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Study of Inhibition DNA Polymerase by Phenolic Compounds in Traditional Food Spices on the Polymerase Chain Reaction Process

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Abstract. Polymerase Chain Reaction (PCR) is a repeated cycle process including denaturation, annealing and extension using the DNA polymerase enzyme. This identification method is based on specific DNA which increases the number of DNA strands millions of times. The aim of this study was to identify and classify phenolic group compounds in traditional food spices that have the potential to inhibit DNA polymerase and their mechanism of action in the PCR process. The observation made was the Literature Review study as an analytical test method regarding the study of DNA polymerase inhibition by phenolic group compounds in traditional food spices in the PCR process. The results of this study were analyzed from several appropriate literature studies showing that most of the spices in traditional foods contain secondary metabolites of phenolic group compounds. These compounds have been shown to inhibit DNA polymerase in the PCR process, one of the mechanisms is to bind to the active site of DNA polymerase which causes decreased activity and deactivates DNA polymerase. Moreover, it can degrade and denaturate DNA polymerase. This makes it difficult to identify haram substances in processed food products using PCR.

Keywords: PCR, Inhibition of DNA polymerase, Phenolic compounds, Traditional spices.

1. Introduction

In line with the development of science and technology, there are issues regarding food products which were originally halal food, but there are doubtful substances contained in these products [1]. Public awareness of the importance of authentic food products which may be mixed with counterfeiting purposes to gain economic benefits is very much needed [2]. In particular, the Muslim community demands a halal guarantee for any product they consume [3]. The halal guarantee intended to protect the rights of Muslims from non-halal products has been regulated in Law No. 33 of 2014 concerning Guarantees for Halal Products (JPH) [4]. One such case is the addition of pork-derived substances, namely the fat found in food, to add to the taste of the food. This is because lard or lard is usually used like butter with a distinctive savory taste in enhancing the quality of the taste [5].

Following up on this case, various analytical methods have been developed to detect pig-derived substances such as DNA-based analytical methods. DNA-based analytical methods are believed to be very sensitive, valid, assessable, and accurate in detecting pig-derived substances, one of which is the PCR method [6]. The Polymerase Chain Reaction (PCR) method is an identification method based on molecular genetics with repeated cycle processes including denaturation, annealing and extension using DNA polymerase enzymes [7].

However, the PCR method involves enzymatic reactions in the process. So that PCR is sensitive to inhibitors that come from samples or through extraction or purification processes. PCR inhibitors can interfere with several steps in the PCR process causing reduced sensitivity or even leading to false negative results through several modes of inhibition [8]. One of them is the inhibition of DNA polymerase activity which causes a

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decrease in the activity of the enzyme [9]. Inhibition of DNA polymerase has been shown to occur from contamination by plant secondary metabolites including phenolic group compounds [10].

This has been proven based on research conducted Glökler et al. [11] by conducting analysis studies of PCR inhibitors, one of which targets DNA polymerases such as urea and phenol compounds which can degrade these enzymes. Another study was by removing PCR inhibitors including polyphenols in various types of Ocimum basilicum tissue using three dissociation techniques namely Bead milling, Cryo-grinding and Rotor-stator [12].

Based on these problems, it is necessary to carry out an analytical test on pork-derived haram substances mixed in traditional food. Traditional food is rich in plant-based spices which contain secondary metabolites of phenolic compounds, namely polyphenols, phenolic acids, flavonoids, tannins, and so on [13]. In addition, the food is widely sold and consumed by the Indonesian Muslim community. Therefore, this research was conducted by analyzing several research journals related to the study of inhibition of DNA polymerase by phenolic compounds in traditional foods in the PCR process.

2. Method

2.1 Data from the Company

The design of this research is Literature Review or Literature Review. A literature review study is a method used to collect data sources related to inhibition of DNA polymerase by phenolic group compounds in Indonesian food spices in the PCR process. Literature reviews are carried out from several sources, namely national and international journals and relevant e-books or handbooks regarding research results.

