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Phylogenetic Analysis of Bengkulu Citrus Based on DNA Sequencing Enhanced Chemistry Students' System Thinking Skills: Literature Review with Bibliometrics and Experiments

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A B S T R A C T

The study aims to enhance students' system thinking skills through phylogenetic analysis based on DNA sequencing results. The study is carried out through three steps: (i) pretest: evaluate students' knowledge, (ii) treatment: analysis of affinity relationships through phylogenetics using DNA sequence data, and (iii) postest: measurement of improvement in systems thinking skills after implementation. Evaluations are conducted using written tests as well as worksheets and supported by surveys, questionnaires, and interviews for qualitative analysis. The experiment began with the isolation of citrus DNA, determination of concentration and purity, amplification, reading of sequencing results, and phylogenetics. The results of the teaching analysis show that there is a significant improvement in students' mastery of conceptual and system thinking skills, and there is a high correlation between the pretest and posttest. The problem analysis of interviews and questionnaires is also done to see the enthusiasm for activities as well as the effectiveness of the worksheets used. The study comes with new information on determining the relationship between local citrus in Bengkulu and improving the students' system thinking skills.

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1. INTRODUCTION

Fruit plants in Bengkulu Province are scattered throughout the districts/cities with potential and market opportunities that are bright to develop. According to the central statistical authority of agriculture horticulture, the total production of Bengkulu citrus in 2019 was 140.704 kW/quiz, and in 2020, it was 115.137 kW/quiz. This citrus production includes the second-highest fruit commodity. Bengkulu local citrus is being cultivated with gerga and calamari. The availability of fruit is produced throughout the year because, in a tree, there are 4-6 generations, which means in one tree, there is a flower and young fruit until the fruit is ready to harvest. With the enormous potential that these two citrus fruits possess, they have an impact on improving people's well-being. In developing strategies for conservation, breeding, and sustainable crop management, information on the genetic diversity level is needed (Zulfahmi, 2013). With the information of diversity, relationships between plants can be detected.

Family connections can be determined based on the extent of shared characteristics. Plants with more identical features are more closely related, whereas plants with more distinctions are more distantly related (Agustina & Hasanuddin, 2021). Phylogenetics is a technique employed to examine the genetic links among organisms. In the field of phylogenetics, animals that share comparable features or characteristics are regarded as having a close relationship (Bayer *et al*., 2009; Sun *et al*., 2015). Family relationships can be determined by analyzing DNA molecular markers, starting from the process of isolating DNA to examining the sequencing results (Luro *et al*., 2022).

DNA isolation is a crucial step in the process of acquiring genetic information and conducting genetic analysis. The process of DNA isolation involves three main stages: cell wall and membrane lysis, DNA separation or purification, and DNA precipitation. The biochemistry practicum incorporates DNA isolation. The Biochemistry Practicum is an obligatory seminar that all chemistry students must attend. The purpose of the biochemistry practicum is to equip students with the necessary knowledge and abilities to plan, execute, and evaluate laboratory experiments. Through engaging in practical activities, students acquire proficiency in understanding topics and develop expertise in conducting diverse assessments using predetermined study materials.

The objectives of the biochemistry practicum course are for students to acquire proficiency in fundamental concepts and to develop expertise in the extraction, characterization, and detection of DNA. The learning outcomes are interconnected among knowledge, attitudes, and skills, as defined by the desired graduate profile. The capabilities of graduates from the chemistry study program are derived from the outcomes of the Indonesian Chemistry Association workshop (2017). These competencies encompass four areas: 1) Attitude, 2) Knowledge, 3) General skills, and 4) Special skills. To get the desired graduate competencies that align with the graduate profile, it is necessary to possess holistic thinking skills, specifically systems thinking skills.

Systems thinking skills are one of the most essential abilities in the 21st century. Systems thinking helps students organize their thoughts in a meaningful way by making connections between seemingly unrelated problems to become interrelated (Miller *et al*., 2019; Rosenkränzer *et al*., 2017). This skill enables students to understand the multilevel structure of several concepts and the relationships between these concepts (Gilbert *et al*., 2019). Systems thinking skills in chemistry education are an approach beyond the reductionist approach that provides knowledge that includes chemical reactions and processes for a more holistic understanding. What is meant by holistic is how chemistry is connected to dynamic and complex social, technological, economic, and environmental systems (Jegstad *et al.*, 2018; Mahaffy *et al*., 2019a; Mahaffy *et al*., 2019b).

