Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

Introduction to Plant Metabolism, Secondary Metabolites Biosynthetic Pathway, and In-Silico Molecular Docking for Determination of Plant Medicinal Compounds: An Overview

Risanti Dhaniaputri[1] Hadi Suwono[2,*] Mohamad Amin[3] Betty Lukiati[4]

[1] *Doctoral Program of Biology Education, Faculty of Mathematics and Science (Universitas Negeri Malang, Jl. Cakrawala 5, Malang City, East Java, Indonesia 65145)*
[2,3,4]*Department of Biology, Faculty of Mathematics and Science (Universitas Negeri Malang, Jl. Cakrawala 5, Malang City, East Java, Indonesia 65145)*
**Corresponding author. Email: hadi.suwono.fmipa@um.ac.id*

ABSTRACT
Natural ingredient produced by plants are widely used for therapeutic treatment, because they are believed to have fewer side effects and are cheaper than synthetic drugs. Plants used as treatment media contain natural secondary metabolites compounds derived from primary and secondary metabolism. Primary metabolism is the basic stage in the formation of complex molecules in plant medicinal products, while secondary metabolism form more advanced products in synthesize the phytochemicals. Plant secondary metabolites involved three chemically basic group of compounds: terpenoids, phenolics, and alkaloids. Biosynthetic pathways of secondary metabolites are conducted through the Shikimic-acid, Malonic-acid, Mevalonic-acid, and Methylerythritol-phosphate pathway. Glucose is the main molecule for the metabolism of these secondary metabolites. This paper discuss about the plant metabolism process, biosynthetic pathway of primary and secondary metabolite products, column chromatography techniques for identification, screening, and analysis of secondary metabolites, and *in-silico molecular docking* procedures to determining 3D protein structures, predicting target proteins, and validating ligan-compound interactions in medicinal plants. The qualitative review was carried out by analyzing the literature references books, research results of indexed journals from Scopus, Google Scholar, Research Gate, and softwarecomputational tools of *in-silico* methods, including PubChem, Protein Data Bank (PDB), PyMol, AutoDock Vina, BLAST. Determination and development of plant medicinal compounds can be started by learning the plant physiology and metabolic process, biosynthesis pathway and degradation of metabolite products, extraction and separation methods using chromatography, and in-silico molecular docking, includes the process of protein and ligand preparation, molecular docking study, drug like-ness and toxicity prediction. In-silico procedures are used for discovering and developing the actions of unknown secondary metabolites by identification their molecular targets using bioinformatics and chemoinformatics characteristics along with the biological system.

***Keywords**: In-silico molecular docking, Plant metabolism, Secondary metabolites.*

1. INTRODUCTION

Plants are photoautotrophic organism that involved the water vapor, sunlight, chlorophyll pigments in mesophyll cells and obtain inorganic nutrient from soil mineral to synthesize their own organic molecules [1]. Plants convert the inorganic to organic compounds through the process of photosynthesis. Photosynthesis means the synthesis of molecules using light, which utilize the complex carbon molecules. In photosynthesis, energy from the sun is captured and transformed from

light energy into biochemical energy, produces the sugar molecules or glucose and oxygen. These glucose molecules are involved in the biosynthesis of amino acids for protein, fatty acids for lipids, and other cell constituents [2].

Photosynthesis takes place in green organism organs which contain the mesophyll cells and chlorophyll pigment. Plant absorbs the water (H_2O) from soil and carbon dioxide (CO_2) present in the air,with the electron molecules emitted from the sunlight. The molecular binding of water and carbon dioxide will produce the carbohydrate single molecules ($C_6H_{12}O_6$, glucose) in mesophyll cells, and then releases the oxygen gas (O_2) that diffuse out into theatmosphere.

These complex macromolecules will be break down into energy and simple inorganic molecules again, through the process called cellular respiration. Cellular respiration converts oxygen and glucose into water and carbon dioxide, it takes place in mitochondrial organelles. Photosynthesis and cellular respiration are responsible for the exchange of oxygen and carbon dioxide between the living organisms, and these processes are part of plant metabolisms. Metabolism defined as the essentialbiochemical processes that takes place in living organism, correlated with the enzymatic process, require energy to occur and produce macromoleculesin plant storage cells [3].

This paper describes about the interconnected between plant metabolism, secondary metabolites biosynthetic pathways, and in-silico molecular docking belong to determine the structure of plant medicinal compounds. Introduction the study of plant metabolism, secondary metabolites, and in-silico approach are needed to understand the process of predicting and inventing the plant drug potential compounds generally.

2. METHODS

The qualitative review was carried out by analyzing the literature references books, previous research results of indexed journals from Scopus, Google Scholar, Research Gate, and software computational tools of *in-silico* molecular docking methods.

3. RESULT

3.1. Plant Metabolism

Plant metabolism has been broadly distinguished into two stages: primary and secondary metabolism. The primary metabolism pathway is the stage for synthesizing primary macromolecules such as carbohydrates, proteins, lipids, nucleic acids, and hormones [1]. Primary metabolism plays a vital role for plant growth and development directly. Primary metabolism is the basic stage in the formation of complex molecules, while secondary metabolism forms the more advanced and unique products in the phytochemical compounds [2], [4].

Primary metabolism occurs in all living cell organisms, produce glucoses, amino acids, fatty acids, and nucleotides, as well as complex molecules such as carbohydrate, protein, lipid, cell membranes, hormone, and nucleic acids (DNA and RNA). Primary metabolites found in seed and plant vegetative organs, and they play an essential role in plant growth, development, reproduction, and signal transduction. Photosynthesis, respiration, protein synthesis, solute transport, and nutrient assimilation are types of primary metabolism process [3], [5].

