RESEARCH ARTICLE

Bioinformatics approach to enhance the undergraduate biology students' understanding of plant terpenoid

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Abstract: Plant metabolite compounds have been applied on plant cellular metabolism, produces organic and inorganic compounds, primary and secondary bioactive molecules, such as glucose, amino acids, fat acids, alkaloids, flavonoids, and terpenoids. Undergraduate biology students learn about the plant terpenoid assisted by bioinformatics to store, manage, and interpret the molecular information about these compounds. This research aims to observe the students' understanding of terpenoid through implementing bioinformatics approach. Besides, to investigate how practice the bioinformatics technology in learning terpenoid may impact students' comprehension of plant metabolism domain and their acquisition of bioinformatics inquiry skills. Bioinformatics is a computational database that relies on digital repositories of molecular biology informations. Data analysis was in the form of quantitative and qualitative descriptive using module learning resources based on terpenoid research and assisted by bioinformatics. The results indicate that students' comprehension of learning and understanding terpenoid has improved, identification and analysis processes of article reviews showed that students were able to discuss and interpret research finding in silico bioinformatics using molecular docking procedures. Assessment of bioinformatics skills showed that all undergraduate biology students could follow the direct instructions well, answer the questions, practice the dry-lab experimental, and formulate the conclusion correctly.

Keywords: bioinformatics; terpenoid; undergraduate biology students

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Introduction

Recently, metabolomics has been a growing field applied to study in-depth information on plant cellular metabolism, a group of the small molecular mass of compounds in plant extracts. Usually, plants produce numerous metabolites for their developmental growth and environmental responses (Tan et al., 2022; Vinay et al., 2021). The plant kingdom contains numerous metabolites compounds, including primary and secondary metabolites. These primary metabolites are responsible for developmental growth and are structurally conserved, whereas secondary metabolites are specialized, utilized under duress conditions, and vary between plant species. Plant sintesize a wide selection of metabolites, including hydrophilic carbohydrates, hydrophobic lipids, organic compounds, ionic inorganic compounds, amino acids, fat acids, flavonoid, alkaloid, and terpenoid (Courdavault et al., 2021; Xiao et al., 2022).
Plants contain more than 50,000 different terpenoid compounds with different structures and types. In interactions between plants and their surroundings, with insects, and with pathogens, terpenoids are crucial. The vegetative parts of plants, such as the roots, flowers, leaves, seeds, and fruit, are where terpenoids are formed. Terpenoids act the crucial role in the growth and development process, for instance carotenoid compounds (C_{40}) as a photosynthetic pigment, abscisic acid (C_{15}) and gibberellins (C_{20}) as a growth regulator hormone (phytohormone), a compound that makes up the structure of cell

membranes (sterol C_{30}), phytoalexins as self-defense mechanisms against predators, ubiquinone involved in mitochondrial electron transport, protein degradation, modification of genetic transcription and translation processes, cell wall biosynthesis, as well as allelopathic compounds in inter-plant competition. Terpenoids' physical structure includes a low molecular weight, high vapor pressure, and the ability to act as a messenger molecule to and from cells (Abbas et al., 2017; Cheng et al., 2007; Frank & Groll, 2017).

The presence of complex metabolites in plant terpenoid poses a challenge for analytical instruments designed to separate and characterize the compounds. Terpenoid analysis by in-vitro can be continued to bioinformatics approach, to determine and predict the potential pharmacological effects. Bioinformatics methods that rely on computational methods include molecular docking, drug-likeness prediction, ADMET analysis, toxicity assessment, and simulation molecular dynamics (MD), which all refer to digital biological databases. Research demonstrates that the bioinformatics computational approach is applied to look for phytochemical compounds that can successfully bind to the active site of target proteins, can be applied as investigation materials and the discovery of new drugs, as well as to provide scientific proof in identifying traditional medicinal ingredients. Bioinformatics approaches can be used to identify and analyze the plant's molecular taxonomy, management of plant diseases, the amount of plant tolerance to environmental stress, and plant terpenoid biosynthetic pathway (Babar et al., 2017; Chapman et al., 2006; Robinson et al., 2010; Sharma & Sarkar, 2013).

Plant terpenoids are the focus of bioinformatics-based learning and research in the field of biology major. Further investigation into terpenoids' potential as drug candidate molecules is being conducted as part of the drug-design process using bioinformatics technique (Ludwiczuk et al., 2017; Zhang et al., 2022). Predicting and analyzing process of terpenoid as potential drug candidates has already reveals through recent international literatures, that some of the plant terpenoid compounds have potential therapeutic agents for certain diseases, based on bioinformatics (Giofrè et al., 2021; Gualdani et al., 2016).

