like chloroquine are no longer effective due to *Plasmodium sp* resistance. Artemisinin and its derivatives, such as artesunate, have replaced chloroquine [4]. However, there are indications of resistance to artemisinin-based combination therapy (ACT) in *Plasmodium falciparum*, the most dangerous and prevalent plasmodial type in Indonesia [5]. Therefore, new antimalaria drugs that are effective and affordable are urgently needed.

The traditional treatment of malaria by the Tetun people in East Nusa Tenggara has been used for hundreds of years to treat symptoms like fever, chills, swollen spleen, headaches, and muscle and joint pain [6]. These treatments include both herbal and non-herbal remedies[8]. One such herbal remedy is *kroti metan*, also known as black pulai (*Alstonia spectabilis*), which is a potential new antimalaria drug[9].

Analyzing the metabolite content of *A. spectabilis* requires a metabolomic approach to profile its secondary metabolites[9]. Methods such as ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-

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QToF-MS/MS) and gas chromatography-mass spectrometry (GC-MS) are used for metabolite profiling[10]. UPLC-QToF-MS/MS provides comprehensive metabolite profiles, improving compound separation efficiency and analysis speed[11]. GC-MS offers accurate compound identification based on molecular structure [12].

There are three approaches to determine the antimalarialactivity of *A. spectabilis*: *in silico, invitro*, and *in vivo*. *In silico* methods like network pharmacology provide insights into complex biological systems by identifying significant proteins involved in disease treatment and targeting compounds that interact with these proteins [13]. This study aims to use network pharmacology to predict the antimalaria activity of *A. spectabilis*[14].

2. Experimental

Materials

Computing studies were conducted using Dell computers with Windows 11, Intel® Xeon(R) W-2223 CPU @ 3.60 GHz octa-core, 16 GB RAM, and NVIDIA Quadro P2200 GPUs. Metabolite profiling results from UPLC-QToF-MS/MS and GC-MS were obtained from preliminary research.

Methods

Collection and Screening of Target Proteins Based on Active Compounds:

Predicting malaria-related genetic targets through active compounds of *A. spectabilis* metabolite profiling was done using SMILES data inputted into databases such as BindingDB, TargetNet, Similarity Ensemble Approach (SEA), and SwissTargetPrediction. The gene targets were standardized using the Uniprot database to avoid inconsistencies and duplicates were removed [16,17].

Malaria-Related Target Protein Collection and Filtration:

Target proteins involved in malaria pathogenesis were identified using GeneCards®: The Human Gene Database, DrugBank Online Database, and Online Mendelian Inheritance in Man (OMIM) by searching for "Malaria" and "Plasmodium." Standardization and de-duplication of target proteins were done using the Uniprot database to ensure each protein appeared only once in the final dataset [16-18].

Protein-Protein Interaction Network Design and Enrichment Analysis:

Interactions between target proteins of active compounds and malaria targets were predicted using GeneCard , DrugBank,OMIM,DisGeNET, and the Therapeutic Target Database. Protein-protein interactions were analyzed using the STRING database with high confidence (0.900) and FDR stringency of 5%. Networks were processed with Cytoscape 3.9.1, and enrichment analysis was performed using MetaScape[19], WebGestalt[20], and Enrichr[21] to understand the biological functions within the network.

3. Results and Discussion

Identifying malaria-related target genes

The study looked at a group of genes related to malaria using the words "Malaria" and "Plasmodium" in several databases, such as Gene Cards, Drug Bank, DisGeNet, Therapeutic Target Database, and Online Buying Inheritance in Man (OMIM). It also looked at genes related to drug activity in BindingDB, TargetNet, Similarity Ensemble Approach (SEA), and Swiss Target Prediction. Target genes were subsequently standardized based on the HUGO Gene Nomenclature Committee (HGNC) to prevent overlapping gene identities between databases. After removing the duplicate gene, the analysis proceeded to identify genetic similarities between the predicted target and the known malaria therapeutic target from the database **(Figure 1).** The procedure produced 2,707 genes from four pharmacological activity databases based on the profiling metabolite structure, as shown in **Figure 2(A)** (blue), and 6,802 therapeutic targets for malaria, as indicated in **Figure 2(A).** The chosen candidate gene was then placed in the Venn diagram. This showed that 657 target genes were similar to each other in a slice between genes related to malaria and genes based on the plant structure found by the profiling metabolite, as shown in **Figure 2 (B).**