The secondary metabolites compounds, both biosynthetic and evolutionary pathway, derived from primary metabolism. The certain secondary metabolites are restricted only in specific plant species, influenced by the biotic and abiotic factors; whereas primary metabolites are found throughout the plant kingdom. Products of secondary metabolite compounds are not directly involved in plant development, but contribute to the species adaptability for survival. Secondary metabolites are related to the plant competitions as allelopathic agents, they have signaling functions to control the activities of other cell organism as phytoalexin compounds, UV protector, flower pollinator attract, defend plants against the variety of Nematoda, bacteria, fungi, and pathogenic microbes from structural and physiological damages, and being eatenby mammals or herbivorous. In natural biological system, plant could not avoid these predators by moving away, they must adapt and protect themselves to survive [1], [6]. Secondary metabolite compounds can be identified by the process of plant tissues extraction techniques.

3.2. Secondary Metabolites Biosynthetic Pathway

Environmental factors such as water deficit, flooding and soil compaction, chilling, freezing, hightemperature, salinity, trace element toxicity and high light intensity can affect the physiological and biochemical during the metabolism process, and impacts the production of secondary metabolites. Secondary metabolites are known as bioactive compounds or plant phytochemistry [6]. The distribution of secondary metabolites is taxonomic restricted and influence the interaction betweenliving organism and its environment [7].

Albrecht Kossel, a Nobel Prize winner, invented the concept of secondary metabolite compounds in 1910, and thirty years later, Czapek described them as ‘the end-products. According to him, secondary metabolites products are derived from nitrogen metabolism such as the deamination process. Recently, the advanced research of chromatography procedure is used for

identification and analysis plant secondary metabolites [5].

Plant bioactive secondary metabolites can be divided into three chemically basic group of compounds: terpenoids, phenolics, and nitrogen-containing compound (alkaloids) [8]. The terpenoids or terpenes, are the largest class of secondary metabolites. Most of the diverse terpenes are insoluble in water, and synthesized from acetyl-coenzyme-A molecules or its glycolytic intermediates. The basic structure of terpenes are isoprene units and classified by the number of 5 carbon units (C_5). Phenolics compounds involve a various group of biomolecules, including flavonoids, tannins, antioxidants, and lignin. The basic flavonoid structure is two aromatic rings and linked by three carbon units. Most of the phenolics are water soluble, some are soluble in organic solvent, and others are large insoluble polymers. Alkaloids consist of nitrogen-containing compounds. The nitrogen atom in alkaloid is usually part of a heterocyclic ring, a ring that contains both nitrogen and carbon atoms. Some types of alkaloid compounds have pharmacological effects on vertebrate animals, and most of them are water soluble [1], [4], [8].

In general, plant secondary metabolites have been classified based on their composition (containing nitrogen or not), chemical structure (presence of ring carbon or sugar), their solubility in water or organic solvents, and the biosynthetic pathway. Biosynthetic pathways of secondary metabolites are conducted through four types of metabolic pathways: *Shikimic-acid pathway*, *Malonic-acid pathway*, *Mevalonic- acid pathway*, and *MEP (methylerythritol-phosphate) pathway*. These biosynthetic pathways are conducted using the glucose molecules that produced in photosynthesis. Glucose is the main molecules for the metabolism of secondary metabolites [2], [3]. Glucose will be further degraded into aromatic amino acids, tannins, flavonoids, lignin, phenols, alkaloid, and terpenes through these four major biosynthetic pathways in cell compartments [3].

Secondary metabolite compounds that contain in various plants could act as antioxidant, such as phenolics, carotenoids, and anthocyanins that accumulate in vegetables and fruit. Antioxidants could reduce the oxidative and structural damages caused by free radical molecules, play the important roles in preventing cancer, delaying aging, UV protector, and reducing tissue inflammation in human health [3], [9].

Primary metabolism is interconnected to the secondary metabolism by way of the production of secondary metabolite compounds, through the biosynthetic pathway. Types of biosynthetic pathway in plant secondary metabolism are shikimic acid (shikimate), malonic acid (malonate), mevalonic acid (mevalonate), and methylerythritol-phosphate pathways. Figure 1 shows the secondary metabolite products which derived from photosynthesis as primary carbon metabolism process through the four biosynthetic pathways [4].

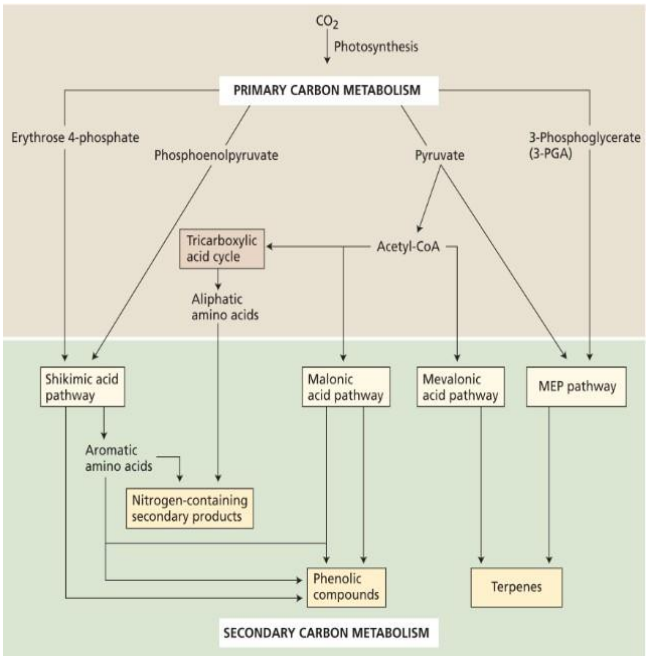

Figure 1. Integrated between plant primary and secondary metabolism through the shikimic acid, malonic acid, mevalonic acid, and methylerythritol phosphate biosynthetic pathways [4]