Technique-based determination of drug substance can save more time and expenses; hence it is extensively employed in the drug and pharmacology industries (Khater et al., 2016; Vardhan & Sahoo, 2020; Yi et al., 2018). Bioinformatics is a relatively new branch of biology that arose in response to the increasing demand for efficient, flexible, and intelligent methods of storing, managing, and interrogating large and complex biological data sets. It is an interdisciplinary science with significant ties to mathematics, statistics, and computer science, as well as the life sciences. Using sequencing technology, plant biologists have deciphered the genetic architecture of various plant and microorganism species, including their proteome, transcriptome, metabolome, and metabolic pathway (Madden et al., 2020; Moradi et al., 2022; Tan et al., 2022; Vinay et al., 2021).

Bioinformatics is a crucial area of study for biology students and researchers since it is a component of the computational technology that supports biology research and education. The education system has not fully included bioinformatics into the teaching and learning process in the classroom, thus an approach and activities are required that can enhance students' understanding of and proficiency with bioinformatics methods. The curriculum that is taught in the school setting should be integrated with this activity (Bain et al., 2022; Martins et al., 2020). Bioinformatics competencies are representing what biology students can do with their proficiency in knowledge (cognitive), skills (psychomotor), and attitude (affective) toward learning biology materials used the bioinformatics technology. These three competencies help the students in learning biology materials, exercising creativity and critical thinking, capable in analyzing the big data biology based on computer, and solving the scientific problem with the technology approach (Chapman et al., 2006; Tractenberg et al., 2019).

The Network for Integrating Bioinformatics into Life Sciences Education (NIBLSE; pronounced "nibbles") is a global research organization that concentrates on the advancement of the fields of biology, bioinformatics, and computer science, formulated the creation of basic bioinformatics capabilities for the science curriculum, challenges to integrating bioinformatics into classroom instruction, and methods for creating, assessing, and evaluating bioinformatics learning tools. The formulation of the curriculum, design of the syllabus, and the creation of the assessment all include the execution of the bioinformatics core skills (Dinsdale et al., 2015; Toby et al., 2022).

NIBLSE standards for bioinformatics competency assessment and indicators cover 7 (seven) Competencies, including: Describe how data and computational methods are utilized in the life sciences to respond to both hypothesis-driven and hypothesis-generating queries (C1); Present an overview of essential computational concepts and relational databases, as well as their applications in the life sciences (C2); Examine the complex biological issues utilizing bioinformatics techniques (C3); Locate, retrieve, and organize diverse categories of biological data (C4); Exploring the biological interactions and data integration (C5); Analyze and describe the categories of data, their structure, and reproducibility (C6), and Interpret biological data's ethical, legal, medicinal, and social consequences (C7) (Toby et al., 2022; Wilson Sayres et al., 2018).

Learning about plant terpenoids is carried out in higher education, particularly for biology majors. The terpenoid group, molecular structure, physical and chemical activity, natural bioactivity, qualitative and quantitative tests, and pharmaceutical activity are all covered in terpenoids. Further investigation into terpenoids' potential as drug candidate molecules are being conducted as part of the drug-design

process using bioinformatics (Ludwiczuk et al., 2017; Zhang et al., 2022). Undergraduate biology who learning the plant terpenoid and its biosynthetic pathway, can implements their knowledge about the terpenoid biosynthesis into their capability using bioinformatics to predict the terpenoid as therapeutic agents or drug candidates. Bioinformatics have been developed in accordance with evaluation in biology molecular study and utilized to represent terpenoid compounds through the genetic tracing stage (Giofrè et al., 2021).

This study aimed to investigate how learning plant terpenoids through bioinformatics approach may impact students' comprehension of the plant metabolism domain and their acquisition of bioinformatics inquiry skills. The findings suggest that undergraduate biology students proficient in utilizing bioinformatics technology can make valuable contributions to the advancement of expertise in the field of biology research and education. Specifically, it can assist students in analyzing the concept of plant primary and secondary metabolisms, understanding the plant metabolites biosynthesis, and exploring the development of metabolite compounds as medicinal products.

Method

The research was conducted at Department of Biology, Universitas Negeri Malang, East Java Indonesia, with the 25 respondents of third year students in Biology major. These students were enrolled in Plant Metabolism course that focuses on plant primary and secondary metabolism, terpenoid compounds, and implementation of bioinformatics techniques to predicting the potential therapeutic agents of terpenoid. Bioinformatics approach on terpenoid material was taught over the course of three meetings using teaching module based on research results and molecular docking procedures. Assessment of cognitive aspects and inquiry skills through pretest and posttest, article review of bioinformatics research, and bioinformatics skills.