2.2 Data collection technique

A literature study data collection consisting of journal articles, e-books, and handbooks is a method for solving problems by tracing written sources that have been made before. Researchers used literature review data collection in thematic structural sequence, namely grouping and discussing sources according to the theme or topic. Journal search using Google Schoolar, PubMad, Mendeley, and Publish or Perish.

3. Result and Discussion

3.1 Principles of Pig Derivative Analysis Using the PCR Method

Polymerase Chain Reaction (PCR) is one of the most powerful and sensitive analytical methods, because PCR can increase the number of DNA strands millions of times from the original number. PCR is used to amplify nucleotides in vitro in an enzymatic synthesis process that uses the enzyme DNA polymerase [14]. This method is needed in identifying components in small and limited quantities. The PCR technique is a repeated cycle technique of 30-40 cycles consisting of 3 stages including denaturation, annealing and extension [15].

PCR-based DNA analysis is most widely used to test for the presence of DNA such as the presence of pork DNA in various products, especially in food because of the specific nature of the PCR [16]. The specificity of the amplified DNA fragment is determined by the specific (primary) DNA sequence of an organism. In the detection of pig derivatives, a pair of cytochrome-b primers has been widely used to amplify mitochondrial DNA encoding the pig cytochrome-b gene, the two primers are forward (5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3') and reverse (3 '-ATG CCA GTA GTG TTT AGA TAG TCG-5') [16].

DNA is the target of analysis because it contains genetic information in organisms. DNA analyzed only certain species-specific genes identified using PCR [17].

3.2 Conventional PCR

Analysis of processed food contamination has been carried out using DNA-based techniques such as the PCR method. This method is known for its effectiveness and high specificity in detecting even a minimum amount of specific DNA [18]. This is because this method can multiply specific DNA up to millions of times in the process. Based on literature studies from several previous studies, it has been proven that the PCR method using primers specific to the porcine leptin gene is recommended for analysis of the possibility of contamination with other meat (pork) in beef or chicken products [19]. Furthermore, analysis of pig DNA in three meatball food samples showed that two positive samples contained pork DNA from PCR and electrophoresis results, while other samples were detected by sequencing methods showing 100% similarity between pork primers and Sus scrofa species (wild boar) [20]. In addition, the identification of pork derivatives contained in bread was analyzed using PCR amplification of the mitochondrial cytochrome b gene region which showed positive results [21].

3.3 Real-time PCR

In contrast to conventional PCR, RT-PCR can analyze DNA products produced during reactions in real time. This method eliminates the electrophoretic process because it is visualized directly using fluorescent staining or probes. The quantity of DNA product is proportional to the fluorescence intensity of the probe that appears [21]. Based on research [22], the RT-PCR method is an analytical method with a high degree of accuracy in identifying pig DNA in processed products. The results of his observations found that all three meatball samples were positive for pig DNA shown from the amplification curve formed. Furthermore, observation [23] with the results of the validity analysis shows that the RT-PCR method has advantages in terms of accuracy, reproducibility and sensitivity. Observations were made to determine the level of DNA residues in pig host cells.

3.4 DNA polymerase enzymes

DNA polymerase is an enzyme used in the process of DNA replication and also as a polymerization catalyst for deoxyribonucleotide chains into new DNA strands. In the PCR process, the DNA polymerase used comes from Escherichia coli or known as the klenow fragment [24]. However, this enzyme can only reach 37°C as its maximum temperature, above that will make the enzyme denatured. Then a new enzyme was developed that was resistant to temperatures of 100°C with maximum activity around 92-95°C, namely Taq DNA polymerase extracted from the bacterium Thermus aquaticus (Taq) [25].

3.5 Enzyme Inhibition

Enzyme inhibition is the inhibition of enzyme work activity caused by the attachment of other molecules. The molecule is called an inhibitor whose mechanism of action is in accordance with the type of the inhibitor in inhibiting the enzyme. There are two types of enzyme inhibition, namely irreversible inhibition and reversible inhibition. Irreversible inhibition is characterized by inhibitors that form covalent bonds with the enzyme so that it is difficult to release (permanent), while reversible inhibition is characterized by inhibitors that dissociate rapidly so that the enzyme does not undergo permanent changes [26].