3.2.1. Shikimic-acid (shikimate) pathway

The shikimic acid pathway (shikimate pathway) isthe basic process for biosynthesis of phenoliccompounds. It takes place in chloroplast plant cells and have the phenylpropanoid precursors. These aromatic compounds are type of secondary metabolites that abundant in plant, and the expression of phenolics are triggered by environmental stresses, such as pathogens and herbivores attack,inappropriate pH and temperature, UV radiation, saline stress, and heavy metal stress.

Phenolic plays the important roles as antioxidant agent, electron transport, pigment molecules, signaling agents communication within plant and animals, and as defense mechanism. Shikimic compound is named after the first isolated of highly toxic Japanese shikimi flower (*Illicium anisatum*) in 1885. Moreover, the seeds of Chinese star anise (*Illicium verum*), the leaves of the sweetgum tree (*Liquidambar styraciflua*), and sample extraction from bark and wood of the sweetgum has been reported contain the shikimic acid [10], [11].

Phenolic compounds found in flavonoids is an essential part of plant secondary metabolites, and the most common of these are flavones, flavonols, and anthocyanins. The skeleton structure of flavonoids includes a chroman ring bearing an aromatic ring, which based to the oxidation level of the central ring (ring C). The recent research reported that the medicinal drugs

based of flavonoids found in roman chamomile (*Chamaemelum nobile*), ginkgo (*Ginkgo biloba*), and liquorice root (*Glycyrrhiza glabra*) [5].

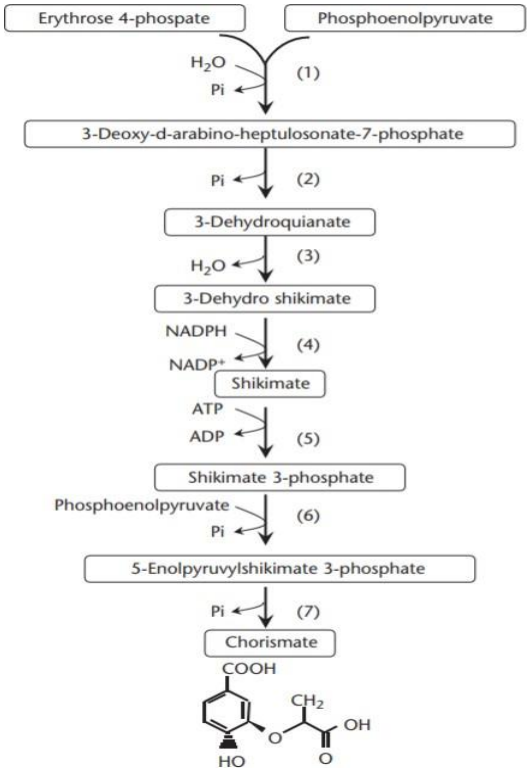

Figure 2. Shikimate pathway [11]

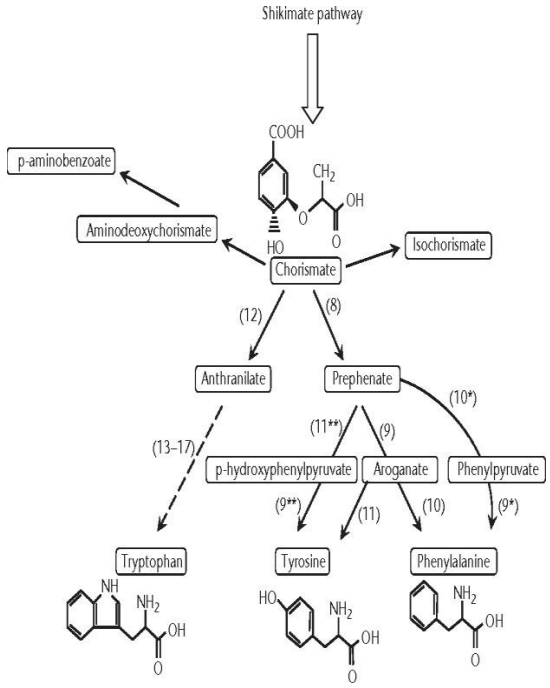

Figure 3. The synthesize process of three aromatic amino acids asprotein building blocks produces through shikimate (chorismate) biosynthetic pathway [12]

Shikimate biosynthetic pathway is also known as the chorismate pathway. This pathway provides the precursors for fungi, bacteria, and plants. Figure 2 shows the overview of shikimate pathway with the enzymatic process, and the next phase is aromatic amino acid synthesis that produced by shikimate pathway in Figure 3: Tryptophan (L-Trp), Tyrosine (L-Tyr), and Phenylalanine (L-Phe), as molecular building blocks for protein biosynthesis. The shikimate pathway is being a metabolic pathway that connecting central and specialized metabolism in the plant cell and carbon degradation during the synthesis of secondary metabolite compounds. This pathway utilizes the basic structure molecules: erythrose-4-phosphate and phosphoenolpyruvate to formed the chorismic acid [11], [13].

