Chapter

Monoglycerides as an Antifungal Agent

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Abstract

Monoglyceride is a part of a lipid group compound. As a derivative of triglycerides, monoglycerides could be produced from renewable resources like fat or vegetable oils. Structurally, monoglyceride has lipophilic and hydrophilic properties in its molecule. Lipophilic properties could be donated by an acyl group from fatty acid and hydrophilic properties from two hydroxyl residues. Therefore, it was referred to as an organic amphiphilic compound. Monoglycerides have potency as antifungal agents. Based on its chemical structure, monoglyceride allows to bind to lipid bilayer and other components on the cell membrane of fungal microorganism and damage it. In this chapter, we will describe the structure and classification, physical and chemical properties, as well as reaction path synthesis of monoglyceride from vegetable oils and mechanism of action of monoglyceride as antifungal agents.

Keywords: monoglyceride, amphiphilic, antifungal, vegetable oil

1. Introduction

Monoglyceride or monoacylglycerol is a part of lipid compounds. Lipid compounds can be derived from two sources, vegetable oils and animal fats. The main component in vegetable oil or animal fats is triglycerides or triacylglycerides that can be converted to monoglycerides. In this case, triglycerides from vegetable oils or animal fats are losing two acyl groups to produce monoglycerides via the formation of diglycerides.

Based on their structure, monoglycerides are monoester compounds from glycerol and fatty acid. The position of the acyl group can determine the types of monoglycerides, whether it is 1-monoglyceride or 2-monoglycerides. A type of fatty acid chain also defines the type of monoglycerides. For example, lauric acid (saturated fatty acid) can be derived into monolaurin, while oleic acid (unsaturated fatty acid) into monoolein.

Monoglycerides have two hydroxyl groups (OH) and anion ester from fatty acids that bound to the propane chain. These structures make monoglyceride an amphiphilic compound with a lipophilic and hydrophilic part in the molecules. Amphiphilic properties of monoglyceride are used as an emulsifier and antibacterial, antifungal, antiviral, antioxidant, and anti-arteriosclerosis agent [1, 2]. Monoglycerides are also widely used in food, cosmetics, pharmaceutical, detergents, and plasticizer industries [3].

Amphiphilic properties of monoglycerides are similar to the amphiphilic properties of polyene that have been proven to have antifungal properties.

An amphiphilic drug such as a polyene compound could interact with the cell membrane of fungi via chemical bonding that led to the damage of the cell [4]. Monoglycerides such as monolaurin were reported to be active against *Candida albicans* [5]. Monocaprin and monomiristine were also proven to have antifungal activity because of their amphiphilic properties.

In this chapter, chemical structure and classification of monoglycerides, as well as their physical and chemical properties, are described. Furthermore, the reaction pathways to produce monoglycerides from vegetable oils and the mechanism of action of monoglycerides as antifungal agents are presented.

2. Chemical structure and classification of monoglycerides

Monoglycerides are lipid compounds that have a wide range of applications in human life. As a part of lipid biomolecule, monoglycerides are primarily composed of carbon (C), hydrogen (H), and oxygen (O) atoms. Monoglycerides can be referred to as the derivatives of triglycerides or tri-acylglycerol or triester glycerol. The lysis process of triglycerides into monoglycerides can be carried out via chemical reaction in methanol or ethanol and catalyzed by base catalysts or lipase enzymes. This reaction is referred to as a transesterification reaction because it converts an ester into another ester compound.

Monoglycerides can be afforded as the intermediate from the transesterification reaction of triglycerides of vegetable oils with short-chain alcohol. This reaction produced methyl esters or ethyl esters and glycerol as the by-products. In other words, monoglycerides are intermediates in the production of biodiesel from vegetable oil through the lysis of triglyceride using methanol in the presence of base catalysts. Limiting the amount of methanol and the reaction time is expected to produce a large amount of monoglyceride, which is referred to as a partial transesterification reaction [6].

The position of the acyl group attached to the glycerol skeleton can define the type of monoglycerides. Based on the acyl group position, monoglycerides can be classified into two types, i.e., 1-monoglycerides (α-monoglycerides) and 2-monoglycerides (β-monoglycerides) as seen in **Figure 1**.