Based on the mechanism of enzyme inhibition, there are two types, namely irreversible and reversible inhibition. The mechanism of irreversible inhibition occurs when the inhibitor binds permanently to the enzyme [27]. In the literature study from the study Arqué et al. [28] explained that irreversible inhibition was demonstrated in

specific tyrosinase inactivators which are also called suicide inactivators, where the kinetics of inactivation of tyrosinase suicide with o-diphenolic substrates, ascorbic acid, L- and D-dopa with aminophenol and different o-diamines. The reversible inhibition is classified into competitive and non-competitive inhibition. In competitive inhibition, tyrosinase inhibition is produced by inhibitory compounds such as metalloenzymes, phenolic and polyphenolic compounds, and some non-aromatic compounds, namely by imitating their substrate [29].

3.6 DNA polymerase inhibition

The thermostable Taq DNA polymerase is a vital component of PCR whose variants have been chemically enhanced. Taq DNA polymerase is widely used because of its high temperature resistance and good processivity [30]. DNA polymerase enzymes can be inhibited. The principle of attack of inhibitors PCR occurs when a substance reacts with a nucleic acid during the extraction process so that another substance inhibits reverse transcription and degrades the DNA template. It was at that time that DNA polymerase experienced inhibition and changed [31]. Even though the inhibitor compounds contained in small amounts, they can also slow down the reaction and weaken the sensitivity of PCR [24]. Inhibitors that interfere with the DNA amplification process can be caused by one of the phenolic group compounds which are generally found in plants. Although phenol is also needed in the process of cell lysis or preparing pure nucleic acids, it can also cause PCR inhibition at certain concentrations. Phenol can stimulate DNA release through hydrogen bonding into single-stranded DNA [32]. Phenol which binds to DNA weakens the hydrogen bonds between bases due to a lowering of the melting point resulting in denaturation and inhibition of the PCR reaction [9].

Discrimination of DNA polymerase errors in the synthesis process can occur as research Kusnadi et al. [23], shows five steps in biochemical reactions of DNA polymerase inhibition. In addition, observations were made by [8] who conducted a DNA polymerase inhibition test of fifteen samples of phenolic compounds based on the added natural product P. corylifolia, using the docking method which showed the results of binding to potential amino acids in DNA polymerase thereby deactivating DNA polymerase activity such as which is shown in Figure 1.

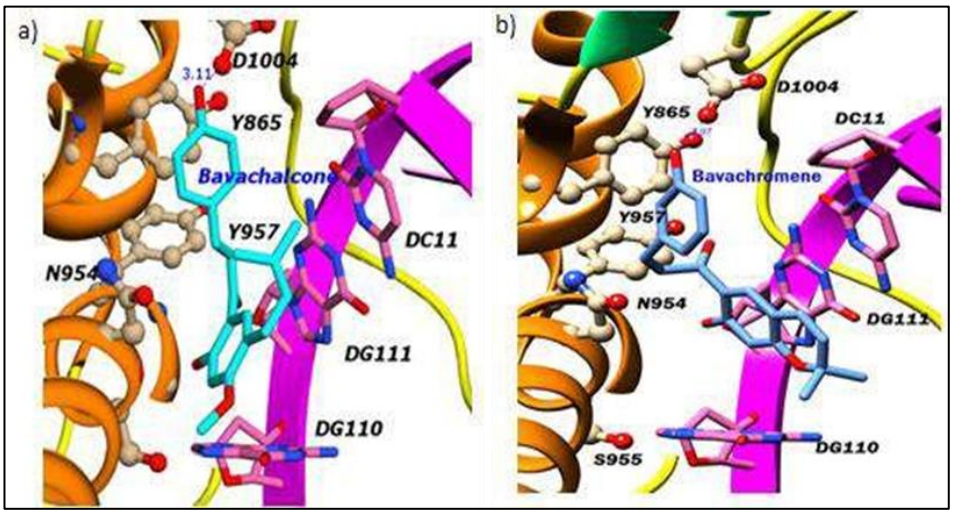

Figure 1. Docking results of (a) Bavachalcone and (b) Bavachromene in the active site of DNA polymerase [50]

3.7 Potential Counterfeiting of Illegal Pork Derivative Products in Foods Using Traditional Spices

Pork derivatives are components produced or derived from pigs such as fat or oil, meat, pork gelatin and so on [17]. These components are still used by some people as food ingredients, medicines and cosmetics. Counterfeiting of products such as lard which

is added to food as a substitute for butter, pork gelatin used for medicine capsules, even glycerin or lard used in making cosmetics, can cause spiritual harm to some people, especially the Muslim community because these ingredients are non-toxic lawful [23].