The shikimate pathway also involves seven enzymatic steps to catalyze the phosphoenolpyruvate (PEP) and erythrose 4-phosphate into chorismate molecules. These essential enzymes are 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS), 3-dehydroquinate synthase (DHQS), 3- dehydroquinate dehydratase/shikimate dehydrogenase (DHQ/SDH), shikimate kinase (SK), 5- enolpyruvylshikimate 3-phosphate synthase (EPSPS), and chorismate synthase (CS). The expression of most of these enzymes are depend on the environmental condition and organism developmental stages [14].

3.2.2. Malonic-acid (Malonate/Acetate) pathway

Most of the phenolics and polyphenol compounds are produced by the shikimate pathway in higher vascular plants, while the phenolic compounds are synthesized in bacteria and fungi by the malonate pathway. Chloroplast in plant cell converts the carbondioxide from the air into glyceraldehyde-3-phosphatethrough the Calvin cycle. The product of these binding sites accumulated into carbohydrate, and then degraded by glycolysis process in cytosol.

The shikimate pathway occurs in the secondary metabolites biosynthetic process, including antioxidant properties and the diverse group of phenolic compounds, provides the precursors for cell structure, growth hormone production, and electron transport chain building block. Otherwise, the role of malonate pathway in phenolic biosynthesis is less essential in higher plants. The shikimate pathway yields the phenylalanine, a basic amino acid, and their derivatives including phenols, phenolic acids, coumarins, lignans, and phenyl propane, while the malonate pathway provides quinones and xanthones. The case of terpenoid process is the integrated of those two biosynthetic pathways combined with the specific precursors [7], [15]. Figure 4 represents the phenolic compound

biosynthesis process through the shikimate pathway linked with the malonate pathway.

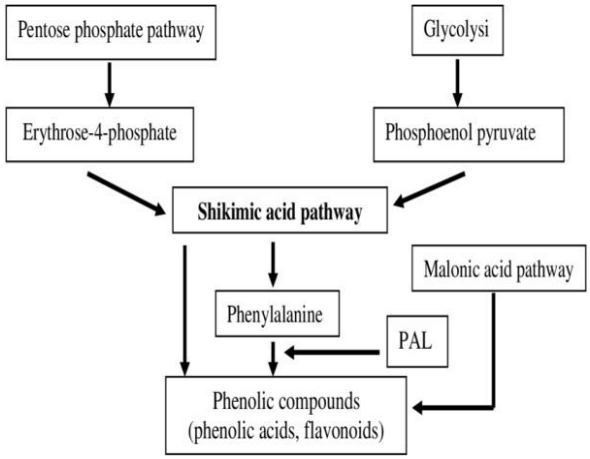

Figure 4. Phenolic compounds biosynthetic pathway through the shikimic and malonic acid [15]

3.2.3. Mevalonic-acid (Mevalonate) pathway

The mevalonic acid (MVA) pathway or mevalonate pathway also known as the isoprenoid pathway that involves the synthesis of 3-hydroxy-3- methylglutaryl-CoA reductase (HMGCR). Moreover, the MVA pathway is the core of metabolic pathway for multiple cellular metabolisms in eukaryotic, archaea, and some bacteria organisms, including cholesterol biosynthesis and protein prenylation. Cholesterol is produced as the molecules that used to build the membrane cell structure, steroid hormones, myelin sheets in neuron system, precursors of vitamin D, formation and release of synaptic vesicles [16], [17].

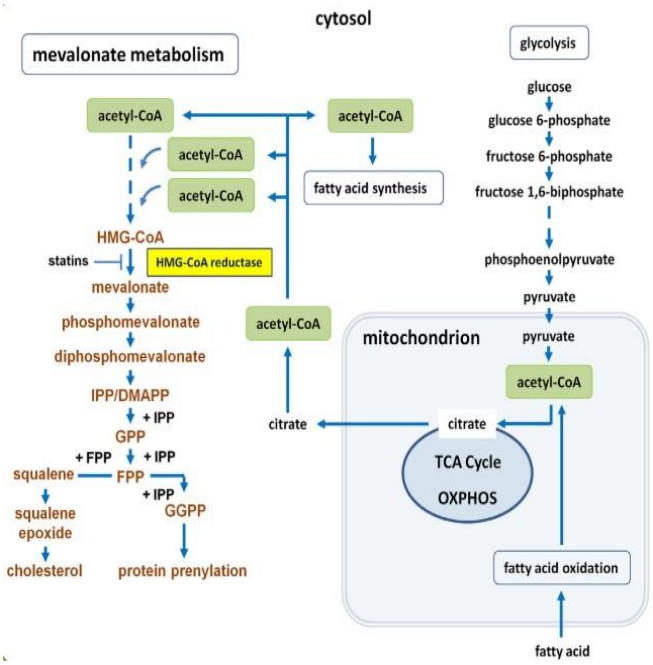

Figure 5. Mevalonate (MVA) pathway [17]

Figure 5 shows the synthesis of fatty acid and cholesterol during the mevalonate metabolism process in cytosol and mitochondria of eukaryotic cells. Mevalonate pathway utilize the acetyl-CoA and glucose to synthesize the cholesterol and fatty acid, through the enzymatic steps and tricarboxylic acid (TCA) cycle. Besides the cholesterol, MVA pathway is alsosynthesize the terpenoid compounds connected along with the Methylerythritol-phosphate (MEP) pathway, using the basic isoprenes molecules [18].