1-Monoglycerides or α-monoglycerides can be identified by the acyl groups attached to the C_1 or C_3 atom of glycerol while 2-monoglycerides (β-monoglycerides) on the C_2 atom. The type of acyl group can be differentiated by the type of the alkyl (—R) groups bound with fatty acid (**Figure 1**). If the —R groups are derived from saturated fatty acid, then it will produce saturated monoglycerides. Monolaurin, monocaprin, monomiristine, monopalmitin, and monostearin are saturated monoglycerides in the form of solid [7–10]. On the other hand, if the –R groups are from unsaturated fatty acid, it could generate unsaturated

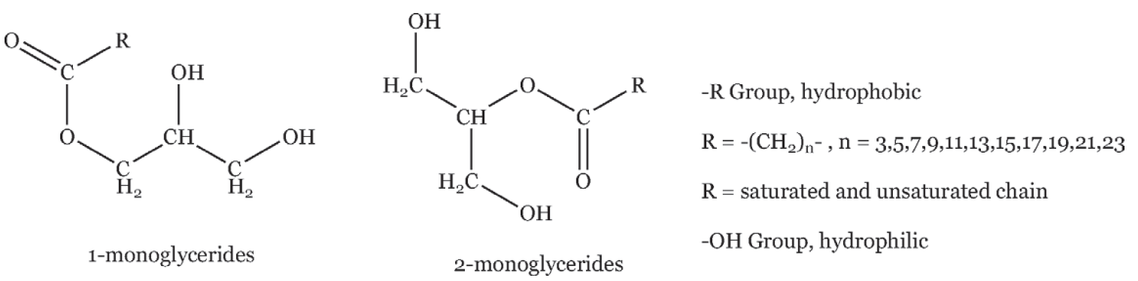


Figure 1.
The structure of 1- and 2-monoglycerides.

monoglycerides such as monoolein and monolinolein that are tend to be in the form of thick liquid [11].

Based on the length of the acyl groups on the glycerol, monoglycerides can be categorized into medium- and long-chain monoglycerides. Monoglycerides with the carbon chain length ranging from 8 to 14 atoms in the acyl groups are considered as medium-chain monoglycerides, for example, monocaprylin, monolaurin, monocaprin, and monomiristine [3]. Meanwhile, monoglycerides with a carbon chain length greater than 14 atoms are classified as long-chain monoglycerides. Monopalmitin, monostearin, and monoolein are the examples of the long-chain monoglycerides.

Previously, it was mentioned that the type of fatty acid has an essential role in determining the type of monoglyceride. Therefore, knowledge about fatty acids is important to discuss. Fatty acids can be divided into saturated and unsaturated fatty acids. Some essential saturated fatty acids are lauric acid and stearic acid, while the unsaturated fatty acids are oleic and linoleic acid. There are also two types of polyunsaturated fatty acids (PUFAs), namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1]. Fatty acids can also be classified based on the chain lengths, namely, medium- and long-chain fatty acids. Caprylic acid, capric acid, lauric acid, and myristic acid are some examples of medium-chain fatty acids. On the other hand, palmitic acid, stearic acid, and oleic acid are included as long-chain fatty acids. The chemical structure of some fatty acids from vegetable oils sample is presented in **Table 1**.

Vegetable oil is one of the raw material sources for producing monoglycerides because it contains triglycerides. Triglycerides from each type of vegetable oil are different, and it depends on the type and composition of fatty acids. Certain fatty acids that construct a triglyceride in vegetable oil tend to present as a major component besides other minor fatty acids. The major fatty acid in coconut oil (*Cocos nucifera L.*) is lauric acid (54%) [12], in castor oil (*Ricinus communis L.*) is ricinoleic acid (93%) [13], in olive oil is oleic acid [14], in sunflower oil is oleic and linoleic acid [15], and in palm oil is palmitic acids [16].

Vegetable oils with certain high levels of fatty acids can be used as raw materials for the synthesis of monoglycerides through various chemical reaction approaches such as partial alkaline-catalyzed transesterification, glycerolysis, and alcoholysis. An alternative reaction approach is by firstly isolating the fatty acid methyl esters or ethyl esters before carrying out the synthesis of monoglyceride. The other reaction pathways in the synthesis of monoglycerides from vegetable oils are esterification reactions of fatty acid and glycerol, transesterification of the fatty acid methyl ester with glycerol, and transesterification of fatty acid methyl ester with protected glycerol reactions followed by deprotection reactions using an acid resin. Hence, in the production of monoglycerides, vegetable oil samples are the primary source because it is rich in triglycerides and fatty acids.