This study aims to use phylogenetic analysis based on DNA sequencing data to enhance the conceptual knowledge and systems thinking abilities of chemistry students. At the undergraduate level, a biochemical experiment focusing on DNA isolation has been conducted, reaching the step of DNA isolation but still needs to progress to the DNA sequencing stage. The sequencing stage offers students the opportunity to observe the correlation between locally grown citrus, particularly Bengkulu citrus, and other commonly consumed citrus fruits. The students' systems thinking abilities will advance with this practical level. The following aspects are novelty in this research. We added bibliometrics in this writing, and several articles are presented in **Table 1.**

Table 1. Previous studies on bibliometric

Title	Result	Ref
The use of simple spectrophotometer in STEM education: A bibliometric analysis	The study, which used the VOSviewer tool, discovered that modified spectrophotometers are commonly used in chemistry and STEM education, presenting opportunities for future research and resolving concerns.	(Shidiq *et al.*, 2021)
A bibliometric analysis of management bioenergy research using vosviewer application	The study used VOSViewer to analyze 180 publications published between 2017 and 2021 that focused on the management of bioenergy. The objective was to identify research themes and propose potential integration with other fields of study.	(Soegoto *et al.*, 2022)
Bibliometric analysis for understanding the correlation between chemistry and special needs education using VOSviewer indexed by Google.	An examination of articles about chemistry and special education, utilizing VOSviewer and Publish or Perish, revealed a decrease in publications in 2017, followed by an increase in 2021.	(Bilad, 2022)
Implementation of Biotechnology in Education towards Green Chemistry Teaching: A Bibliometrics Study and Research Trends	The bibliometric analysis of research trends on biotechnology in education identified four prospective study concepts, highlighting the need to incorporate green chemistry into school curricula. Journals were found to be the primary source of information for this study.	(Riandi *et al.*, 2022)
A bibliometric analysis of materials research in Indonesian journal using VOSviewer	An analysis of scholarly papers on Indonesian materials was carried out using VOSviewer. The findings revealed that the topic of "acid" garnered the highest level of interest between 2016 and 2021, with a total of 43 articles and eight international collaborations.	(Nandiyanto *et al*, 2021)
Sustainable development goals (SDGs) in science education: Definition, literature review, and bibliometric analysis.	Bibliometric analysis is an essential tool in science education that provides a comprehensive understanding of the subject. It highlights the significant role it plays in promoting research on the Sustainable Development Goals (SDGs).	(Maryanti *et al.,* 2022)
Bibliometric analysis of briquette research trends during the Covid-19 pandemic.	Bibliometric analysis, data mapping, and VOSviewer were used to examine 973 briquette-related papers. Research activity has decreased in the previous three years as a result of the COVID-19 outbreak, according to the data.	(Al Husaeni, 2022)

Table 1 (Continue). Previous studies on bibliometric

Title	Result	Ref
How to calculate bibliometric using VOSviewer with Publish or Perish (using Scopus data): science education keywords	VOSviewer is a powerful tool for analyzing bibliometric data, providing a straightforward and systematic approach to understanding research progress in scientific education. It encompasses a collection of 200 papers spanning from 2013 to 2023.	(Al Husaeni, 2022)
Bibliometric computational mapping analysis of publications on mechanical engineering education using VOSviewer	An analysis conducted using VOSviewer revealed a significant increase in research focused on nanoparticles, propolis, and propolis in the field of nano propolis during the past decade.	(Al Husaeni & Nandiyanto, 2022)
Global trend of ethnoscience research: a bibliometric analysis using Scopus database	An examination of the Scopus database revealed a substantial growth in ethnoscience research over the last five decades, indicating promising avenues for further investigation. This bibliometric analysis has highlighted prospective avenues for future research in the field of ethnoscience.	(Supriyadi *et al.*, 2023)
Particulate matter emission from combustion and non-combustion automotive engine process: review and computational bibliometric analysis on its source, sizes, and health and lung impact	This study examines the upward trajectory of scientific publications on the subject of particulate matter, categorized by factors such as citation count, publisher, author, nation, and affiliation.	(Nandiyanto *et al.*, 2023)

2. LITERATURE REVIEW
2.1. Phylogenetic Analysis of Citrus

Citrus (*Citrus sp*), belonging to the *Rutaceae* family, is a genus with significant commercial importance. Citrus is a nutrient-dense fruit, particularly abundant in vitamin C, which is crucial for maintaining good health. Additionally, oranges include antioxidants that safeguard the body's cells against harm caused by free radicals. The quality of citrus fruit can be evaluated by analyzing the purity and concentration levels of different chemicals found in oranges. 100 g of oranges include 0.9 g of protein, 0.2 g of fat, 11.3 g of carbohydrates, 23 mg of phosphorus, 33 mg of calcium, 0.4 mg of iron, 190 International Units of vitamin A, 0.08 mg of vitamin B1, 49 mg of vitamin C, and 87.2 g of water. Citrus fruits, particularly those from the Citrus sp species, contain important components such as vitamin C, folic acid, carotenoids (especially β-carotene), flavonoids, limonoids, and dietary fiber. Citrus fruits contain sixteen carotenoids that operate as important sources of vitamin A (Bayer *et al.*, 2009; Luro *et al.*, 2022; Ramadhani *et al.*, 2022).