The word halal is often shown in food and beverage products that are legalized or do not contain haram substances for consumption by the Muslim community [4]. Haram substances that are often found in products such as processed food are pork and its derivatives which are clearly prohibited by Islamic law. The addition of pork-derived substances such as fat found in traditional foods such as rawon, soup, curry, sayur lodeh and others may occur to add to the taste of these foods. This is because lard or lard is usually used like butter with a distinctive savory taste in enhancing the quality of the taste.

As we know and we have found that many traditional foods that are sold and loved by Indonesian people are rich in plant-based spices which contain secondary metabolites of phenolic group compounds. One of the traditional foods that uses traditional spices is rawon which is made from basic spices such as kluwak, turmeric, garlic, shallots, galangal, lemongrass, candlenut, coriander, and others [4].

3.8 Secondary Metabolite Content of Spices in Traditional Foods

Traditional spices are parts of plants that are commonly used as medicines, antimicrobials, cooking spices, and traditional drinks such as herbs, sekoteng and others [33]. The spices that are often used in traditional food include kluwak, black cumin, galangal, lemongrass, candlenut, coriander, star anise, bay leaves, cloves, cinnamon, cardamom, kaffir lime leaves and so on. The compound content of these spices mostly contains phenolic group compounds, namely polyphenols, phenolic acids, flavonoids, tannins, and so on [34].

Many traditional plant-based food spices are known to contain secondary metabolites [35]. The content of these spice compounds has also been extensively developed based on their functional phytochemical properties of the various compounds found in Table 1. Observations from several literature studies Sharma et al. [36] demonstrated the phytochemical characteristics of finding tannins and flavonoids, carotenoids, and others in kluwak, 5-Hydroxyferulic acid and 3-Hydroxybenzoic acid in black cumin through antimicrobial activity tests [37]. Furthermore, the effectiveness test of galangal extract Sut et al. [38] and lemongrass [39] found the content of phenolic compounds. Subsequent research is candlenut, coriander, star anise, bay leaves, cloves, cinnamon, cardamom and lime leaves in Table 1 shows that the most secondary metabolites in these spices are phenolic group compounds (polyphenols, phenolic acids, flavonoids, and tannins).

Table 1. Compound Content of Traditional Food Spices

Spices	Compound Content	Ref
kluwak	Tannins, flavonoids, carotenoids, anthocyanins, hidnocarpat acid, cyanide acid, khaulmograt acid, gloric acid, and beta-carotene.	[33]
Black cumin	5-Hydroxyferulic acid, 3-Hydroxybenzoic acid, Ferulic acid, Sinapinic acid, Salicylic acid, Quercetin, Kaempferol, Tannins, Amino acids, Proteins, Carbohydrates, Essential oils, lkaloids, and saponins	[40]
Galangal	Phenolic compounds, flavonoids, and essential oils.	[41]
Lemongrass	Alkaloids, flavonoids, tannins, quinones, steroids and terpenoids	[42]