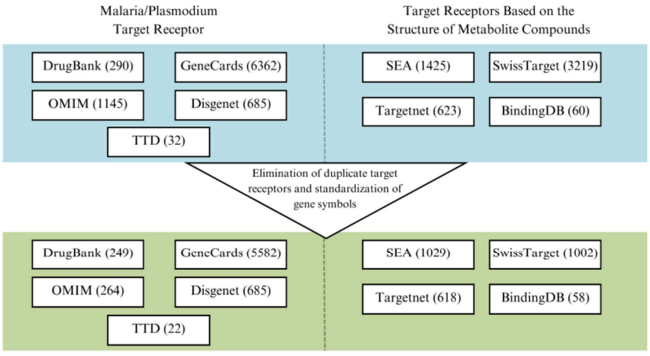

Figure 1: The process involves collecting malaria-related target proteins and target receptors based on the structure of the metabolite compound, as well as eliminating duplicate targets.

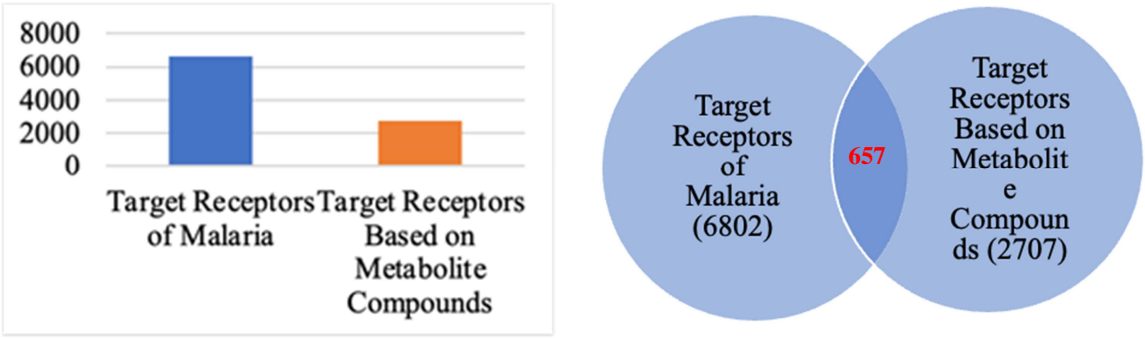

Figure 2: Comparison the number of target receptors associated with malaria disease and target receptors based on the structure of the metabolite compound (A), the Venn Diagram shows the number of targets that overlap between the target malaria receptor and the targets based on metabolite structure. Network pharmacology analyses rely on the number of target receptors in this sequence (B).

Protein-protein interaction construction and tissue topology analysis

The STRING database and Cytoscape software are used to build a protein-protein interaction network (PPI) on 657 selected genes with the highest confidence level of 0.900 **(Figure 3).** On the other hand, the CytoNCA panel in the Cytoscape software calculates the PPI topology. A cluster analysis is then carried out to cluster genes with similar pharmacological activity. In this study, the entire cluster has a $p<0.05$ value which indicates that each protein in a cluster had a significant relationship of protein interaction in the same pharmacogic activity[20], But we chose cluster 6 based on the gene predisposition that has been linked to the pathogenesis of malaria. **Table 2** lists the results and parameters of the cluster topology analysis. Based on the degree parameters shown in **Table 2** and **Figure 4**, the top five genes are ITGB2 (Integrin beta-2), ITGB1 (Integrin beta-1), ITGAL (Integrin alpha-L), ITGA4 (Integrin beta-4), and ITGB3 (Intengrin bet-3). The ITGB2-gen is in its early stages, so we hypothesized that it has a strong correlation with malaria disease. The leukocyte integrin family has a member called integrin beta 2 (ITGB2), which is also known as CD18/LFA-1 that helps integrin heterodimer binding. ITGB2 is involved in lymphocyte binding to tissue[23]. ITGB2 expression is often associated with immune responses, such as inflammation and adaptive immunity. Lack of ITGB2 expression can lead to problems with lymphocyte cell adhesion, which makes the immune system less able to fight off antigens[24,25]. ITGB2 affects malaria by causing a syndrome called malaria-associated acute respiratory disorder. This shows that it is a key molecule in the inflammatory effects of *P. berghei*-caused malaria in living organisms. Acute lung inflammation correlates with genetic suppression of the ITGB2 subunit[26]. Therefore, ITGB2 modulation has the potential to make it an antimalarial target through improved immune systems. Our findings reveal that no significant changes were observed when ranking targets based on other parameters.