The genetic variety of oranges is extensive, as seen by the abundance of taxonomic classifications, including various species and hybrids (Liu *et al.*, 2012). Phylogenetic analysis can be employed to classify citrus. Phylogenetic Analysis is described as a taxonomic classification of an organism based on its evolutionary history (Luro *et al.*, 2022). Phylogenetic Analysis based on molecular data (nucleotide or amino acid) is an important area in sequence analysis. Sequence analysis determines how the family descended during the evolutionary process. The evolutionary relationship between sequences is described by placing the sequence as the outer branch of a tree. The branch relationship on the inside of the tree

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reflects the level at which different sequences interact. Two similar sequences will be located as neighboring outside the branches and interacting in the general branch. The steps of phylogenetic analysis are described in **Figure 1**.

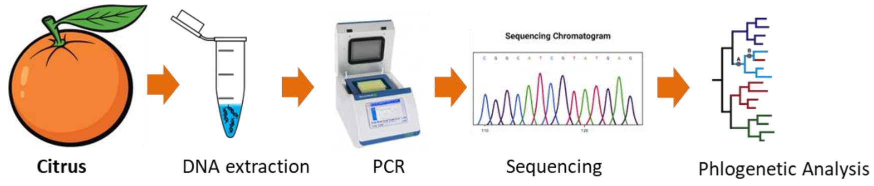

Figure 1. Steps of phylogenetic analysis.

The phylogenetic tree shows a graph of species related specifically between genetic sequences. Sequences that have proximity can be identified by placing adjacent branches on the tree. Molecular markers are used to identify sequence data. DNA barcoding is a molecular marker used in taxonomy to identify genetic changes in a species using a short sequence of DNA (Sun *et al*., 2015). DNA isolation is a crucial step in obtaining genetic information and doing genetic analysis. High-quality DNA is used for tasks such as molecular profiling, constructing genome libraries, and sequencing processes. Common challenges in plant DNA separation include the presence of polysaccharides, polyphenols, proteins, RNA, and secondary metabolites that might impede the DNA extraction process.

Extracting DNA from the nucleus, mitochondria, or other organelles by extraction or smoothing is usually done by adding an extraction buffer or smooth buffer to prevent DNA damage. In certain sample conditions, to help smooth the cell membrane/nucleus/organelle or cell walls, the sample is inserted into liquid nitrogen, which is immediately mixed before the extract buffer is added. The compounds used to maximize the result of pure DNA isolation were added: phenols and chlorophores (Gokarn *et al*., 2016). The subsequent step involves isolating DNA from other cellular components or unwanted impurities. Centrifugation is used to separate DNA from other components, such as cell debris. After extraction, the process is continued by precipitation DNA using absolute ethanol or isopropanol. Apart from DNA, all other materials will dissolve in cold ethanol. Thus, the DNA will sink and separate from the compounds or other materials when centrifugated.

The Doyle-Doyle approach is enhanced by the inclusion of CTAB (Cetyl Trimethyl Ammonium Bromide) is a method commonly used in plant genomic DNA isolation that contains a lot of polysaccharides and polyphenol compounds (Triani, 2020). They established a technique to enhance the application of CTAB, which other researchers had previously employed. This method has the benefit of requiring a reduced number of samples.

CTAB is a positively charged surfactant that disrupts cell membranes, denatures proteins, and isolates carbohydrates from nucleic acids. When tissue damage happens, polyphenol chemicals from the vacuole undergo oxidation and form covalent bonds with proteins and nucleic acids, causing the extracted DNA solution to change color to brown. The CTAB method offers the benefits of being simple, cost-effective, and rapid, resulting in DNA separation with high purity and concentration when compared to alternative plant isolation techniques (Chanu *et al*., 2021). Accurately identifying species by conventional methods can be time-consuming due to a lack of plant knowledge and the necessary traits of flowers and fruits for identification.

2.2. System Thinking Skills

Systems thinking is a comprehensive framework and set of tools that have been developed over the last seventy years to enhance our understanding of complex patterns and facilitate effective change. Utilizing systems thinking principles in chemistry education can facilitate a transition from a reductionist to a holistic viewpoint. Systems thinking involves "(i) visualizing the interconnections and relationships between the parts in the system; (ii) examining behaviors that change over time; and (iii) examining how systems-level phenomena emerge from interactions between the system's parts". Systems thinking approaches have the potential to enhance students' understanding of chemistry by emphasizing the relationships between chemical phenomena, increasing awareness of chemistry's effects on global and societal matters, and equipping students to tackle intricate global issues in the 21st century (Flynn *et al*., 2019).

Systems thinking skills in chemistry education are important for comprehending the scientific method and attaining a comprehensive understanding of chemistry-related issues. Proficiency in systems thinking necessitates that students fully understand the scientific process, encompassing methods, data, and conclusions, to effectively tackle a problem (Jegstad *et al*., 2018; Sensibaugh *et al*., 2017). Intensive research is underway to enhance systems thinking skills in chemistry education (Orgill *et al*., 2019). This research includes various approaches such as introducing systems thinking to the community, utilizing application systems to promote systems thinking (Kim *et al*., 2019), implementing project and problem-based learning models (Nagarajan & Overton, 2019), and integrating systems thinking into the green chemistry curriculum (Aubrecht *et al*., 2019; Hutchison, 2019; Mammino, 2019). Through the advancement of this research, pupils are anticipated to get a piece of more comprehensive and profound knowledge and comprehension. Students engage in the learning process by attending lectures and participating in practicums. Engaging in chemistry practicum enhances students' comprehension of fundamental ideas by providing direct interaction with real-world materials, facilitating the connection between microscopic and macroscopic domains (Irby *et al*., 2020; Loveys & Riggs, 2019; Vachliotis *et al*., 2021). **Figure 2** provides an overview of many attributes of system complexity that hold significance in chemical system analysis.