3. Physicochemical properties and applications of monoglycerides

Monoglycerides consist of a lipophilic and hydrophilic part in the molecules (**Figure 1**). Acyl groups from the fatty acid contribute to the lipophilic properties, while the two hydroxyl groups are responsible for the hydrophilic properties of monoglyceride. Because of its unique structure, monoglycerides are also known as an amphiphilic compound, which are widely used as surfactants. Surfactants are an active compound with lipophilic tail and hydrophilic head in a molecule that has a function to decrease the surface tension of molecules. Hydrophilic properties of —OH groups enable monoglycerides to interact with a water molecule, while the lipophilic from acyl groups make it possible to interact with oil or lipid.

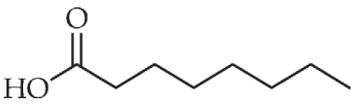
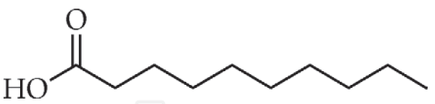
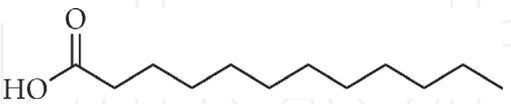
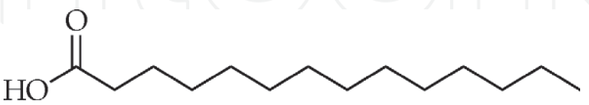
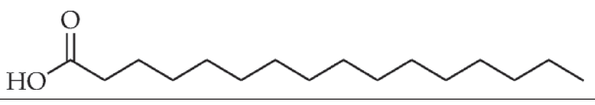
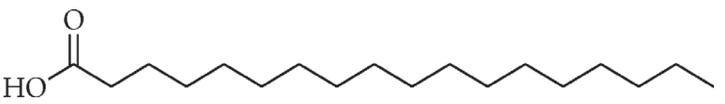
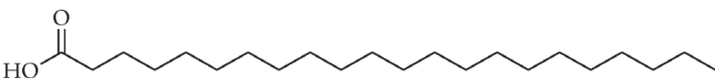
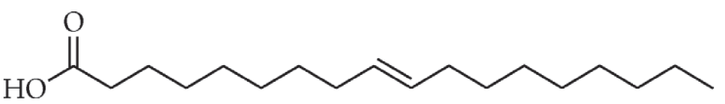
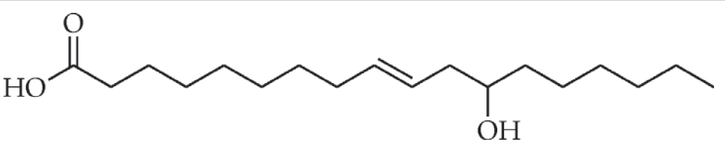
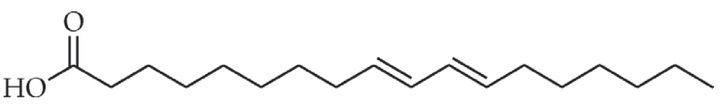
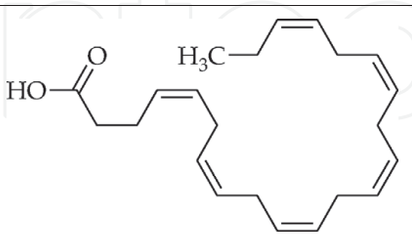
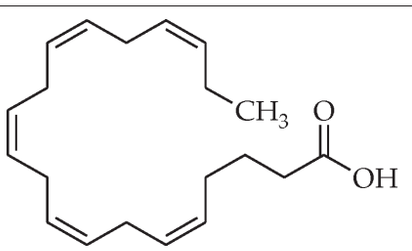
Fatty acid structure	**Name**
Saturated fatty acid	
	Caprylic acid
	Capric acid
	Lauric acid
	Myristic acid
	Palmitic acid
	Stearic acid
	Behenic acid
Un*saturated fatty acid*	
	Oleic acid
	Ricinoleic acid
	Linoleic acid
Polyunsaturated fatty acid (PUFA)	
	Docosahexaenoic acid
	Eicosapentaenoic acid

Table 1.
Fatty acids from vegetable oils.

Monoglycerides are classified as nonionic surfactants because the acyl groups do not have any charges. Based on its properties, monoglycerides are suitable to be used as an emulsifier to mix oils and water. Monoglycerides from vegetable oils are expected to be nontoxic emulsifier. Therefore, monoglycerides have broad applications in human life. Some studies supported the claim that monoglycerides have crucial and excellent applications as a safe emulsifier. This fact makes monoglycerides to be widely used in the food industry, detergents, plasticizers, cosmetics, and pharmaceutical formulations [3]. Naik et al. [15] have reported that almost 75% of emulsifiers in the food industry are using monoglycerides, making them the main food emulsifier. About 85,000,000 kg monoglycerides are purchased in the United States each year [17]. The long-chain monoglycerides (monopalmitin and monostearate) have an excellent emulsifier properties compared to medium-chain monoglycerides (monolaurin and monocaprin). Moreover, the saturated monoglycerides have better emulsifying ability than unsaturated monoglycerides [3].