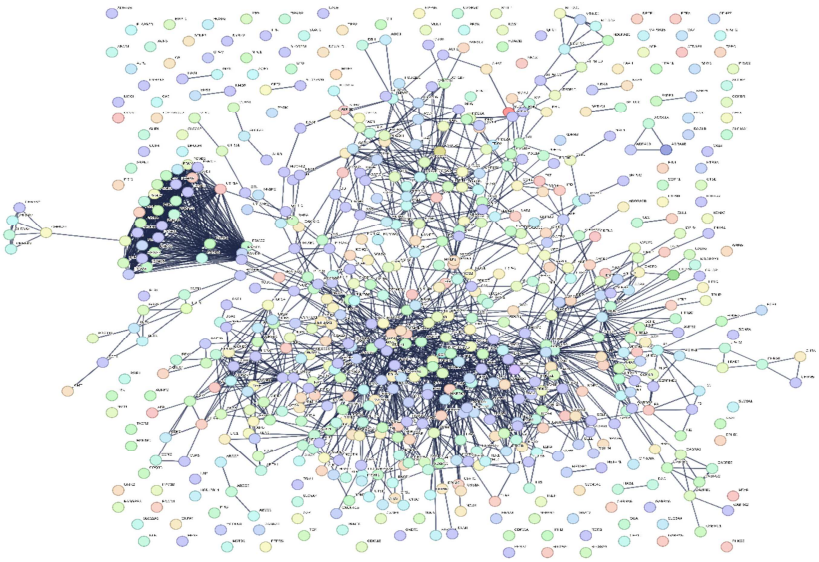

Figure 3: Protein-protein interaction networks (PPI) of 646 selected target genes were built using a STRING database with the highest confidence (0.9).

Table 1: Clusterization analysis in Protein-Protein Interaction Networks

Kluster	Protein	p-value	Quality
1	USP14, PSMD8, PSMD7, PSMD6, PSMD4, PSMD3, PSMD2, PSMD14, PSMD13, PSMD12, PSMD11, PSMD1, PSMC6, PSMC5, PSMC4, PSMC3, PSMC2, PSMC1, PSMB9, PSMB7, PSMB5, PSMB4, PSMB3, PSMB2, PSMB1, PSMA8, PSMA6, PSMA5, PSMA4, PSMA3, PSMA2	0.000	0.983
2	ALOX15, CYP1A2, CYP26B1, CYP2C19, CYP2C9, CYP2E1, CYP2J2, CYP3A4, EPHX1, GSR, GSTA2, GSTM1, GSTO1, GSTP1, HPGDS, PPIG, PTGS1, UGT2B7	9.885×10^{-7}	0.67
3	UGT2B7, PTGS2, PTGS1, PPIG, HSD11B1, HPGDS, GSTP1, GSTO1, EPHX1, CYP3A4, CYP2J2, CYP2E1, CYP2D6, CYP2C9, CYP2C19, CYP1A2, ATP12A, ALOX5, ALOX15	3.574×10^{-6}	0.664
4	AURKA, CCNA2, CCNB1, CCND3, CCNH, CDC25C, CDK1, CDK2, CDK4, CDK6, CDK7, NEK2, PCNA, PLK1, TOP2A, WEE1	6.293×10^{-6}	0.612
5	AURKA, CCNA2, CCNB1, CDC25C, CDC45, CDC7, CDK1, CDK2, CDK7, CHEK1, CHEK2, NEK2, PLK1, TOP2A, WEE1	4.372×10^{-5}	0.632
6	ICAM1, ILK, ITGA2, ITGA2B, ITGA4, ITGA5, ITGAL, ITGAV, ITGB1, ITGB2, ITGB3, PRKD1, PTK2B, SELE, SELP, VCAM1	1.139×10^{-4}	0.544
7	ICAM1, ITGA2, ITGA2B, ITGA4, ITGA5, ITGAL, ITGAV, ITGB1, ITGB2, ITGB3, PRKD1, PTK2, PTK2B, PTPRC, SELL, SELP, VCAM1	1.734×10^{-4}	0.526
8	EGFR, ERBB2, FGFR2, FLT1, GRB2, HGF, IGF1R, IL6ST, KDR, KISS1R, LYN, MET, PDGFRB, PIK3CD, PIK3R1, PLCG1, PTK2, PTPN11, SRC, STAT3, SYK	0.007	0.452