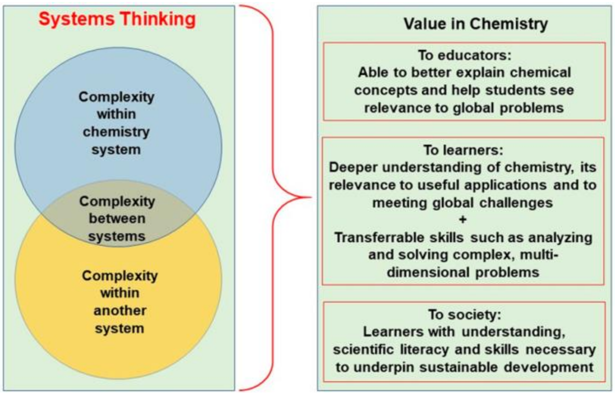

Figure 2. Overview of system thinking and value in chemistry (Constable *et al*., 2019).

3. METHODS

3.1. Bibliometric Analysis Method

Bibliometric analysis is a recognized mathematical and statistical method used by the scientific community to quantitatively analyze the performance and productivity of publications. It can help identify emerging thematic areas, important gaps, and opportunities for future research. The data utilized in the paper was derived from research published in Scopus articles. Scopus is a digital database that is used for bibliographic purposes. It offers scholars access to a wide variety of high-quality publications and is regarded as the most

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popular and comprehensive data source (Ding & Yang, 2022). Detailed information regarding how to use bibliometrics is explained elsewhere (Al Husaeni & Nandiyanto, 2022).

The Scopus article data search is used to filter publications containing the keyword "system thinking in science education" based on the publication's title requirements. The following keywords were used in the Scopus search: (system AND thinking AND in AND science AND education) TITLE-ABSTRACT-KEY. The referenced publications were published between 2014 and 2023. All information was collected in February 2023. The data acquired by sorting back corresponds to similar topics in chemistry education. The research information system (.ris) and comma-separated value format (*.csv) file formats are then used to export the articles that have been gathered following the criteria of this research analysis. VOSviewer can also be used for visualizing and assessing trends through bibliometric maps. Next, data from the source database is shown. VOSviewer is used to create three forms of publishing mapping: network visualization, density visualization, and overlay visualizations based on co-citation networks between existing articles. When creating bibliometric maps, the keyword frequency threshold is set to a minimum of three occurrences. Therefore, 105 irrelevant terms and keywords have been eliminated. **Figure 3** summarizes the process of selecting studies.

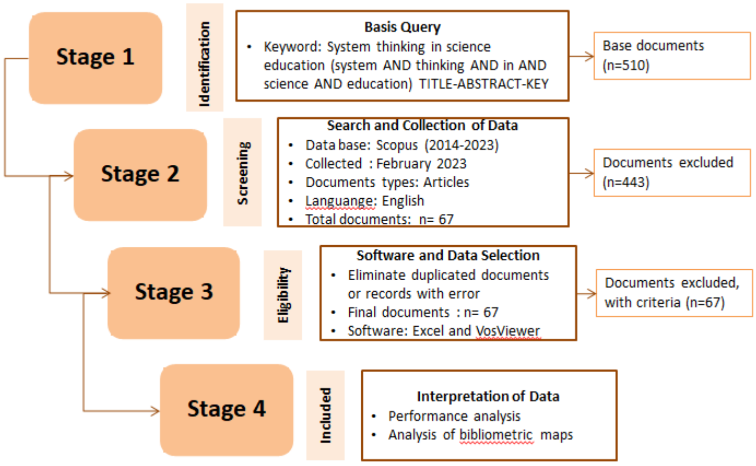

Figure 3. Overview of the study selection process.

3.2. Research Subjects

The participants in this study consisted of 62 chemistry students who enrolled in a biochemistry practicum course at a state university in Bandung, Indonesia.

3.3. Teaching Methods

The research was carried out in three steps, and each step was thoroughly assessed with direct observation.

(i) **Pretest**. Students are assessed through a pretest to determine their mastery of concepts and systems thinking skills before implementing treatment.
(ii) **Treatment**. Students are given a module and then carry out experiments to determine kinship relationships by making a phylogenetic tree of local Bengkulu citrus. This experiment began by isolating citrus DNA, determining DNA concentration and purity, DNA amplification, reading DNA sequencing results, and making phylogenetics. During the practicum, students complete a worksheet.
(iii) **Posttest**. At the end of the experiment, students were given a posttest which aimed to measure the increase in mastery of concepts and systems thinking skills after implementing phylogenetic analysis of local Bengkulu citrus based on DNA sequences.