Some studies have reported that monoglycerides are promising antibacterial, antifungal, and antiviral agents [2, 9, 11, 18]. Medium-chain monoglycerides are revealed to have better antimicrobial activity than long-chain monoglycerides. The high antimicrobial activity of monoglycerides is contributed by its unique chemical structures and excellent amphiphilic properties. The amphiphilic nature of monoglycerides enables the formation of effective interactions with various chemical compositions that construct the membrane cell of pathogenic bacteria, fungi, or viruses, paralyzing these microorganisms.

Monoglycerides from polyunsaturated fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), play an important function for human health. Wang et al. [1] have reported that monoolein has antioxidant and anti-atherosclerotic activity. Naik et al. [15] have described the antibacterial properties of monoglycerides, allowing them to be used as drug coating agents. Various applications of monoglycerides that have been presented previously prove that the nature and chemical structure of monoglycerides have an important role in determining their wide range of applications in human life.

The compound of 1-monoglycerides can exist in four different polymorphic forms at different temperatures [3]. In the polymorphic form α, 1-monoglyceride is unstable but has active chemical properties and excellent emulsion ability. The transition of 1-monoglycerides from the polymorphic form α to sub polymorphic α is observed in a lower temperature condition (35–50°C). If 1-monoglycerides are stored for a long time at room temperature, it will change to a more stable polymorphic form β. The 1-monoglyceride compound can also crystallize rapidly in certain solvents and form a stable β'-form. For example, 1-monocaprin, 1-monolaurin, and 1-monomiristine will crystallize quickly to form white solids from n-hexane solvent [6]. Unsaturated 1-monoglyceride compounds such as 1-monoolein also exist in the β-polymorphic form [19]. The symmetrical molecular structure of 2-monoglycerides causes this compound to always be in the form of polymorphic β [19]. This fact can be seen in 2-monolaurin that can crystallize rapidly in n-hexane solvents to form white solids and is very stable at room temperature [18].

The saturated monoglycerides can be found in three physical appearances, such as thick liquid (monocaprylin), fatty solid (monocaprin, monolaurin, monomiristine, monopalmitin), and waxy solid (monostearate and monobehenate). The longer carbon chain length of the saturated monoglyceride leads to an increase in the melting point. Monocaprylin has a melting point of 40–42°C. As the Carbon chain length increases from monocaprylin to monobehenate, its melting point increases to 65–77°C. Unsaturated monoglycerides such as monoolein and monolinolein are present in liquid form with melting points below 35°C. Regarding

the solubility, almost all of the saturated monoglycerides with the acyl carbon chain length of C_{10}–C_{18} are soluble in ethanol but not soluble in water. Only monocaprylin (C_8) is slightly soluble in water but completely soluble in ethanol. That means the hydrophilic properties of monoglycerides are inversely proportional to the carbon chain length of the acyl groups [3].

4. Preparation of monoglycerides as antifungal agents

Almost all monoglycerides are conventionally made from vegetable oils or animal fat through a lysis reaction approach using alcohol from glycerol. The reaction to break down triglycerides in vegetable oils or animal fats to monoglycerides using glycerol is called glycerolysis reaction. The glycerolysis reaction is a transesterification reaction that needs base catalysts, such as inorganic bases NaOH, KOH, and Ca $(OH)_2$, and sodium alkoxide [20, 21]. The glycerolysis reaction of vegetable oils to produce monoglycerides always takes place at a high temperature around 220–260°C and under inert N_2 gas conditions [17]. However, there are some weaknesses from the production of monoglyceride through the glycerolysis reaction of vegetable oils, i.e.:

a. Monoglycerides are obtained in a dark color and burnt smell.

b. It requires high energy consumption.

c. High reaction temperatures are not suitable for the production of heat-sensitive monoglycerides such as monoglycerides from EPA and DHA.