The learning resource used was module based on phytochemical research and assisted by bioinformatics methods. The module includes learning materials on phytochemical compounds, an introduction to bioinformatics techniques, and in-silico molecular docking procedures. Plant sample was mahogany (*Swietenia* sp.) leaves, and an in-silico approach used to analyze the potential of terpenoids as medicinal compounds. The computer device is laptop with Intel-core i5, 1.6Ghz Turbo 3.9Ghz specifications, Intel VGARUHD Graphics 620, Windows 10 Home 64bit. Ligands and target protein databases used are PubChem (https://pubchem.ncbi.nlm.nih.gov/), Protein Data Bank (www.rcsb.org), Pass-Server Online (http://www.way2drug.com/passonline/), and Uniprot (https://www.uniprot.org/). Molecular docking softwares used for free-access are BIOVIA Discovery Studio (https://www.discngine.com/discovery-studio); PyMol (https://pymol.org/) and PyRx (https://pyrx.sourceforge.io/).

Quantitative data includes pretest and posttest, which consist of 20 multiple choice questions, content-based on plant primary and secondary metabolisms, secondary metabolites/phytochemical compounds, content-knowledge about bioinformatics technique, and implementation of molecular docking bioinformatics approach. Qualitative data includes the article review of research result about the implementation of bioinformatics approach to studying the plant phytochemistry. Students are divided into eight groups, each group analyzing bioinformatics articles, including plant samples, research methods, phytochemical compounds and target proteins involved, online databases and bioinformatics software, analytical techniques and pharmacological effects of phytochemical compounds, writing the discussions, and conceive the conclusions. Assessment of bioinformatics skills used the instrument and observation sheet which refers to the bioinformatics competency rubric. Students work on the molecular docking process through the direct instruction using laptop devices based on database online and bioinformatics softwares.

Results and Discussion

Students' understanding of terpenoid and bioinformatics through pretest and posttest

To study how the bioinformatics learning environment influences undergraduate biology students' comprehension of plant metabolism and terpenoid analysis, we examined the students' acquisition of cognitive and skill domain using the pretest, posttest, and observation sheet of terpenoid concepts and bioinformatics approach.

Pretest and posttest questions involves the following topics: plant metabolism process, anabolism and catabolism reactions, primary and secondary metabolite products, classification and biosynthesis pathway of secondary metabolites (phytochemicals) compounds, the role and function of secondary metabolites for plants, introduction to bioinformatics technology, definition and purpose of bioinformatics, bioinformatics approach in biological research, databases and software used in bioinformatics analysis,

and stages of bioinformatics procedures in predicting phytochemical compounds as potential drugs.
The pretest and posttest questions were obtained from the module material, showing the difference results in learning outcomes. The posttest scores of all students increasing significantly, especially on the topic of secondary metabolites and bioinformatics. Statistical analysis using paired sample t-test to examined the distinction between pretest and posttest.
Table 1 showed the average pretest and posttest scores for each content knowledge topic, to observe the students' understanding.

Table 1. Pretest and posttest scores of content-knowledges

Content knowledge	Question number	Pretest average score (N=25)	Posttest average score (N=25)
Primary and secondary metabolism	1, 2, 3	49.33	66.67
Secondary metabolites products	4, 5, 6, 7, 8, 9, 10	42.29	65.71
Introduction to bioinformatics technique	11, 12, 13, 14, 15	70.40	89.60
Implementation of in-silico bioinformatics to biology material	16, 17, 18, 19, 20	52.80	71.20

Referred from Table 1 and statistical calculations, the average score on pretest was 53.0, SD 17.97, and for posttest was 73.20, SD 11.54, $P < 0.05$ (Table 2). It can be stated descriptively that there is a difference of the average scores between pretest and posttest, despite the fact that the average values of the pretest and posttest are identical.

Table 2. Paired sample statistics of pretest and posttest

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pretest	53.00	25	17.970	3.594
	Posttest	73.20	25	11.536	2.307

These differences in learning outcomes of pretest and posttest were then examined for their significance level to see whether there was an effect of using teaching modules (Table 3). Based on the table, the value of Sig. (2-tailed) is $0.00 < 0.05$, implying that there is an average difference between pretest and posttest learning outcomes and that using teaching modules has an effect on students' content-knowledge of plant metabolism, phytochemical metabolites, and bioinformatics materials.

Table 3. Paired differences samples test of pretest and posttest

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	Pretest - Posttest	-20.200	10.555	2.111	-24.557	-15.843	-9.569	24	.000

Analysis of student responses revealed an increase in the topic of bioinformatics introduction. The lowest results were the topic of secondary metabolites and the implementation of bioinformatics. The identification of each item showed that the majority of students answered queries incorrectly about primary metabolic products, secondary metabolite biosynthetic pathways, plant organ locations, and phytochemical ligands of in-silico (numbers 4, 6, 10, and 16).
Posttest result presented that more than 20 students correctly answered questions about anabolic processes, types and functions of secondary metabolites, introduction to bioinformatics technique, the role of bioinformatics in biology learning, and plant metabolism materials that can be integrated with in-silico bioinformatics (number 2, 5, 7, 8, 11, 12, 13, 14, 15, 17, 18, and 20). These results indicate that there were significant differences from pretest to posttest, where in the pretest for the number of questions above, only one number was answered correctly by the most students (number 13). This

question explores about the role of bioinformatics technique in empowering students' digital literacy based on computing technology at higher education.