3.2.4. Methylerythritol-phosphate pathway

The Methylerythritol-phosphate (MEP) pathway, that integrated with the Mevalonate (MVA) pathway, produces the terpenoid compounds in cytosol and plastid cell compartment. Terpenoid derived from the basic unit isoprenes, which are the largest classes of natural products with over 55000 molecules. Terpenoids are found in all organism kingdom, and essential for cell-wall biosynthesis, plant growth regulation, antimicrobial properties, UV protection, photosynthesis metabolism, intracellular signaling, protein degradation, and have biological activities as pharmaceutical drug compounds. Anticancer drug. Taxol (Taxus brevifolia) and antimalarial medicine Artemisinin (Artemisia annua) are reported as the most renowned terpenoid-based medicinal drugs [8], [19].

Biosynthesis of terpenoid through the MEP pathway are divided into four steps: Formation of IPP (*isopenthenyl pyrophosphate*) and its isomer DMAPP (*dimethylallyl diphosphate*); addition of IPP by prenyl transferases to form a *prenyl diphosphates*; folding and cyclization of *prenyl-diphosphates* by terpene cyclases enzyme; and modification of secondary enzymatic to the basic skeleton [1]. Figure 6 shows the synthesis of terpenoid compound types through the connection between MVA and MEP biosynthetic pathways.

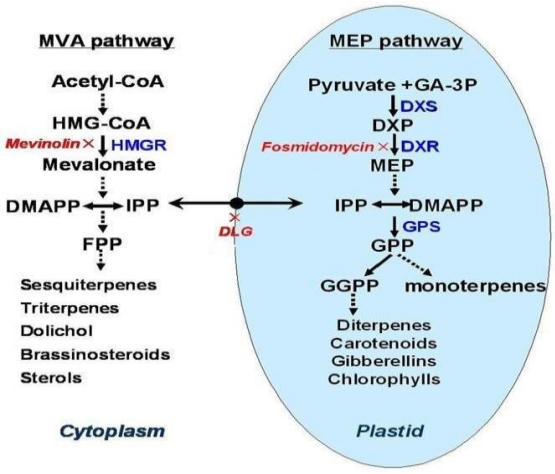

Figure 6. Methylerythritol-phosphate (MEP) pathway [18]

3.3. *In-Silico Molecular Docking*

Identification, visualization, and analysis of the certain secondary metabolites can be processed through the way of *in-vitro* qualitative screening and *in-silico* procedures. *In-vitro* analytical method encompasses the extraction, isolation, and purification of secondary metabolites extracts. The organic and aqueous solvent used for extraction process, are chosen based on their solubility and polarity of the solute. The different types of these solvents are ethanol (ethyl alcohol), methanol hexane, chloroform, or ethylacetate [9], [20].

Extraction is the first process to separate and isolate the secondary metabolite compounds from the raw material in plant samples. Separation is the further step to purify and obtain the active fraction or pure secondary metabolites from the extraction technique above. The various extraction methods are maceration, decoction, reflux extraction, percolation, Soxhlet extraction, pressurized liquid extraction/PLE, ultrasound assisted extraction/USE, microwave assisted extraction/MAE, pulse-electric field extraction/PEF, supercritical fluid extraction, enzyme assisted extraction/EAE, hydro distillation and steam distillation [21].

The further steps of separation are purification, identification, and analysis of extract compounds using column chromatography. Various types of column chromatography techniques are *Thin Layer Chromatography* (TLC), *Liquid Chromatography Mass Spectrometry* (LCMS), and *High-Pressure Liquid Chromatography* (HPLC). Moreover, LCMS and HPLC are the most familiar methods to identify and analyze the specific secondary metabolites in plant samples [21].

The application of LCMS procedure has increased to define the molecular structures, metabolic pathways, and chemical structure of the plant secondary metabolites. Identification of diverse compounds genes and their interaction will be leads to the creation of new medicinal drugs in genetic engineering, which would affect to the human health. Furthermore, LCMS is the most popular method to analyze plant medicines because it is not limited by the sample's volatility, easy to learnt, and could be proven to detectthe quantification of secondary metabolites groups inplant sample [3], [9], [21].

Screening, identification, exploration, and analysis from plant medicinal sample extracts will be continued to the *in-silico molecular docking* procedure. Secondary metabolites that have been identified through extraction and separation methods can be analyzed using *in-silico molecular docking* bioinformatics, to predict the potential drug target inextract plant sample. The study of in-silico molecular docking is integrated of organic biochemistry, biological science, statistical, computer, and engineering study. In-silico (computational) molecular docking approach is the further machine-learning technique for analysis from the protein databases with computational software, classification the specific secondary metabolites, developing and supporting for the drug discovery based on the plant bioactive compounds. Drug discovery and development is a very complicated process and time consuming, requires the study of biology and genetic molecular approach based on digital technology and computational tools [22].

In-silico molecular docking is one of the bioinformatics methods that could be used for visualizing and discovering the actions of un-known plant secondary metabolites compounds by identification of their molecular targets using chemo informatics and bioinformatics connected with the biological system, analyzing the orientation of single molecule to another when bound to each other to form a stable complex, predicting the drug molecules which could bind to a specific target known to be involved in causing a disease, and frequently used to determine the binding affinity of small molecule to their proteintargets. [20], [22]. Figure 7 represents the application of molecular docking in drug discovery based on plant secondary metabolites, including the process of target drug selection, ligand-enzyme selection, collecting the information into the database, scoring, visualization, and evaluating the docking results.