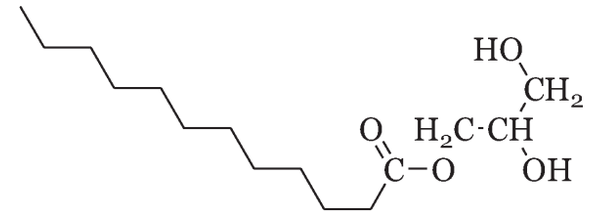
d. It requires purification of monoglyceride products with molecular distillation.

The development of alternative reaction pathways for the synthesis of monoglyceride is essential, considering the high demand for monoglycerides as safe emulsifiers, antimicrobial agents (antibacterial, antifungal, antiviral), and other important applications. In this chapter, some reaction routes of monoglycerides are summarized, and their antifungal activities are also discussed.

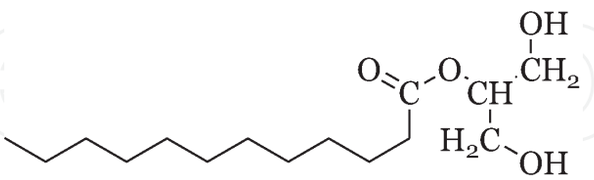
4.1 Monolaurin

Monolaurin is a saturated monoglyceride from lauric acid (C12: 0) with 12 carbon atoms in the acyl group (**Table 1**). Lauric acid is a saturated fatty acid that can be found in coconut oil. Nitbani et al. [12] have reported that the percentage composition of lauric acid in coconut oil was 54% as methyl laurate by GC–MS analysis. Generally, derivatization of lauric acid and fatty acids are needed because their boiling points are too high to be detected by gas chromatography unless converted into their esters such as methyl laurate. From this brief explanation, it can be concluded that monolaurin can be developed or derived from the raw ingredients of coconut oil, lauric acid, or methyl laurate. Monolaurin can be classified into 1-monolaurin and 2-monolaurin based on the position of the acyl group, as can be seen in **Figure 2**.

Monolaurin is a well-known monoglyceride for its killing activities of various species of fungi, especially *Candida albicans* [22]. Monolaurin has also been reported to be very active against *Penicillium* spp., *Aspergillus* spp. [23], and *Fusarium* spp. [24], the dominant food spoilage organisms. Those fungi species contribute to the decrease of the economic value of some foods such as meat, fruits, and cereals. Burhannuddin et al. [25] have reported that monoglyceride from virgin coconut oil (VCO), with monolaurin as the dominant compound, showed good



1-monolaurin



2-monolaurin

Figure 2.
The structure of monolaurin.

antifungal activity against *C. albicans* fungus. The ability of monolaurin to inhibit the growth of *C. albicans* was also reported by Chinatangkul et al. [26]. Monolaurin was also able to be used against common foodborne pathogens, including *C. albicans* with a minimum inhibitory concentration (MIC) value of 0.64 mg/mL and a minimum bactericidal concentration (MBC) value of 5.0 mg/mL [27].

Various literature presented the synthesis routes of 1-monolaurin through three reaction pathways that are summarized below. The reaction routes are esterification reaction of lauric acid and glycerol using either homogeneous or heterogeneous acid catalysts or a lipase enzyme catalyst and transesterification reaction of methyl laurate with glycerol.

4.1.1 Esterification reaction of lauric acid and glycerol catalyzed by acid catalysts

Monolaurin can be synthesized via esterification of lauric acid and glycerol in the presence of acid catalysts such as sulfuric acid (H_2SO_4) and *p*-toluenesulfonic acid (*p*TSA). The disadvantage of homogeneous catalyst is that it cannot be reused and needs to be neutralized. Utilization of heterogeneous acid catalysts in the esterification reaction of lauric acid and glycerol is expected to fulfill the drawbacks of homogeneous catalyst. The advantage of heterogeneous acid catalysts is that it can be easily separated from the reaction mixture and is reusable.

Table 2 summarizes the conditions of the esterification reaction of lauric acid and glycerol in the presence of homogeneous and heterogeneous acid catalysts from several publications. From these data, it can be concluded that the *p*TSA (homogeneous acid catalyst) shows excellent catalytic activity in the synthesis of monolaurin with a moderate yield (43.54%) and high purity (100%). Among the heterogeneous acid catalysts, sulfated zirconia-loaded SBA-15 displays an excellent catalytic ability in the esterification reaction to produce monolaurin with high yield (79.1%) and selectivity (83.42%).

4.1.2 Esterification reaction of lauric acid and glycerol catalyzed by lipase enzymes

Various types of enzyme catalysts, especially lipase enzymes, have been applied to synthesize monolaurin via the esterification reaction of lauric acid and glycerol.