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Phylogenetic analysis was carried out using DNA sequencing data. To obtain this, students first isolate DNA. The samples used were leaf originating from young leaves of local Bengkulu citrus plants, namely Gerga and Calamansi. Gerga leaves are obtained from plantations in Lebong Regency, Bengkulu Province. Calamansi leaves come from plantations in Muko-muko Regency, Bengkulu Province.

3.3.1. DNA isolation, amplification, and PCR sequencing

DNA extraction is done using the CTAB (Cetyl Trimethyl Ammonium Bromide) method modification by Doyle and Doyles. The primary used in amplification is ITS2 (Internal Transcribed Spacer 2), which is 5’ GCTGCGTTCTTCATCGATGC 3’ in 20 µL PCR reactions. The reaction components for effective PCR amplification are 10 µL gotaq green 2x master mix, 1 µL primary forward (10 µM), 1 µL primary reverse (10 µM), 2 µL DNA templates, and 6 µL nuclease free water. PCR amplification is performed using a set of programs: 1x initial denaturation cycle at 95^{0}C for 3 minutes; 35x denaturing cycle at 95^{0}C for 30 seconds, annealing at 56^{0}C for 30 seconds, extension at 72^{0}C for 15 seconds; and final extension at 72^{0}C during 3 minutes. The PCR products that have been obtained are then sorted in LabTex XI ITB, Bandung Institute of Technology.

3.3.2. Sequence editing and alignment

To edit and assemble consensus strings using the Geneious By Dotmatics application program. Analogue sequence and nucleotide sequence comparison was detected using the GenBank Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/).

3.3.3. Phylogenetic analysis

Assessment of intraspecific genetic divergences by using pairwise distance calculations. DNA sequence data to determine affinity with phylogenetic analysis using the MEGA XI application (https://www.megasoftware.net/).

3.4. Research Instrument

The research instrument used was a written test, the test consisted of 15 multiple choice questions and 5 essay questions that had been validated by experts and were developed according to appropriate indicators, apart from that a worksheet was also used to determine students' mastery of concepts and systems thinking skills. Surveys, questionnaires, and interviews were also conducted for qualitative analysis.

4. RESULTS AND DISCUSSION

4.1. Bibliometric Analysis Results

4.1.1. Publication data search results

Bibliometric analysis is done to look at current research trends and obtain results. The reference manager tool that is included in the Scopus database was used to search data. The search resulted in the discovery and collection of 510 data articles that are indexed in Scopus and deal with the subject of system thinking in science education. System thinking in chemistry education was searched for utilizing the keywords that were applied, and as a result, 67 publications that satisfied the requirements for the research were acquired. The metadata of the article was retrieved, and this information comprises the name of the author, the title, the year, the name of the journal, the publisher, the number of quotes, the article link, and the URLs related to the piece.

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4.1.2. Research development in the field of system thinking in chemistry education

The progression of research in the field of system thinking in chemistry education, as shown in **Figure 4**, which was published in the indexed journal Scopus, is presented here. The information that is presented in **Figure 4** reveals that there have been 67 publications published on the topic of system thinking in the field of Chemistry Education between the years 2014 and 2023. 2019 has the most articles, with 31 total articles. There were three articles published in 2014. 2015 saw the publication of a single piece. There was one article published in 2016. There were no papers published in 2017, but there were three pieces published in 2018, thirty-one articles published in 2019, nine articles published in 2020, seven articles published in 2021, eleven articles published in 2022, and one article published in 2023. According to the number of publications, research on system thinking skills is still researched only sometimes each year, particularly in the most recent decade (2014-2023).

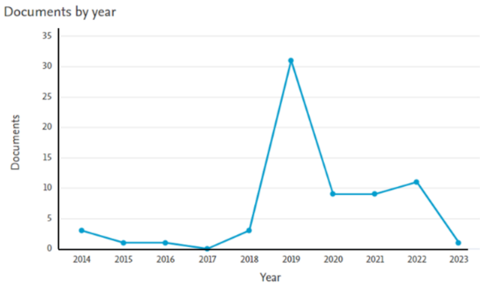

Figure 4. Development of system thinking in chemistry education research.

Researchers from various countries compared the number of documents retrieved from Scopus for this study. These nations are the United States, the United Kingdom, Canada, Germany, Australia, Belgium, Israel, Brazil, Sweden, India, and Indonesia. Based on **Figure 5,** which illustrates the geographic distribution of system thinking literature in chemistry education research, the United States exports the most goods. At the same time, Indonesia is still ranked below India.

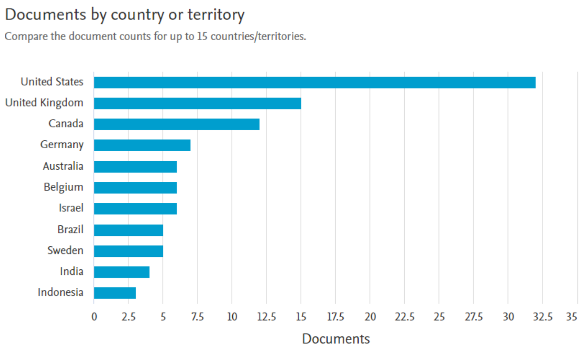

Figure 5. Global Distribution of literature on system thinking in chemistry education, 2014-2023 (n=67).