Candlenut	Linoleic acid, palmitic, stearic, protein, vitamin B1, fatty substances, saponins, flavonides, and polyphenols.	[43]
Coriander	Alkaloid compounds, saponins, tannins, flavonoids, phenolics, triterpenoids, and glycosides.	[44]
Star anise	Phenolic compounds namely orthidine E; Adriadysiolide is a terpenoid compound, 8-Decene-4,6-diyne-1,2,10-triol, chrysogedone A, Egomaketone/perilla ketone and Lepalone/5-(3-Furyl)-2-methyl-1-penten-3 -one; and flavonols, namely quercetin and kaempferol	[45]
Bay leaf	Alkaloids, flavonoids, saponins, tannins, and steroids	[46]
Clove	Tannins, saponins, phlobatannins, phenolic compounds, reducing sugars, terpenoids, steroids and glucosides	[43]
Cinnamon	Polyphenols, catechin, epicatechin, procyanidin B1, procyanidin B2 and protocatechuic acid	[41]
Cardamom	2,9-dihydroxy-1,8-cineol and 2,4-dihydroxy-1,8-cineol, -pinene, -pinene, camphene, limonene, -cymene, -terpineol and –humulene, alkaloids, tannins, polyphenols, saponins , flavonoids and triterpenoids	[46]
Lime leaves	Alkaloids, flavonoids, polyphenolics, quinones, monoterpenoids and sesquiterpenoids	[47]

3.9 Classification of Traditional Food Spices from Contained Inhibitor Compounds

Classification of inhibitor compounds in traditional food spices based on the secondary metabolites contained. As previously explained, inhibitor compounds derived from plants, namely phenolic group compounds. The classification of these inhibitor compounds is divided into four groups including polyphenols, phenolic acids, flavonoids, and tannins which are shown in Table 2. This aims to facilitate research in analyzing the potential of inhibitor compounds in some of these traditional food spices.

Table 2. Classification of Phenolic Compound Inhibitors Contained in Traditional Food Spices

Inhibitors	Traditional Food Spices	Ref
Phenolic	Galangal, Candlenut, Coriander, Star anise, Cloves, Cinnamon, and Cardamom	[41]
Polyphenols	Black cumin, star anise and kaffir lime leaves	[39]
Phenolic acid	Kluwak, Black cumin, Lemongrass, Coriander, Star anise, Bay leaves, Cloves, and Cardamom	[32]
tanins	Kluwak, Black cumin, Galangal, Lemongrass, Candlenut, Coriander, Star anise, Bay leaves, Cardamom and Kaffir lime leaves	[48]

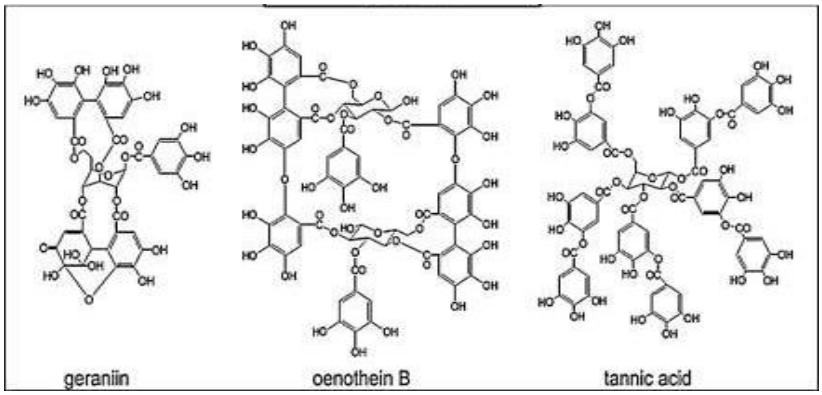

Figure 2. Structure of polyphenolic compounds [35]