Type of catalyst	Reaction condition	Yield	References
H_2SO_4	Temperature 130°C, 6 h, molar ratio of 1:1 (glycerol/lauric acid), 5% H_2SO_4 (w/w from lauric acid), solvent-free	31.05% yield of monolaurin, 91% purity	[28]
pTSA	Temperature 60°C, 6 h, molar ratio of 8:1 (glycerol/lauric acid), 2.5% pTSA (w/w from lauric acid), solvent-free	43.54% yield of monolaurin, 100% purity	[7]
Silica gel-coated with propyl sulfonic acids	Temperature 112°C, 8 h, molar ratio of 1:1 (glycerol/lauric acid), 5 wt% catalyst (relative to glycerol), solvent-free	51% yield of monoglyceride	[29]
Sulfated zirconia-loaded SBA-15	Temperature 160°C, 6 h, molar ratio of 4:1 (glycerol/lauric acid), sulfated zirconia-loaded SBA-15 catalyst with 16 wt.% zirconium oxychloride loading, solvent-free	79.1% yield and 83.42% selectivity of monolaurin, lauric acid conversion 94.9%	[30]
Zeolite Y CBV 712	Temperature 120–140°C, 7 h, molar ratio of 8:1 (glycerol/lauric acid), 15 wt% zeolite Y with dealumination, solvent-free	Reaction conversion: 97.8%, selectivity of 65%, and 59.5% yield of monolaurin	[31]

Table 2.
Reaction conditions in the synthesis of monolaurin from lauric acid and glycerol catalyzed by an acid catalyst.

Table 3 presents some publications related to the synthesis condition of monolaurin. The Novozym 435 lipase enzyme produced by Novozym Inc. has the highest catalytic activity to catalyze the esterification reaction of glycerol and lauric acid to produce monolaurin with high yield and selectivity (up to 100%).

4.1.3 Transesterification reaction of methyl laurate and glycerol

Monolaurin can be produced from methyl laurate and glycerol via transesterification reaction. The methyl laurate can be obtained from the transesterification reaction of coconut oil [12]. Glycerol itself is a by-product of the biodiesel production process. A mass yield of glycerol produced from the biodiesel production from vegetable oils is around 10% [37–39].

The reaction conditions and the results from the reaction of glycerol and methyl laurate to produce monolaurin can be seen in **Table 4**. Based on the data, 1-monolaurin compounds can be produced in high yield (almost reached 83%) in a binary solvent of *tert*-butanol/*iso*propanol (20:80; wt/wt). The solvent plays an important role in creating effective collisions between methyl laurate molecule and glycerol so that the activation energy is reached and the product of monolaurin is obtained. The presence of the lipase enzyme also determines the success of the formation of monolaurin products via a transesterification reaction of methyl laurate and glycerol.

Compound 2-monolaurin can be synthesized through the ethanolysis reaction of coconut oil using the Lipozyme TL IM catalyst [42]. Coconut oil is rich in lauric acid so that it can be used as a raw material for synthesis 2-monolaurin. In this case, ethanol can break down the triglycerides from coconut oil into monoglycerides, catalyzed by the sn-1,3-specific lipase enzyme. Because it uses an sn-1,3-specific enzyme, the acyl groups released are in positions 1 and 3 of triglycerides. This reaction is referred to as the alcoholysis reaction of coconut oils. Nitbani et al. [18] have successfully synthesized 2-monolaurin with high purity through the

Type of catalyst	Reaction condition	Yield	References
Lipozyme IM-20	Temperature 55°C, 6 h, molar ratio of 1:1 (glycerol/lauric acid), 3% (w/w) Lipozyme IM-20, solvent-free	45.5% yield of monolaurin, 26.8% of dilaurin	[32]
The partially purified lipase from *Rhizopus* sp.	Temperature 50°C, 72 h, stirring at 200 rpm, the molar ratio of 1:1 (glycerol/lauric acid), 100% (w/w) molecular sieve, 2 mg of partially purified lipase from *Rhizopus* sp., solvent-free	17.52% yield of monolaurin	[33]
CALB lipase (*Candida antarctica B*) immobilized on polysiloxane-polyvinyl alcohol particles (POS-PVA)	Temperature 45–60°C, 6 h, stirring at 200 rpm, molar ratio of 3:1 (glycerol/lauric acid), 0.5 g CALB L immobilized on POS-PVA (from the total weight of reactant), solvent-free	36% yield of monolaurin	[34]
Lipase G (*Penicillium camembertii lipase*) immobilized on epoxy SiO_2-PVA composite	Temperature 60°C, 6 h, stirring at 200 rpm, molar ratio of 8:1 (glycerol/lauric acid), 5% (w/w) lipase G immobilized on SiO_2-PVA loading, solvent-free	59.45% yield of monoglycerides, 62.91% selectivity	[17]
Lipozyme RM IM (*Rhizomucor mayhem lipase*)	Temperature 60°C, 3 h, molar ratio of 4:1 (glycerol/lauric acid), 4% (w/w) Lipozyme RM IM, solvent-free	50% yield of monolaurin, 34.6% yield of dilaurin	[35]
Novozym 435	Temperature 60°C, 8 h, stirring at 200 rpm, molar ratio of 4:1 (glycerol/lauric acid), Novozym 435 (60mg per mmol of carboxylic acid), nonaqueous reaction media: ionic liquid $[C_{12}mim][BF_4]$	100% selectivity and 100% yield of monolaurin	[36]