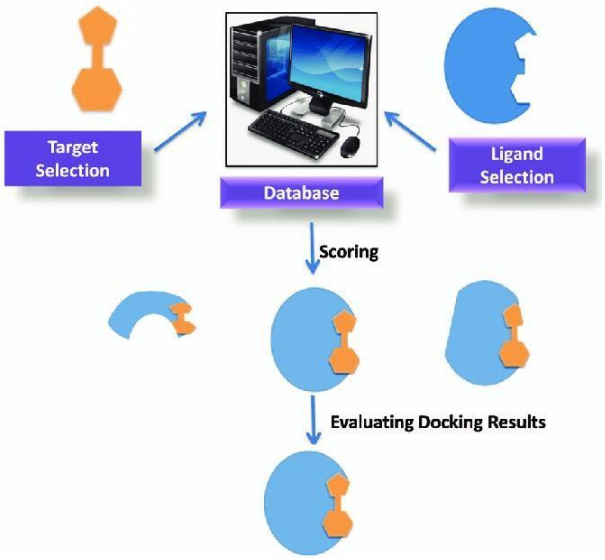

Figure 7. The overview of in-silico molecular docking in drug discovery [23]

The field of study *in-silico* molecular docking contain of polypharmacology, drug repositioning, ligand-target binding rationalization, virtual screening, target fishing and profiling, and prediction of adverse drug reaction [24]. Drug discovery processstart from the knowledges about the databases of plant secondary metabolites. Databases provide the specific information about these availability compounds, such as: name of the local and vernacular of plant, types of secondary

metabolites compounds, basic chemical structures or chemo informatics, pharmacological and biological activities, and toxicity effects [20].

In-silico molecular docking methods in drug discovery based of plant secondary metabolites are consist of four steps generally: protein preparation, ligand preparation, molecular docking study (validation of molecular docking methods, analysis of molecular docking with computational software, analysis of molecular docking result and structure visualization) drug-likeness and toxicity prediction. Besides, application of in-silico in drug design and development consist of protein-protein interaction, machine learning, molecular dynamic, virtual screening, docking simulation (visualization), and homology modelling [25]– [27].

3.3.1. Protein preparation

Protein and enzymes macromolecule target in plant secondary metabolites are searched and downloaded from biological databases, such as PubChem at https://pubchem.ncbi.nlm.nih.gov/ or Protein Data Bank at https://www.rcsb.org/. PubChem is the public repository that storing the largest collection and chemical information of biological molecules, freely accessible for chemical and physical structures, biological pathway and biomolecules activities, protein and genes structures, toxicity impacts and disease discovery, medicinal chemistry, safety molecules, patents and literature citations from around the world. PubChem first launched in 2004 as the Molecular Libraries Roadmap Initiatives of the US National Institutes of Health (NIH). PubChem organizes the integrated data from three sources databases: Substance, Compound, and BioAssay. The Substance database (SID: SubstanceID) stores the contributed and recent chemical information. This information is collected and stored in Compound database (CID: CompoundID), and then the BioAssay database (AID: AssayID) are keep the descriptions of these biological assays on chemical substances [28].

Protein Data Bank (PDB) is the global achieve of molecule biological database which maintained by Research Collaboratory for Structural Bioinformatics (RCSB) and Worldwide Protein Data Bank Organization. Moreover, RSCB PDB create tools and resources for research and education of biological system, and also collect the information about 3D protein structure, enzyme, nucleic acid, complex macromolecules from all aspects of biology, biomedicine, agriculture, energy science, and health. This software used for browsing, analyzing, and molecular visualization based of PDB ID, name, sequence, and ligand SMILES. Furthermore, the PDB database receives the depositor and information including nuclear magnetic resonance spectroscopy, macromolecular crystallography, 3D electron microscopy and micro-electron diffraction. Besides that, metabolic pathway of biological activity that yields the metabolites as the product of chemical reaction, are submitted to the PDB [26], [29], [30].

3.3.2. Ligand preparation

The ligand structures are downloaded from https://pubchem.ncbi.nlm.nih.gov/ in 2D model, or prepared using the Sybyl 7.3 Molecular Modeling Suite of Tripos, Inc. Conformation of 3D ligand aredetermined using Concord 4.01. The structures of 3D ligand are extracted from the PubChem database, The PubMed, Google Scholar, and the other Phytochemical literature databases, which used to describe the secondary metabolites compounds present in plant [25], [26], [31].

3.3.3. Molecular docking study

Molecular docking study is explained the validation of molecular docking methods, computational software to analyze the structure visualization of the compounds. PyRx Virtual Screening Tool is used to explain the possible orientation, binding affinity, and the structure conformation for the ligand with the potential binding site. The potential ligand is converted to PDBQT format, and continued for docking process in AutoDock Vina. Molecular docking is used for determine, predict, visualize, and evaluate the interaction of binding affinity between the target proteins (enzymes) and certain ligands, recognition the rapid of the potential inhibitors through the binding assessment of candidate ligands [26].

Visualization of 3D protein structure shows agraphic comparison of PDB sequence with UniProt and other annotation. Jmol, an open source for chemical structures (http://www.jmol.org/) is the 3D viewer for the RCSB PDB website and other web resources. Besides that, browser WebGL as the new technology for 3D graphics, also provides support for visualization of 3D structure. JavaScript browser enable to analyze the graphics applications within a web browser. AutoDock Tools, PyMol, LigPlot, PLANTS and YASARA are used for the docking analysis. The project of visualization the protein structures utilize online computational tools such as BLAST, MarvinSketch, and Clustal Omega, completed with UCSF camera as powerful and user-friendly visualization target [30], [32]– [34]. Furthermore, positions and orientations of the ligandson the active sites of receptors, can be visualized and performed in BIOVIA Discovery Studio v. 2020 software, as well as the interactions of the amino acidare displayed in 2D and 3D conformation structures [35].