The posttest on the topic *Introduction to bioinformatics technique* delivered the highest scores, with ≥ 20 students answering each of the five questions correctly: number 11 on the role of general bioinformatics (23 students); number 12 on the scientific definition of bioinformatics (23 students); number 13 on the role of bioinformatics technique in empowering students' digital literacy (25 students); number 14 on biology research and learning assisted by bioinformatics (20 students); and number 15 on plant metabolic materials integrated with in-silico bioinformatics (21 students).

Overall, based on the statistical results and data analysis of the pretest and posttest, the learning outcomes using modules based on phytochemical research and assisted by in-silico bioinformatics indicate that the students' cognitive abilities to encompass the plant metabolism, secondary metabolites products, and introduction to bioinformatics, have been increasing.

Students' understanding of terpenoid through articles review

The findings of the pretest and posttest, as well as a review of articles on the results of bioinformatics research, were used to assess students' cognitive capacities in learning terpenoids and bioinformatics. Students worked on the pretest and posttest questions using teaching modules based on the findings of phytochemical research and assisted by bioinformatics techniques.

In addition to assessing learning outcomes through pre- and posttest, students review the article pertaining to bioinformatics procedure in phytochemical research. Students identify and analyze the research article encompass plant samples, select the phytochemical compounds and target proteins, distinguish between the databases online and software, write down the analytical techniques and pharmacological effects of phytochemicals, and construct the conclusions based on research findings. Table 4 presents of each group's student analysis.

Table 4. Results of identification and analysis stage of research articles

No	Question	Answer
1.	Plant sample	Mahogany stem bark (*Swietenia macrophylla*, King.)
2.	Phytochemistry (Ligand)	α-D-glukopiranosa, Catechin, Evodionol, Swietenin K
3.	Target protein	α-glukosidase enzyme (PDB: 3A4A) from *Saccharomyces cerevisiae*
4.	Database online	PubChem, Protein Data Bank (PDB)
5.	Software	Autodock Tools 1.5.6, Autodock 4.2.6, Discovery Studio 2016
6.	Analytical technique	a. Extraction and fractionation of mahogany stem bark by maceration technique, partitioned with n-hexane-water and evaporated with rotary vacuum evaporator procedure. α-glucosidase inhibition test with absorbance at λ 405 nm and determination of IC_{50} value. b. Compounds isolation and separation by column chromatography containing silica gel 60 as the stationary phase (sample: silica 1:30) and eluted with dichloromethane: methanol (10:1) as the mobile phase. Fractionation results were analyzed by Thin Layer Chromatography (TLC). c. Compounds screening and identification by Liquid Chromatography-Mass Spectrometry (LC-MS) d. *Docking Molecular* • Macromolecule preparation The protein structure of α-glucosidase enzyme was prepared with Autodock Tools 1.5.6 and saved in form (*.pdb). • Ligand preparation The structure of natural ligand α-D-glucopyranose and *acarbose* compound identified in the mahogany bark fraction was made into a 3D structure and its binding affinity was determined. • Pharmacokinetics screening Pharmacokinetics screening with Lipinski test and ADMET. The Lipinski assay adequate the *Rule of Five* requirements, and the ADMET test includes Absorption, Distribution, Metabolism, Excretion, and Toxicity values. • Protein optimization The optimization method is performed by calculating the RMSD (*Root mean sequer deviation*) value. The best RMSD value is shown when it is less than 2 Å.
7.	Pharmacological effect	The phytochemicals present in mahogany stem bark can inhibit the activity of the α-glucosidase enzyme, decreasing the blood glucose levels.
8.	Conclusion	Extracted mahogany stem bark revealed the presence of catechin, evidionil, and swietenin K compounds. These phytochemistry can inhibit α-glucosidase enzyme activity, thereby lowering blood glucose levels, so mahogany stem bark has the potential to be used as an antidiabetic drug.