Figure 6 demonstrates that system thinking in chemistry education divides the subject matter into multiple categories. Social sciences, chemistry, environmental science, computer science, energy, health professions, chemical engineering, Pharmacology, Toxicology, Pharmaceutics, Biochemistry, Genetics and Molecular Biology, Medicine, Psychology, Agricultural and Biological Sciences, and Engineering. The largest proportion of subjects in the social sciences group was 31.1%, followed by chemistry with 29.8% and environmental science with 9.1%.

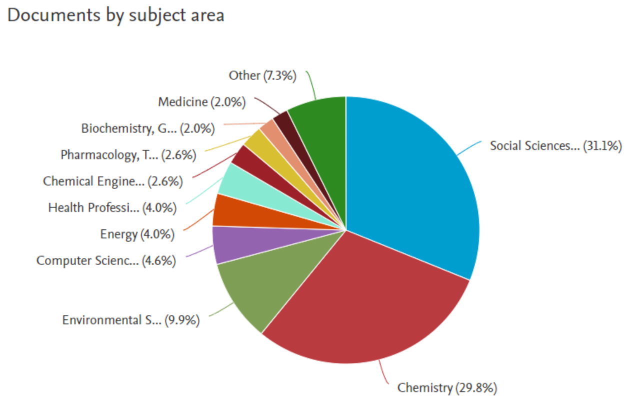

Figure 6. Documents by subject area system thinking in chemistry education research.

4.2. Experimental Apparatus

A phylogenetic analysis was carried out to determine the relationship between local Bengkulu citrus, namely gerga and calamansi, based on DNA sequencing data. In this research, the first step was to isolate the DNA of the two citruses using the Doyle and Doyle method with Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer solution. DNA samples from the two citrus leaves that had been isolated produced white pellets. The DNA pellets were stored using a TE (Tris-EDTA) buffer at -4°C, which aims to ensure that the samples obtained are not damaged. The samples were then tested for purity and concentration quantitatively using a nanodrop spectrophotometer. The results of the NanoDrop test can be seen in **Table 2**. DNA with a purity (A260 /A280) of 1.8 to 2.0 and a concentration of 100 µg/µL is considered to be of good quality according to the nanodrop test (Chanu *et al*., 2021). According to **Table 2**, the DNA extracted from leaf samples of both citruss indicates that the concentration and purity of gerga are superior to those of calamansi. The elevated DNA concentration of the two citruses acquired can be utilized for subsequent investigation.

Table 2. DNA spectrophotometer results.

Sample	Concentration (µg/µL)	Purity (A_{260}/A_{280})
Calamansi	2561.0	1.22
Gerga	2674.4	1.33

The use of ethanol in the washing process can have an impact on the concentration and purity of DNA. Increasing the frequency of ethanol washing of the sample will result in higher DNA purity, but it will also lead to a decrease in DNA concentration. The solubility of complex molecules, including carbohydrates, proteins, and nucleic acids, decreases as the concentration of ethanol increases (Gokarn *et al*., 2016). Nucleic acids exhibit the highest solubility among polymers when dissolved in ethanol. During the DNA isolation process, DNA precipitation takes place when the ethanol concentration reaches 60-70%. Consequently, to cleanse the DNA during isolation, it is rinsed with a solution containing 70% ethanol. Elevated levels of sodium ions result in turbid liquids, along with alcohol—a solution with a lower concentration results in a reduced amount of DNA precipitation. Centrifugation is performed to enhance the thoroughness of DNA deposition. DNA sequence data obtained through sequencing was edited using the Geneious Prime 2023.0 application following the Tallei and Kolondam procedure (Tallei *et al*., 2016). The pairwise alignment process was carried out for the sequencing results using the ITS2 reverse primer, then combined with the ITS2 forward

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primer sequencing results using Global Alignment and integrated into generous. Both ends of the sequence were cut during alignment to avoid misreading so that all sequences were cut, leaving 472 nucleotides for calamansi citrus and 390 nucleotides for gerga citrus. The consensus DNA sequence of gerga and calamansi can be seen in **Table 3.**

The DNA consensus sequence obtained based on **Table 3** can be used to search for similar sequences in the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the Kalamansi and Gerga sequences were aligned. Phylogenetic trees were built with the UPGMA method, genetic distances were computed using Kimura's 2-parameter approach, and the MEGA XI application was used to summarise the study selection process (Tallei *et al*., 2016; Tamura *et al*., 2013). According to (Tallei *et al*., 2016), organisms with a reduced genetic distance have a closer relationship. The results of the phylogenetic tree of gerga and kalamansi can be seen in **Figure 7**. Based on the phylogenetic tree, it was found that gerga is closely related to sweet oranges (*Citrus sinensis*) and grapefruit (*Citrus maxima).* Gerga results from crossing sweet oranges (*Citrus sinensis osbeck*) and tangerines (*Citrus reticulate blanco*) (Utari & Ningrum, 2023). Calamansi is closely related to tangerines (*Citrus reticulata*).