3.3.4. In-silico drug-likeness and toxicity prediction

The process of drug-likeness assessment from the certain plant isolated compounds is predicted based on the established concept from Lipinski, known as 'Lipinski's Rule of Five' in 1997 [36]. Lipinski states that medicinal compounds from the plant secondary metabolites have the drug-likeness potential according to these five rules: hydrogen-bond acceptors (HBAs) no more than 10, hydrogen-bond donors (HBDs) no more than 5, log P should be less than 5, molecular mass should be less than 500 Dalton, and total polar surface area (TPSA) should not be greater than 140Å. The rule violations of eachassessment should not be more than 2. Drug-likeness is a prediction concept that a particular organic molecule from plant compounds has consistent potency with being an active drug [35].

The further steps are submitted the certain compounds with the Canonical SMILE structure to the SwissADME (http://www.swissadme.ch/), pkCSM (http://biosig.unimelb.edu.au/pkcsm/), and PreADMET tools to measure the silico pharmacokinetic parameters. This ADMET assessment includes the process of absorption, distribution, metabolism, excretion, and toxicity studies of the isolated compounds [27], [35]. The toxicity prediction of the isolated compounds is defined using OSIRIS Property 2, PreADMET, ECOSAR v2. software and Pro Tox-II (https://tox-new.charite.de/protox_II/). The toxicological prediction gives results such as capability of compounds to carcinogenicity, hepatotoxicity, mutagenicity, and cytotoxicity. The compounds from the extract plant samples are predicted to be carcinogenic, non-carcinogenic and non-irritant. Based on the ADMET assessment and prediction, isolated certain plant bioactive compounds can be confirmed as a good or bad drug candidate [27], [35].

Determination of chemical bioactive compounds derived from plant medicinal products, can be startedby learning the plant physiology and metabolic process, biosynthetic pathway and degradation of metabolite products, qualitative screening and analysis using in-vitro and in-silico procedures. Secondary metabolites in plant medicinal compounds are synthesized from the degradation of glucose molecules through the glycolysis in primary metabolism, and then produced the four majors of secondary metabolites from the essential plant biosynthetic pathways. The potential drug based on plant secondary metabolites can be determined by *in silico molecular docking* bioinformatics procedure. This computational approach provides an essential tool and software for predicting, inventing, visualizing, determining, and developing the plant potential medicinal drugs for human's health.

ACKNOWLEDGMENTS

This research about the study of plant metabolism, secondary metabolites biosynthetic pathway, and *in-silico* molecular docking was funded by PNBP Grant, Universitas Negeri Malang, Indonesia, 2021.

REFERENCES

[1] M. A. Bernards, Plant natural products: A primer, Can. J. Zool., vol. 88, 2010, pp. 601– 614. doi: 10.1139/Z10-035.

[2] H. Ashihara, A. Crozier, A. Komamine, Plant Metabolism and Biotechnology, 2011.

[3] M. E. Saltveit, Synthesis and metabolism of phenolic compounds 5 . 2 structure of somesimple phenolic compounds, vol. 1, 2018, pp. 115–123.

[4] M. P. Norton, D. G. Karczub, Appendix4: secondary metabolites, Plant Physiol.Dev, 2015, pp. 605–606. Available: http://6e.plantphys.net/appendices.html#.

[5] N. Ivanova, V. Gugleva, M. Dobreva, I. Pehlivanov, S. Stefanov, V. Andonova, We are IntechOpen, the world's leading publisher of open access books built byscientists, for scientists TOP 1 %, Intech, vol. 1, 2016, p. 13.

[6] T. Isah, Stress and defense responses in plant secondary metabolites production, Biol. Res., vol. 52, 2019, p. 39. doi: 10.1186/s40659-019-0246-3.

[7] I. F. G. Mera, D. E. G. Falconí, V. M. Córdova, Secondary metabolites in plants: main classes, phytochemical analysis and pharmacological activities, Rev. Bionatura, vol. 4, 2019. doi: 10.21931/RB/2019.04.04.11.

[8] K. Eljounaidi, B. R. Lichman, Nature's chemists: the discovery and engineering of phytochemical biosynthesis, Front. Chem., vol. 8, 2020, pp. 1–10. doi: 10.3389/fchem.2020.596479.

[9] A. Altemimi, N. Lakhssassi, A. Baharlouei, D. G. Watson, D. A. Lightfoot, Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts, Plants, vol. 6, 2017. doi: 10.3390/plants6040042.

[10] S. Ghosh, Y. Chisti, U. C. Banerjee, Production of shikimic acid, Biotechnol. Adv., vol. 30, 2012, pp. 1425–1431. doi: 10.1016/j.biotechadv.2012.03.001.

[11] V. Tzin, G. Galili, A. Aharoni, Shikimate pathway and aromatic amino acid biosynthesis, eLS, 2012, pp. 1–10. doi: 10.1002/9780470015902.a0001315.pub2.

[12] N. F. Sanchez, R. S. Coronado, B. H. Carlos, C. Villanueva, Shikimic acid pathway in biosynthesis of phenolic compounds, Plant Physiol. Asp. Phenolic Compd., vol. 32, 2018, pp. 137–144. Available: http://www.intechopen.com/books/trends-in-telecommunications-technologies/gps-total-electron-content-tec-prediction-at- ionosphere-layer-over-the-equatorial- region%0AInTec.

[13] D. M. Pott, S. Osorio, J. G. Vallarino, From central to specialized metabolism: An overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit, Front. Plant Sci., vol. 10, 2019. doi: 10.3389/fpls.2019.00835.