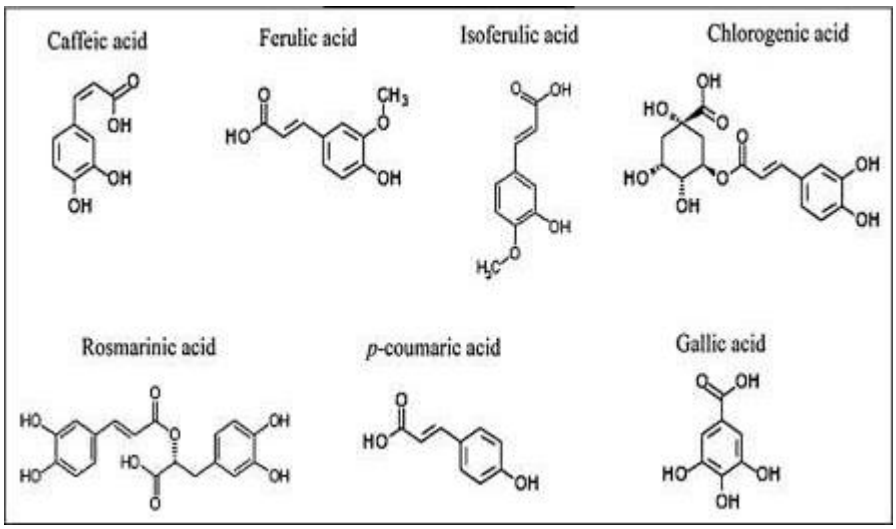

Figure 3. The structure of the phenolic acid compound [35]

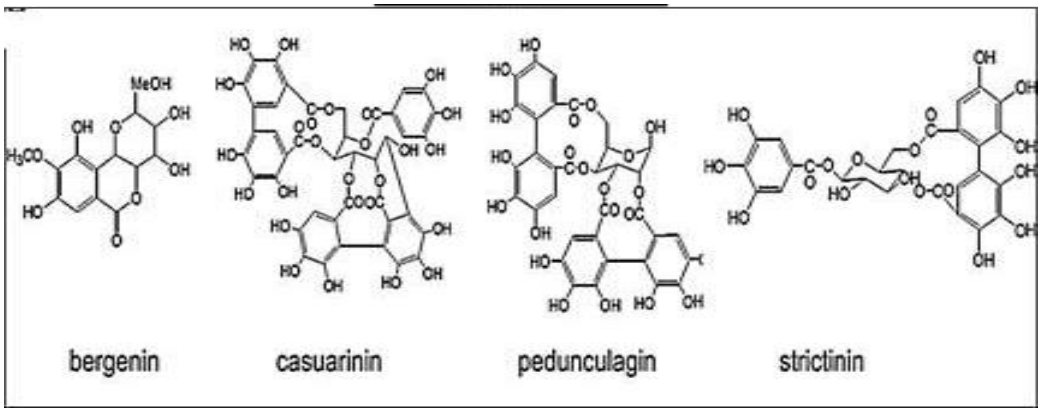

Figure 4. Structure of tannin compounds [37]

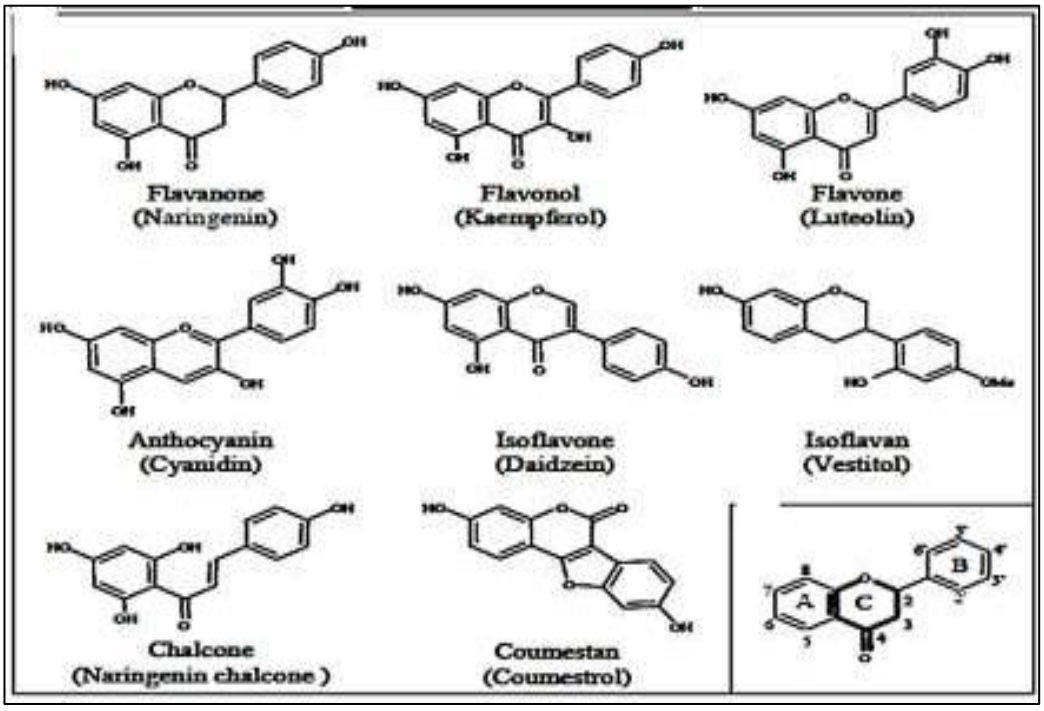

Figure 5. Structure of flavonoid compounds [52]