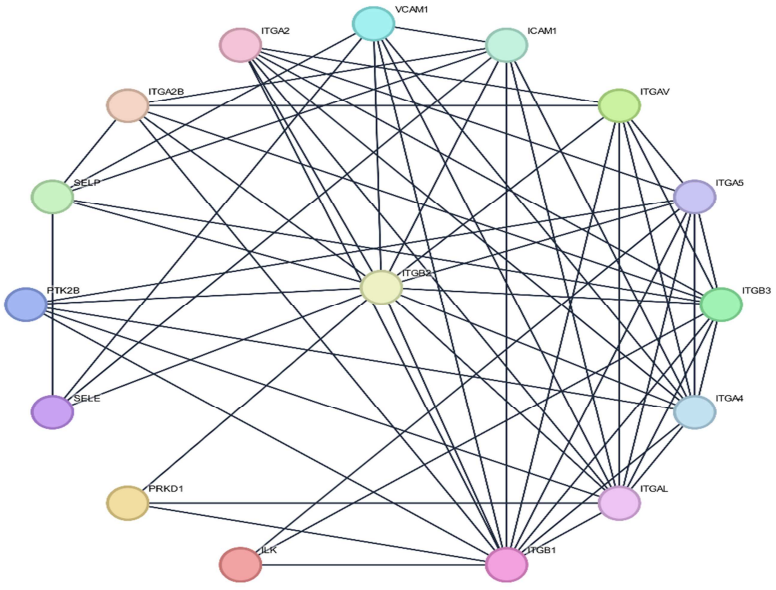

Figure 4: A protein-protein interaction network (PPI) of 16 target genes was selected based on cluster analysis associated with malaria disease.

Table 2: Network topology analysis resulting from clustering of 16 selected target genes

Gene symbol	Degree	BetweennessCentrality	Closeness Centrality
ITGB2	14	0.94	0.18
ITGB1	13	0.88	0.13
ITGAL	11	0.79	0.05
ITGA4	10	0.75	0.03
ITGB3	10	0.75	0.06
ITGA5	9	0.71	0.03
ICAM1	8	0.68	0.03
ITGAV	8	0.68	0.01
ITGA2	7	0.65	0
VCAM1	7	0.65	0.02
ITGA2B	6	0.63	0.01
SELP	6	0.63	0.02
PTK2B	5	0.6	0
SELE	4	0.56	0
ILK	3	0.54	0
PRKD1	3	0.56	0

Protein-protein interaction network enrichment analysis

Based on the cluster results, 16 genes were selected in cluster 6 that were associated with malaria and analyzed using MetaScape. As shown in **Figure 5,** the malaria-target compound network has some paths shown as gene ontology (GO). These include the "Integrin-mediated signaling pathway" "cell-cell adhesion," and "Biocarta monocyte pathway." An important biological process, the Integrin-mediated signaling pathway, links the compounds of medicinal plants and malaria. Enrichment analysis using the Enrichr database (**Figure 6A**) supports the results. At this stage, the observation progresses to the phase of analyzing the physiological roles of each gene selected in cluster 6 using the WebGestalt database. These components include biological processes (BP), cellular components (CC), and molecular functions (MF). In biological processes, genes in cluster 6 have a dominant role in “cell communication”, “cellular component organization”, and “responses to stimuli” (**Figure 7A).** Next, the cellular part tells us where each gene is located, with the main locations being on the "membran", the "protein-containing complex", and the "extracellular vesicle" (**Figure 7B**). Meanwhile, “protein binding”, “ion bonding”, and “molecular transducer activity” become important molecular functions in 16 selected genes (c). Referring to enrichment analysis using web databases Gestalt and WikiPathway, four of the 16 selected genes involved in the pathogenesis of malaria through the Integrin-mediated signaling pathway: ITGAL ITGB2, ICAM1, and VCAM1 are commonly found in liver cells (**Figures 6B and 8**). This supports our conclusion that the results from cluster 6 are closely linked to the development of malaria, with ITGB2 being a key protein based on the highest degree value.