[14] Z. Z. Zhang et al., Three types of ultraviolet irradiation differentially promote expression of shikimate pathway genes and production of anthocyanins in grape berries, Plant Physiol. Biochem., vol. 57, 2012, pp. 74–83. doi: 10.1016/j.plaphy.2012.05.005.

[15] A. Ghasemzadeh, N. Ghasemzadeh, Flavonoids and phenolic acids: role andbiochemical activity in plants and human, J.Med. Plant Res., vol. 5, 2011, pp. 6697– 6703. doi: 10.5897/JMPR11.1404.

[16] Y. Xia et al., The mevalonate pathway is a druggable target for vaccine adjuvant discovery, Cell, vol. 175, 2018, pp. 1059- 1073.e21. doi: 10.1016/j.cell.2018.08.070.

[17] G. Gruenbacherand, M. Thurnher, Mevalonate metabolism immuno in oncology, Front Immunol, vol.8, 2017, pp.1-8. doi: 10.3389/fimmu.2017.01714

[18] D. Yang et al., Different roles of the mevalonate and methylerythritol phosphate pathways in cell growth and tanshinone production of salvia miltiorrhiza hairy roots, PLoS One, vol. 7, 2012. doi: 10.1371/journal.pone.0046797.

[19] A. Frank, M. Groll, The methylerythritol phosphate pathway to isoprenoids, Chem. Rev., vol. 117, 2017, pp. 5675–5703. doi: 10.1021/acs.chemrev.6b00537.

[20] A. Singh, S. Zahra, S. Kumar, Phytochemistry: an in-silico and in-vitro update, Phytochem. An in-silico in-vitro Updat., January 2020, 2019, doi: 10.1007/978-981-13-6920-9.

[21] Q. W. Zhang, L. G. Lin, W. C. Ye,Techniques for extraction and isolation of natural products: A comprehensive review, Chinese Med. (United Kingdom), vol. 13, 2018, pp. 1–26. doi: 10.1186/s13020-018-0177-x.

[22] H. Chikhale, A. Nerkar, G. E. Society, Review on In-silico techniques an approach to drug discovery, Curr. Trends Pharm. Pharm. Chem., vol. 2, 2020, pp. 24–32.

[23] M. Prasad et al., Virus-host interactions: new insights and advances in drug development against viral pathogens, Curr. Drug Metab., vol. 18, 2017. doi:10.2174/1389200218666170925115132.

[24] L. Pinzi, G. Rastelli, Molecular docking: shifting paradigms in drug discovery, Int. J.Mol. Sci., vol. 20, 2019. doi:10.3390/ijms20184331.

[25] R. Hariyanti, A. Rachmania, M. Karinah, H. Sunaryo, In silico analysis of the phytochemical compounds in carica papaya seeds for optimizing the inhibitors of HMG- CoA reductase, 2019, pp. 123–132. doi:10.5220/0008240501230132.

[26] S. Mahmud et al., Plant-based phytochemical screening by targeting main protease of sars-cov-2 to design effective potent inhibitors, Biology (Basel)., vol. 10, 2021, pp. 1–18. doi: 10.3390/biology10070589.

[27] M. Anza et al., Antimicrobial activity, in silico molecular docking, admet and dft analysis of secondary metabolites from rootsof three ethiopian medicinal plants, Adv. Appl. Bioinforma. Chem., vol. 14, 2021, pp. 117–132. doi: 10.2147/AABC.S323657.

[28] S. Kim et al., PubChem substance and compound databases, Nucleic Acids Res., vol. 44, 2016, pp. D1202–D1213. doi: 10.1093/nar/gkv951.

[29] P. W. Rose et al., The RCSB protein data bank: integrative view of protein, gene and 3D structural information, Nucleic Acids Res., vol. 45, 2017, pp. D271–D281. doi: 10.1093/nar/gkw1000.

[30] S. K. Burley et al., RCSB protein data bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences, Nucleic Acids Res., vol. 49, 2021, pp. D437–D451. doi:10.1093/nar/gkaa1038.

[31] K. E. Hevener et al., Validation of molecular docking programs for virtual screening against dihydropteroate synthase, J. Chem. Inf. Model., vol. 49, 2009, pp. 444–460. doi: 10.1021/ci800293n.

[32] G. O. Oduselu, O. O. Ajani, Y. U. Ajamma, B. Brors, E. Adebiyi, Homology modelling and molecular docking studies of selected substituted

benzo[d]imidazol-1-yl)methyl)benzimidamide scaffolds on plasmodium falciparum adenylosuccinate lyase receptor, Bioinform. Biol. Insights, vol. 13, 2019. doi: 10.1177/1177932219865533.

[33] C. R. Terrell, L. L. Listenberger, Using molecular visualization to explore protein structure and function and enhance student facility with computational tools, Biochem. Mol. Biol. Educ., vol. 45, 2017, pp. 318–328. doi: 10.1002/bmb.21040.

[34] E. Mulatsari, T. Martati, E. Mumpuni, N. L. Dewi, In silico analysis of antiviral activity of analog curcumin compounds, J. Jamu Indones., vol. 5, 2020, pp. 114–121. doi: 10.29244/jji.v5i3.173.

[35] S. S. Mousavi et al., In silico evaluation of iranian medicinal plant phytoconstituents as inhibitors against main protease and the receptor-binding domain of sars-cov-2, Molecules, vol. 26, 2021. doi:10.3390/molecules26185724

[36] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv.Rev., vol. 64, 2012, pp. 4–17. doi: 10.1016/j.addr.2012.09.019