Table 3. The consensus DNA sequence.

Name of Citrus	The Consensus DNA sequence
Gerga	TGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCCAAGCCATT AGGCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCCCCACCCCACCCCCCCAAAC CAAGGCGGGGGCCCTGGGGTGCGGGCGGAGATTGGCCTCCCGTGCGCTGACCGCTCGC GGTTGGCCCAAATATGAGTCCTCGGCGACCGAAGCCGCGGYGATCGGTGGTGAAACAA AGCCTCTCGAGCTCCCGCCGCGCGCCCGGTCTCCAAGTGTGGACTCTGCGACCCTGAAGC TCCGCGCAAGCGGCGCTCGCATTGCGACCCCAGGTCAGGCGGGATTACCCGCTGAGTTT AAGCATATCAATAAGCGGAGGAAAAGAAACTTACCAGGATTCCCTTAGTAACGGCGAGC GAACCGGGAAGAGCCCAGCTTGAAAATCGGGCGCCCCCGGCGTCTGAATTGTAGT
Calamansi	AATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCCAAGCCATTAG GCCGAGGGCACGTCTGCCTGGGTGTCACGCATCGTTGCYCCACCCCACCCCCCCAAACCA AGGCGGGGGCCCCGGGGTGCGGGCGGAGATTGGCCTCCCGTGCGCTGACCGCTCGCGG YTGGCCCAAATCTGAGTCCTCGGCGACCGAAGCCGYGGCGATCGGTGGYGAAACAAAAG CCTCTCGAGCTCCCGCCGCGCGCCCGGTCTCCGAGTGGGGACTCTGCGRCCCTGAAGCTC CGCGCAAGCGGCGCTCGCATYGCGACCCCAGGTCAGGYGGGATTACCCGCTGAGTTTAA GCATATCAATAAGCGGAGGAAAAGAAACTTACCAGGATTCCCTTAGTAACGGCGAGCGA ACCGGGAAGAGCCCAGCTTGAAAATCGGGCGCCCCCGGCG

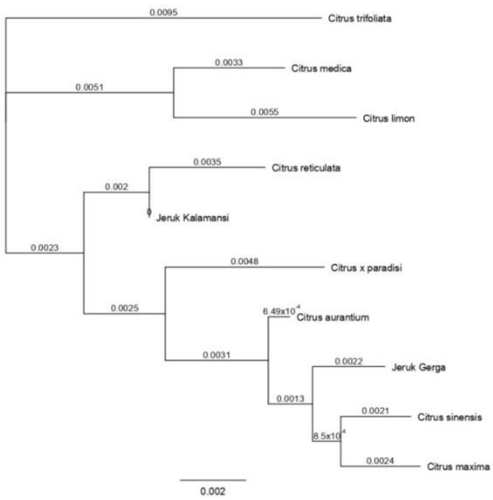

Figure 7. Phylogenetic of gerga and calamansi.

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4.2. Students' Demographics

Biochemistry is the examination of chemical reactions occurring in and connected to living organisms. The biochemistry practicum is one of the branches of chemistry that students must understand. Each student has a different interest in chemistry. The results in **Table 4** show a diversity of student interests. These results are used as initial data in determining students' mastery of concepts and thinking skills, especially in understanding DNA.

Table 4. Students' demographic.

No	Branches of Chemistry	Score (%)
1	Biochemistry	37.80
2	Organic Chemistry	29.70
3	Analytical Chemistry	13.50
4	Inorganic Chemistry	10.80
5	Physical Chemistry	8.10

4.3. Teaching Results

Examination of the demographic data in **Table 5** demonstrates a significant association between students' interest in biochemistry and their understanding of the biochemistry practicum. 37.80% of students exhibit a higher level of enthusiasm and choose biochemistry as their favorite topic. Modifications are implemented in the biochemistry laboratory exercise, namely through the execution of phylogenetic analysis, which is closely related to the extraction of DNA material in biochemistry, to uphold this.

Table 5 presents an overview of the pre-test and post-test values acquired by the students. Questions are provided to assess proficiency in ideas and foster systems thinking abilities along the learning journey. The mean score achieved by pupils on the pre-test is 38.58; however, in the post-test, it has risen to 73.26. There is a range of grades achieved by students. The outcome is derived from the examination of N-Gain values.

Table 5. Summary of student grades.

	n	Pre-test	Post-test
Min score	62	14	32
Max score	62	72	98
Avarage	62	38.58	73.26

Table 6 shows that as much as 29% of students have increased N-Gain scores in the highest category. This increase is up to N-gain scores of 0.96. That means students have a much better post-test score after treatment. A different test analysis is required to see further how the difference in N-gain values is affected based on the treatment performed. The results of the parametric or non-parametric tests are normality tests. Statistical tests are conducted using SPSS. Based on the test results, a significance value of 0.282 is obtained. A significant value greater than > 0.05 means that the class performed is normally distributed so that a parameter test can be performed for further tests. Normally distributed data has the same median, mean, and mode properties (Knief & Forstmeier, 2021; Mishra *et al*., 2019).