3.10 Mechanism of inhibition of DNA polymerase by phenolic compounds

The mechanism of action of DNA polymerase can be disrupted by the presence of inhibitory compounds during the PCR process. In Figure 6, inhibitor compounds can attack DNA polymerase directly or indirectly in the PCR process [24]. One of these inhibitors is a phenolic group of compounds such as polyphenols, phenolic acids, flavonoids, and tannins. The mechanism of inhibition of DNA polymerase by phenolic

group compounds has been extensively studied as shown in Table 3. Several studies have shown that inhibition deactivates DNA polymerase Figure 7 due to the presence of phenolic groups that are oxidized to quinones resulting in the enzyme binding to quinones [10].

There are also those who state that these inhibitors can reduce enzyme activity and cause decreased amplification efficiency [49]. Phenolic compounds can also degrade DNA polymerase and inhibit DNA polymerase or reverse transcriptase activity as shown in studies [50]. Observations made by [32] proved that phenolic group compounds have the potential to precipitate DNA polymerase, causing its activity to decrease from his research. [50] showed that the inhibition of DNA polymerase by polyphenolic compounds forms a secondary structure through the phenol ring. Finally, research [23] found that there was inhibition of DNA polymerase by phenolic compounds so that it was denatured.

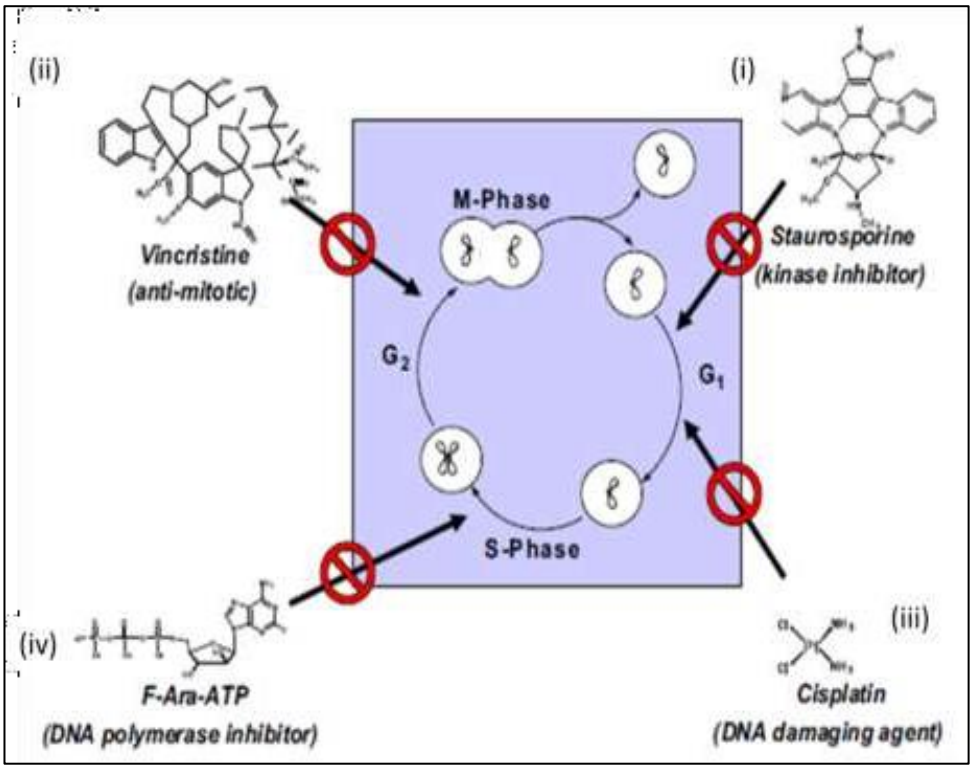

Figure 6. Chemotherapy strategies to inhibit DNA synthesis include, (i) the use of kinase inhibitors to block cell cycle development, (ii) anti-mitotic agents that inhibit the assembly and formation of microtubules, (iii) compounds that induce the formation of DNA lesions, and (iv) antimetabolites that directly or indirectly inhibit DNA polymerase activity [51]