Student's understanding of bioinformatics through molecular docking procedure

The assessment of bioinformatics competence initiates by students' ability in identifying and defining the functions, roles, and fields of bioinformatics, both generally and in the context of biology. These are some examples of bioinformatics roles for biology scientists: 1) As a development and implementation database, exploration of genomic sources, as well as interpretation and conclusion of biological data obtained from researches (Osman et al., 2020); 2) Identify and classify types of phytochemical plants, analyze biosynthetic phytochemical pathway with potential as herbal drugs (Sharma & Sarkar, 2013); 3) Enhance and preserve the level of fruit maturity in biotechnology process and plant breeding (Gomez-Casati et al., 2018); 4) Identify and examine particular biological macromolecule, for instance, a disease may be examined to identify how certain genes contribute to its spread in order to find a cure and preventative measures (Liang et al., 2019); 5). Improve the synthesis of secondary metabolites based on genetic analysis, as a repository of genetic diversity data, as well as new tools design that can be used to study functions of gene in plants (Ren et al., 2020); and 6). Phytochemical ligand and target protein analysis, biological characterization, protein characterization, and phylogenetic taxonomy of organism are applied to predict diseases and effects of medications (Branco & Choupina, 2021).
Assessment of students' skills in studying the terpenoids based on NIBLSE standards was observed from their ability using bioinformatics. The evaluation tool consists of sixteen queries and work methods for identifying phytochemical ligands, target proteins, pharmacokinetics assay, and molecular docking processes. Assessment result of bioinformatics skills for terpenoid analysis showed in Table 5.

Table 5. Assessment of bioinformatics skills based on student activity

Bioinformatics skills	Average score	Competency level
C1: Describe how data and computational methods are utilized in the life sciences to respond to both hypothesis-driven and hypothesis-generating queries	87	High
C2: Present an overview of essential computational concepts and relational databases, as well as their applications in the life sciences	89	High
C3: Examine the complex biological issues utilizing bioinformatics techniques	87	High
C4: Locate, retrieve, and organize diverse categories of biological data	92	High
C5: Exploring the biological interactions and data integration	90	High
C6: Analyze and describe the categories of data, their structure, and reproducibility	88	High
C7: Interpret biological data's ethical, legal, medicinal, and social consequences	84	High

According to Table 5, the students achieved the highest score in competency C4, which involves their ability to locate, utilize, and handle biological data from different sources. Students can utilize certain file formats to access and store terpenoid molecular structures. They can also analyze terpenoid information obtained from databases and identify the structure of target proteins using PubChem, Biovia Discovery Studio software, PyRx, and PyMol.
The student's bioinformatics competency with the lowest score is C7, which states that students can convey research findings in accordance with medical regulations and their influence on the social environment. Competency C9 describes aspects of students' ability to discuss and interpret terpenoid analysis procedures based on laboratory research and in-silico approaches. Students can write discussions, draw conclusions, and present the results of terpenoid compound analysis (Cycloartenol, Fissinolide, Hederagenin, and Swietenin B) as candidate anti-inflammatory drug ingredients.

Searching the terpenoid informations through the PubChem database

The most common terpenoid phytochemicals chosen by students in this activity were Cycloartenol, Hederagenin, Fissinolide, and Swietenin B. Information regarding terpenoids obtained through PubChem included two- and three-dimensional structures, chemical and physical properties, taxonomy, classification, pharmacology and biochemistry, associated disorders and diseases, biological test results, and research literatures.
Students practice to drawing the three-dimensional structure of cycloartenol, hederagenin, fissinolide, and swietenin B after learning about these terpenoids. The 3D structure is the basic phytochemical framework for the molecular docking process, which will later binding to the target protein (Figure 1).