Table 3.
Reaction conditions in the synthesis of monolaurin from lauric acid and glycerol catalyzed by lipase enzymes.

Type of catalyst	Reaction condition	Yield	References
Novozym 435	Temperature 50°C, 24 h, stirring at 250rpm, molar ratio of 1:1 (glycerol/ methyl laurate), 5% (w/w) Novozym-435, solvent-free	47.6% monolaurin	[40]
Lipozyme 435 (immobilized-*Candida Antarctic lipase* on a macroporous acrylic polymer resin)	Temperature 50°C, 1.5 h, molar ratio of 1:6 (glycerol/ methyl laurate), 5% (w/w) Novozym 435, solvent: 15% (wt.) a binary solvent system (*tert*-butanol/*iso*propanol, 20:80, wt./wt.), a continuous flow system at a flow rate of 0.1 mL/min	82.5 ± 2.5 (wt. %) yield of monolaurin	[41]

Table 4.
Reaction conditions in the synthesis of monolaurin from methyl laurate catalyzed by lipase enzymes.

alcoholysis reaction of coconut oil using the Lipozyme TL IM enzyme. The 2-monolaurin compound was obtained in a yield of 30.1% and purity of 100% after purification using TLC preparation with a mixture of chloroform/acetone/methanol (9.5:0.45:0.05) as the eluent solvent. The Lipozyme TL IM enzyme is an sn-1,3-

specific lipase enzyme that has excellent catalytic activity for lipid substrate-related reactions such as hydrolysis, alcoholysis (transesterification), esterification, and acidolysis [42].

4.2 Monomyristin

Monomyristin compound is a medium-chain saturated monoglyceride with a number of carbon atoms in the acyl chain as 14 (C14). Altieri et al. [24] reported that both myristic acid and monomyristin compounds could inhibit the growth of fungi *F. oxysporum* DSMZ 2018 and *F. avenaceum* DSMZ 62161, although they were still weaker than lauric acid and monolaurin. Jumina et al. [9] also reported that 1-monomyristin has high activity against *C. albicans* compared with 2-monomyristin (not active). Therefore, the synthesis route of 1-monomyristin is important to be discussed. The chemical structure of 1-monomyristin is shown in **Figure 3**.

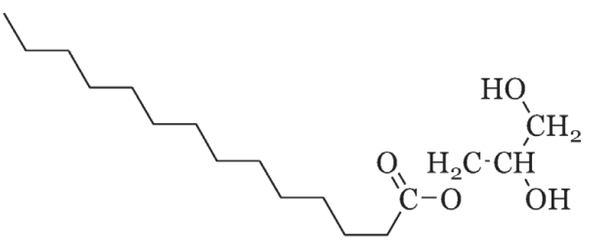
The 1-monomyristin compound can be obtained from the reaction of myristic acid and glycerol in the presence of lipase enzyme catalyst [17]. Another reaction pathway is through the transesterification of ethyl myristate with protected glycerol (1,2-O-isopropylidene glycerol) followed by a deprotection reaction using Amberlyst-15 to produce 1-monomyristin. Some works related to the synthesis of 1-monomyristin are presented in **Table 5**.