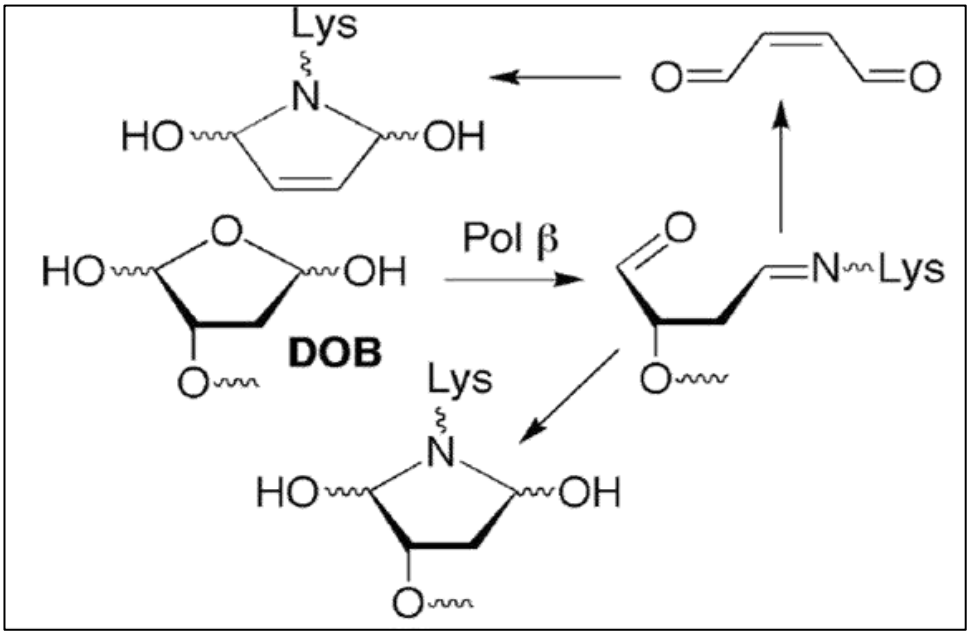

Figure 7. An overview of the mechanism of irreversible inhibition of DNA polymerase by the phenolic compound Dioxobutane (DOB) which can deactivate the enzyme [52]

Table 3 . Effect of Inhibitors of Phenolic Compounds on DNA Polymerase

Inhibitors	Influence on DNA polymerase	Ref
Phenolic group compounds from traditional food spices	• Binding and inactivating DNA polymerase	[51]
	• Decreased activity of DNA polymerase	[50]
	• DNA polymerase degradation	[52]
	• Inhibition of DNA polymerase or reverse transcriptase activity	[53]
	• Precipitation of DNA polymerase and decrease in activity	[24]
	• Formation of the secondary structure through the inhibiting phenol ring	[39]
	• Binding or denaturing DNA polymerase	[23]

4. Conclusion

Research studies on inhibition of DNA polymerase by phenolic group compounds in Indonesian food spices in the PCR process carried out, it was concluded that the PCR method is a DNA-based identification test that has high accuracy, effectiveness and sensitivity. So it is recommended in the analysis of haram substances in processed food products. Most of the secondary metabolites of traditional food spices contain phenolic compounds. These phenolic compounds have been shown to inhibit DNA polymerase in the PCR process. The mechanism of inhibition of DNA polymerase is largely due to the presence of phenolic groups that bind to the active site of the enzyme resulting in a decrease in activity and deactivation of the enzyme.

5. Author's Declaration

Author contributions and responsibilities - The authors made major contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation and discussion of results. The authors read and approved the final manuscript.

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