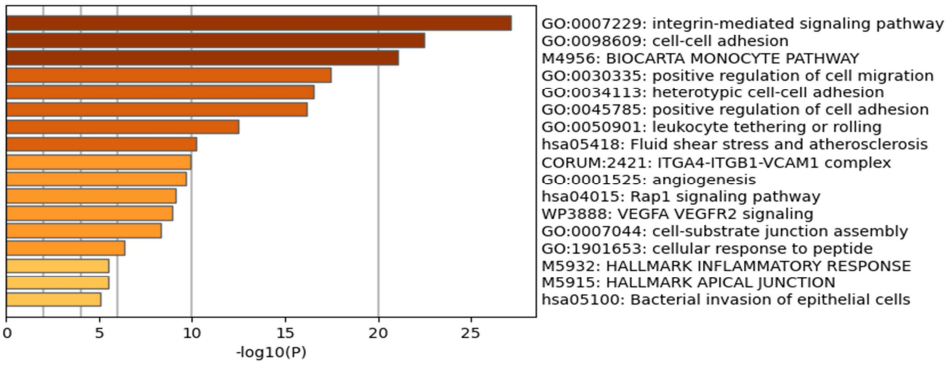

Figure 5: Target path analysis of 16 selected genes using the Metascape database. The results of the analysis show the biological processes involved in malaria.

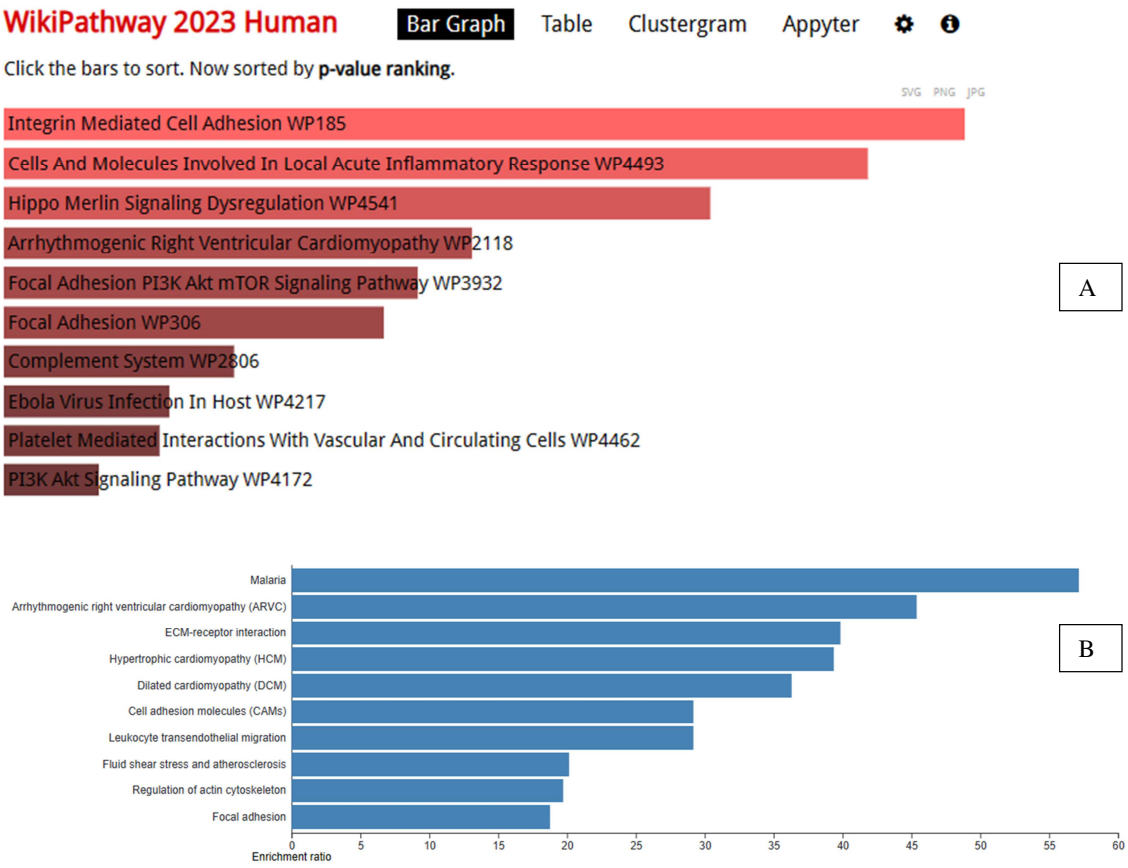

Figure 6: An analysis of the role enrichment of 16 selected genes against biological functions from the Enrichr database and the relationship of the selected 16 genes to disease