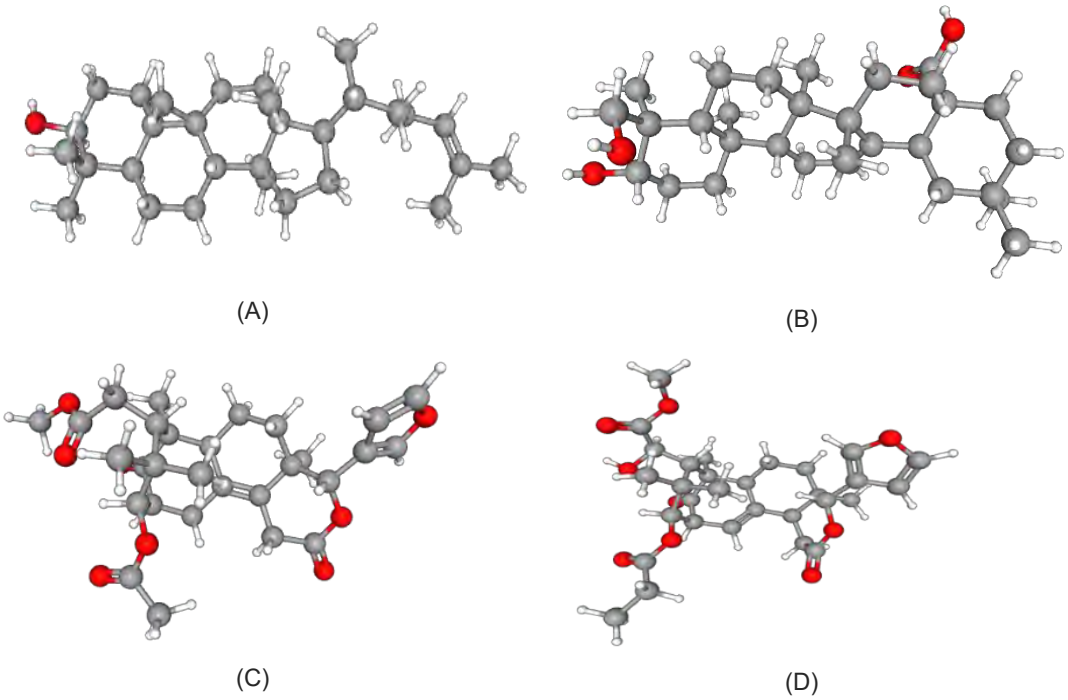


Figure 1. Three-dimensional structure of terpenoids downloaded from PubChem: (A). Cycloartenol, (B). Hederagenin, (C). Fissinolide, and (D). Swietenin B.

Terpenoid as bioactive therapeutic agents based on Probability activity (Pa) and Probability in-activity (Pi) values

In this section, students studying the potential terpenoid as therapeutic agents, by searching and exploring the terpenoid informations through Pass-Server Online website (http://www.way2drug.com/passonline/). Students keep the CANONICAL SMILES structure downloaded from PubChem, and then the results showed that Cycloartenol, Hederagenin, Fissinolide, and Swietenin B compounds belong to the terpenoid class which have therapeutic agents activity on symptoms of inflammation (as anti-inflammation ingredients). These potential terpenoid examined as anti-inflammatory agents based on Pa and Pi values from Pass-Server Online (Table 6).

Table 6. Terpenoids activity as anti-inflammatory agents

Terpenoid	Activity	Pa (*Probability activity*)	Pi (*Probability in-activity*)
Cycloartenol	Anti-inflammation	0,702	0,015
Hederagenin	Anti-inflammation	0,851	0,005
Fissinolide	Anti-inflammation	0,772	0,009
Swietenin B	Anti-inflammation	0,787	0,008

Inflammation is the protective response of mammalian tissue when exposed to infective agents such as bacteria, viruses, and fungi, chemical toxins such as reactive oxygen species, and physical agents such as heat, cold, or mechanical trauma. Our immunity induces inflammation in response to etiologic agents (physical and chemical contaminants), distinguishing inflammation from infection. Inflammation is the union of inflammatory response and healing, whereas infection is the invasion of toxins and the resulting deleterious effects. Chronic inflammatory conditions are involved in numerous diseases, such as atherosclerosis, obesity, type 2 diabetes, asthma, inflammatory bowel diseases, neurodegenerative diseases, rheumatoid arthritis, and cancer, so their significance in the modern era of medical science cannot be overstated (Ahmed, 2011; Chhabra, 2021; Greten & Grivennikov, 2019).

Terpenoid determination as medicinal compounds by Lipinski test

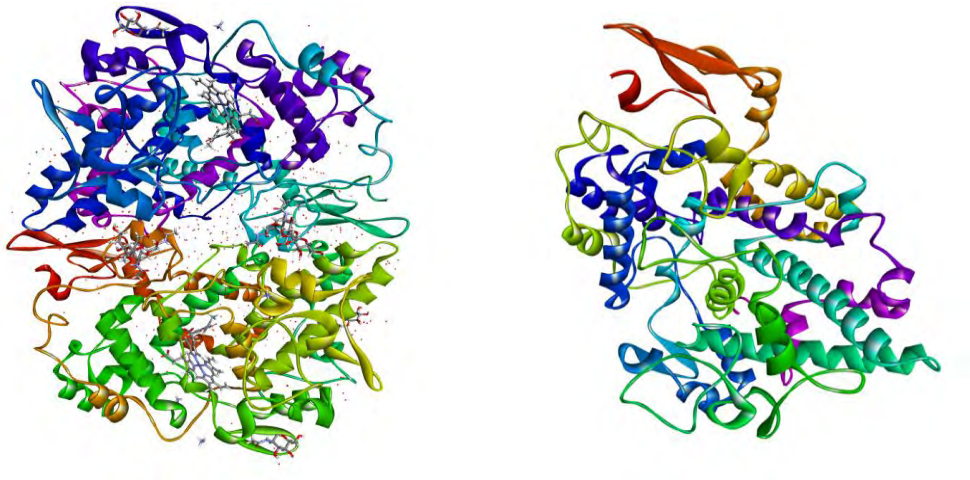
The pharmacological significance of a ligand is determined by its drug ability or drug-likeness, which is evaluated using specific physiochemical and structural characteristics. Therefore, all ligands screened were evaluated for their drug-like properties according to various criteria (Joshi et al., 2020). When studying and evaluating the bioactive compounds, students used the Lipinski's assays to forecast their potential as drugs candidate through http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp.
Terpenoid as plant bioactive compounds are tested with Lipinski to predict their ability as drug candidate. Lipinski 'rule of 5' states that drug likeness for molecules are predict high probability of success or failure if they complying with 2 or more of these following rules (Lipinski et al., 2012): Molecular weight less than 500 Da, less than 5 H-bond donors, less than 10 H-bond acceptors, molar refractivity between 40-130, and high lipophilicity less than 5 (calculated as LogP).
Students perform the Lipinski test on the four terpenoids mentioned above, using the CANONICAL SMILES values received from PubChem. Table 7 shows the Lipinski test findings for the Cycloartenol, Fissinolide, Hederagenin, and Swietenin B.