The parametric test selected in the hypothesis test uses the paired sample t-test. This test is a method to study the effectiveness of treatment, marked by the difference between the average before (pretest) and the average after (post-test) treatment (Orcan, 2020). The results of the paired sample t-test showed a mean value of -34.67742, with a standard deviation value of 12.2632, a standard error mean of 1.557, and a Sig. Value (2 tailed) of 0.000

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< 0.05. That means there is a significant average difference between the learning outcomes of the pretest and the post-test, where the practicum performed improves the mastery of the student's conceptual and thinking skills.

Table 6. Percentage of students' N-Gain.

Category	n	Min N-Gain score	Max N-Gain score	Percentage (%)
High	18	0.71	0.96	29
Medium	37	0.30	0.69	60
Low	7	0.19	0.29	11

The increased value students gain is supported by the treatment given, i.e., the student's efforts to complete the worksheet well. On the worksheet, students perform several stages to improve their thinking. The worksheet includes:

(i) Completing orientation questions corresponding to learning indicators;
(ii) Writing the results of observations performed in an attempt to study the phenetic relationship with DNA;
(iii) Formulating problems based on phenotypes that have been found;
(iv) Formulating hypotheses;
(v) Making experimental designs and observation data in which the procedure is written in the form of flow diagrams and complete with the tools and materials used at each stage;
(vi) Discussing and analyzing the data results of the findings using appropriate references;
(vii) Finding experimental conclusions based on the analysis that has been obtained.

4.3. Qualitative Analyses

We conducted a pretest before giving the students the phylogenetic analysis practice. The pretest results showed that not all students understand phylogenetic analysis based on DNA sequences. Several kids were unable to respond to the provided questions. Phylogenetic analysis determined a relationship between the garage and the calamari. To enhance the students' mastery of concepts and system thinking skills, the practice is conducted by isolating DNA, finding relationships to be more integrated, and training the students' system thinking skills.

Furthermore, we engage in qualitative analysis alongside quantitative analysis. Students were asked to complete the perception sheet using a lift, and in addition to supporting the results obtained, interviews were also conducted. Aspects used to get the desired qualitative data are (i) enthusiasm for practical activities, (ii) use of worksheets-based system-thinking skills, (iii) improvement of system-thinking skills, and (iv) team collaboration of these aspects detailed back to 17 questions with a description of the positive questions of 13 points while the negative questions of 4 points. The questions were given four answers from the criteria: strongly agree, agree, disagree, and strongly disagree. A summary of each aspect of perception can be seen in **Table 7**.

Table 7. Results of students' perception.

Perception's aspects	Strongly agree (%)	Agree (%)	Disagree (%)	Strongly disagree (%)
practical enthusiasm	49.03	48.06	7.26	0.00
use of worksheets-based system-thinking skills	27.42	64.52	7.74	1.61
improvement of system-thinking skills	38.71	59.68	2.42	27.42
team collaboration	39.11	57.26	4.84	1.61

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The student's enthusiasm for the practice is very high. 49,03% of students agreed that the internship was enjoyable and exciting. The training engages students because it uses microscales with rare tools such as micropipette, vortex, centrifuge, electrophoresis, and UV transluminator. Besides, the practice also trains students' thinking skills so that the results are more accurate and can be analyzed well, especially in discussing the relativity of the orange observed using DNA sequence data.

To enhance students' system thinking skills, utilize worksheets designed explicitly by the prescribed indicators. Approximately 64.52% of students agreed that the worksheets used effectively comprehended the sequential steps evaluated personal strengths and weaknesses during the practice, and arranged potential improvements to the practice. 59.68% of students concurred that the implemented practice effectively facilitated the development of system thinking skills, both throughout the learning process and in terms of the achieved outcomes. In addition, it enhanced the cooperation among fellow students.

The qualitative result obtained corresponds to the previously announced quantitative value. This proves that the treatment given can improve student's mastery of concepts by directly comparing macroscopic and microscopic data (Irby *et al*., 2020; Loveys & Riggs, 2019; Vachliotis *et al*., 2021); the understanding acquired by students is more holistic where students go through scientific processes ranging from determining methods, processing data, as well as drawing conclusions (Jegstad *et al*., 2018; Sensibaugh *et al*., 2017).

5. CONCLUSION

Strategies to improve students' systems thinking skills and mastery of concepts have been demonstrated. In contrast to the DNA isolation practicum carried out, this study has reached the stage of determining phylogenetic relationships using DNA sequence data. The teaching analysis results show a significant increase in students' mastery of concepts and systems thinking skills with a Sig. (2 tailed) is 0.000 < 0.05 with the highest increase in N-Gain value between 0.30 to 0.69, namely 60%. Problem analysis from interviews and questionnaires was also carried out to see the enthusiasm for the activities and the effectiveness of the worksheets. This study comes with new information about determining the relationship between local Bengkulu citrus and improving students' systems thinking skills.

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7. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the data and the paper are free of plagiarism.

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