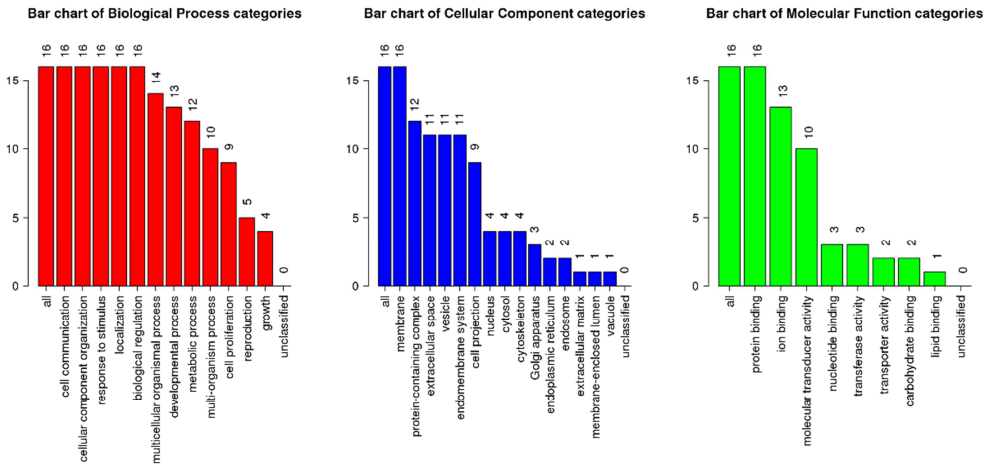

Figure 7: GO analysis of target gene-disease interactions for selected plant compounds shows correlations of (a) biological processes (BP); (b) cellular components (CC); and (c) molecular functions (MF) analyzed with the WebGestalt database. The number of genes involved is shown next to the horizontal bar chart.

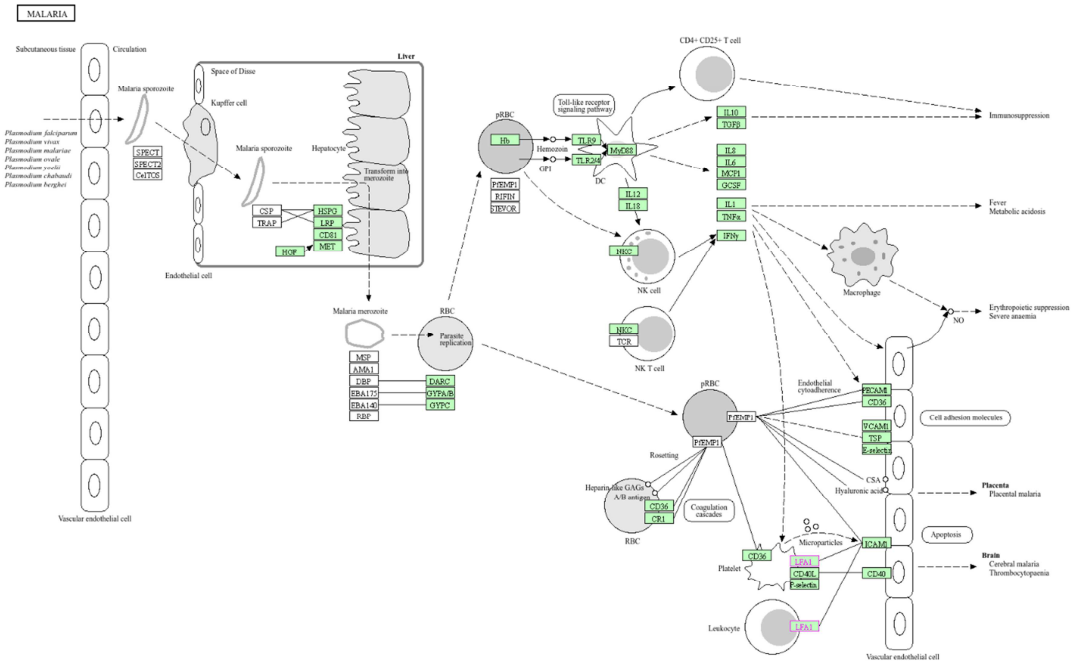

Figure 8: Analysis of the role of the "integrin-mediated signaling pathway" in the life cycle of malaria

4. Conclusions

The research identified 657 target genes, with cluster 6 potentially having strong interconnections with malaria activity. 16 selected genes from cluster 6 were further analyzed to predict their pathways in malaria pathogenesis. The primary gene, ITGB2, is linked to immune responses, indicating that *A. spectabilis* potential antimalaria activity is based on immune system modulation.

5. Conflicts of interest

The authors declare there is no conflict of interest.

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