Table 7. Result of Lipinski assays for the potential terpenoid

Terpenoid	Molecular weight (≤ 500 g/mol)	Hydrogen bond donor count (≤ 5)	Hydrogen bond acceptor count (≤ 10)	Molar refractivity (40-130)	XLogP3 (≤ 5)
Cycloartenol	426	1	1	130,72	8,17
Fissinolide	512	0	8	130,69	4,73
Hederagenin	472	3	4	134,09	6,21
Swietenin B	542	1	9	136,63	3,94

Selection and preparation the target protein

Target protein was retrieved from the Protein Data Bank website (www.rcsb.org) or literature study from the preliminary result research. Characteristics of protein target were adjusted from their capability as the inhibitor or activator enzymes for the disease pathway (Joshi et al., 2020; Taidi et al., 2022). Students at this level identify the specific target protein that contributes to anti-inflammatory symptoms by acting as an enzyme. The target protein used in this procedure is Cyclooxygenase-2/COX-2 (PDB: 5IKV). The three-dimensional structure of COX-2 was downloaded as a (*.pdb) format, and the structure was purified of water and other ligand molecules using the BIOVIA Discovery Studio software (Figure 2).



(A) (B)

Figure 2. Three-dimensional structures of natural COX-2 (A), and pure COX-A (B)

Cyclooxygenase 2 (COX-2) is a well-established target for the development of anti-inflammatory intermediates. COX is an endogenous enzyme primarily responsible for the conversion of arachidonic acid to prostaglandins. This enzyme exists as COX-1 and COX-2, two common isoforms. COX-1 is a constitutive enzyme, whereas COX-2 is an inducible enzyme that is expressed in response to an inflammatory stimulus and is responsible for inflammation. Inhibiting COX-2 enzyme is a proven method for treating inflammation. Anti-inflammatory, analgesic, and antipyretic effects have been linked to the inhibition of prostaglandin synthesis as a result of uncoupling of oxidative phosphorylation (Cruz et al., 2022; Joshi et al., 2020).

Inflammation is a natural protective and defensive response to tissue damage induced by physical trauma, toxic chemicals, and microorganisms. Under these conditions, the arachidonic acid pathway, one of the most essential and fundamental inflammatory mechanisms, is activated. Prostaglandins, produced by the cyclooxygenase (COX) enzyme family via the COX pathway, are regarded as the most prominent inflammatory mediators in this pathway (Sadeghi et al., 2022).

Molecular docking visualization

Terpenoids as the ligand molecules were visualized their three-dimensional conformers (SDF) from PubChem and followed by BIOVIA Discovery Studio. The ligand's energy was minimized with PyRx software and converted to pdbqt format and then input to the molecular docking software. BIOVIA Discovery Studio to visualize 2D structure of complex protein-ligand, as well as PyMol Molecular Graphic system software to prepare complex protein-ligand following the docking and 3D visualizing process (Mahmud et al., 2021; Umar et al., 2021).

Students practice using the PyRx and PyMol software to visualize terpenoid ligands associating with target proteins. If there is a bond between ligand and target protein, the molecular docking is successful. Figure 3 shows the findings of the terpenoid compounds that were successfully attached to the COX-2 target protein as molecular docking process.

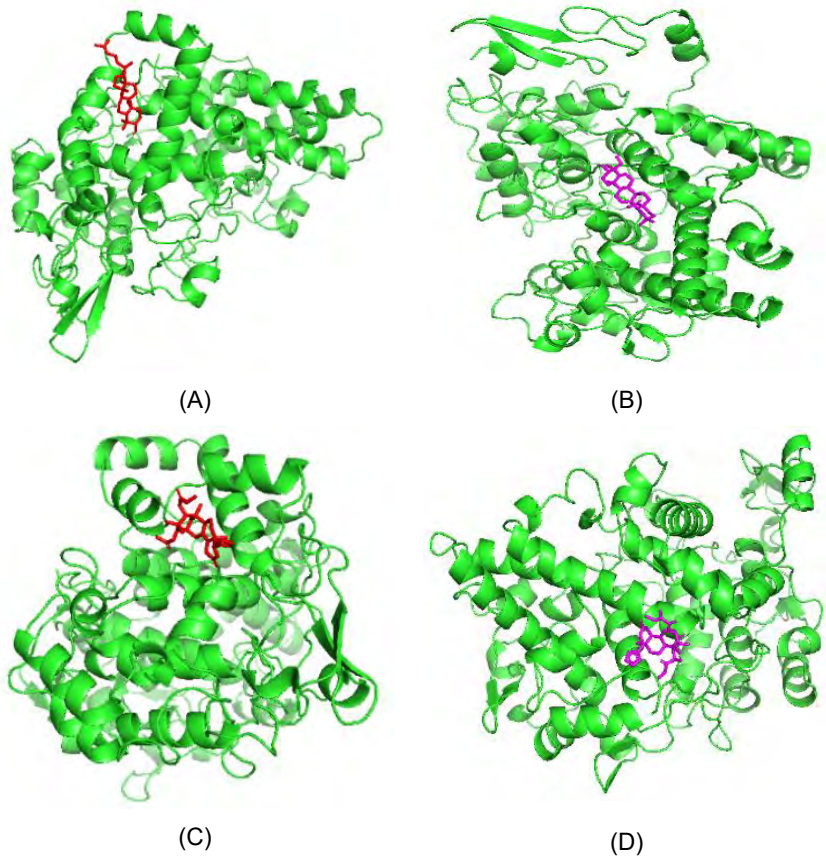


Figure 3. Visualization of docking molecular between terpenoid ligands and target protein COX-2 (green): A). Cycloartenol (red) binds to COX-2; B). Hederagenin (purple) binds to COX-2; C). Fissinolide (red) binds to COX-2; and D). Swietenin B (purple) binds to COX-2.

As the bioinformatics approach are component of the study of molecular interactions, virtual screening and molecular docking techniques are frequently used in complex ligand and receptor analysis in medical therapy (Moradi et al., 2022). Molecular docking was used to predict interactions between ligand (compound/drugs) and receptors (proteins/targets). The findings of docking include docking pose which describes how the ligand interacts with the target protein (residual results), and docking score which describes the binding affinity of ligand-protein (Gogoi et al., 2021). This ligand binding-affinity can be predicted by utilizing electrostatic interactions, hydrogen bonds, hydrophobic interactions, and Van der Waals bonds. The smaller the ligand binding affinity value, the better it bonds with the protein target (Yi et al., 2018).

Conclusion

Terpenoid compounds, as plant metabolites, are studied by undergraduates biology in the Plant Metabolism subject. Students can enhance their understanding and competencies through the test and practice skills with bioinformatics approach. Assessment of students' ability can be represented by the implementation of learning modules based on research, learning outcomes from the results of pretest and posttest, analysis and review of research article on terpenoid and bioinformatics, and practicing molecular docking procedures on terpenoids as bioactive compounds with drug potential. Assessment of learning outcomes includes students' understanding of terpenoid content-knowledge, students' skills to identify phytochemical ligands and target proteins, determine the database online and bioinformatics softwares, represent the terpenoid assays as medicinal compounds, and visualize the three-dimensional docking of terpenoid structures.

The bioinformatics approach to learning terpenoids in this study is limited to increasing students' knowledge and skills in analyzing terpenoid as plant bioactive compounds that have therapeutic agents. Bioinformatics engineering has many functions and roles in various scientific disciplines, but in this research, the prediction and development of plant terpenoid as potential drug compounds are emphasized. Students empowered their content-knowledge of terpenoids by utilizing learning module based on research results and bioinformatics approach, working on queries written on the module, and conducting the activities to trace terpenoid procedures using a molecular docking bioinformatics at the beginner level.

The implementation of bioinformatics approach in this research is also limited to the visualisation of three-dimensional structure of molecular docking between terpenoids (ligands) and target proteins. This research is an early stage for biology students to learning about bioinformatics techniques in plant bioactive compounds. If students have mastered this technique, then the future progress can be directed towards developing and determining the medicinal contituents derived from these compounds.

The future research can be carried out to enhance the students' knowledge and understanding through developing learning resources related to the integration of bioinformatics into biology materials, adding student classes about bioinformatics, strengthening students' skills in accessing databases and bioinformatics software, as well as student competency in analyzing the potential of plant bioactive compounds down to the molecular level (nano). Students' skills in learning plant metabolism that are well integrated with bioinformatics technology in the future can play an important role in research on the discovery and development of new drugs based on plant bioactive compounds.

Conflicts of Interest

No potential conflict of interest was reported by the author (s).

Author Contributions

R. Dhaniaputri: methodology, analysis, writing original manuscript, and editing. **H. Suwono**: analysis, review, and editing. **B. Lukiati**: analysis, and review

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