From **Table 5**, it can be noticed that the Novozym 435 enzyme has displayed extraordinary catalytic activity in the esterification reaction of myristic acid and glycerol to produce 1-monomyristin with 100% selectivity and yield. It should be noted that this reaction takes place in an ionic liquid [C12mim][BF4]. The [C12mim][BF4] is an example of a temperature switchable ionic liquid/solid phase used for the selective synthesis of monoglycerides [36]. Monomyristin can also be produced through two stages of reaction of ethyl myristate with 1,2-O-isopropylidene glycerol (mol ratio 1:8) using K_2CO_3 as a catalyst, which produces an intermediate isopropylidene glycerol myristate as a yellowish liquid with a yield of 32.12% and purity of 95.55%. In the second step, the isopropylidene glycerol myristate compound is deprotected with Amberlyst-15 (mol ratio 1:10) for 30 hours at room temperature to produce 1-monomyristin in the form of white solid in 100% of yield.

4.3 Monocaprin

Monocaprin is a monoglyceride from capric acid comprising 10 carbon atoms (C10) (**Figure 4**).

Monocaprin is produced from vegetable oils that are relatively safe or nontoxic to the body. Monocaprin is known as a safe food additive, so it is widely used as an emulsifier in the food industry. Solutions containing monocaprin can also act as

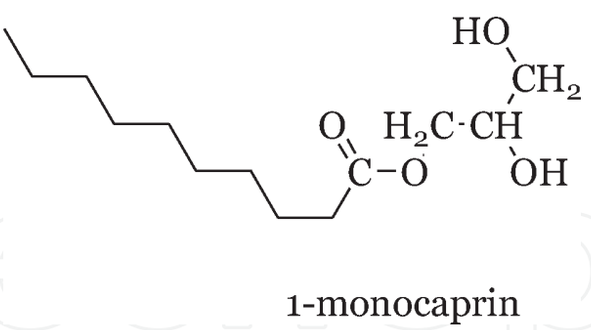


1-monomyristin

Figure 3.
The structure of 1-monomyristin.

Type of catalyst	Reaction condition	Yield	References
Lipase G (*Penicillium camembertii* lipase) immobilized on epoxy SiO_2-PVA composite	Temperature 60°C, 6 h, stirring at 200 rpm, molar ratio 8:1 (glycerol/ myristic acid), 5% Lipase G immobilized on SiO_2-PVA loading (w/w), solvent-free	47.92% MAG, 79.92% selectivity	[17]
Novozym 435	Temperature 60°C, 8 h, stir at 200 rpm, molar ratio 4:1 (glycerol/ myristic acid); Novozym 435 (60mg per mmol of carboxylic acid), nonaqueous reaction media: ionic liquid $[C_{12}mim][BF_4]$	100% selectivity and 100% yield of monomyristin	[36]
Potassium carbonate (K_2CO_3)	a. Step 1: reaction of ethyl myristate and 1,2-acetonide glycerol: temperature 140°C, 30 h, molar ratio 1:8 (ethyl caprate/1,2-acetonide glycerol), 5% K_2CO_3 (w/w), isolation product of isopropylidene glycerol myristate using diethyl ether b. Step 2: reaction of isopropylidene glycerol myristate and Amberlyst-15: room temperature, 30 h, ethanol as solvent, weight ratio 1:10 (Amberlyst-15/ isopropylidene glycerol myristate), isolation of 1-monomyristin by filtration and evaporation	100% yield of 1-monomyristin	[9]

Table 5.
Reaction conditions in the synthesis of 1-monomyristin.



1-monocaprin

Figure 4.
The structure of 1-monocaprin.

microbicidal agents against *Candida albicans* [43]. Lipid compounds, including monoglycerides, are also known as antifungal agents [44]. Monocaprin has been reported to have antifungal activity against three food spoilage fungi *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Penicillium citrinum* with minimum inhibitory concentration values of 0.31, 0.63, and 0.63 mg/mL, respectively. The minimum fungicidal concentrations were also reported to be 1.25, 2.50, and 2.50 mg/mL, respectively [45].

The production of monocaprin also has similarities with monomiristine and monolaurin, which involves esterification reactions of capric acid and glycerol. Another reaction pathway for the synthesis of monocaprin is by utilizing ethyl caprate and 1,2-acetonide glycerol (a protected glycerol compound). A summary of

Type of catalyst	Reaction condition	Yield	References
Candida antarctica (CAL)	Temperature 60°C, 6 h, water (12% in glycerol (w/w)), molar ratio of 1:1 (glycerol/capric acid), lipase dosage (100 U/g) of capric acid, solvent-free, batch reactor	Capric acid conversion as high as 96.9%	[46]
Porcine liver carboxylesterase (PLE)	Temperature 60 °C; 4 h; pH = 7; reverse micelles: isooctane (reaction medium) and bis(2-ethylhexyl) sodium sulfosuccinate (AOT, anionicsurfactant); R-value ([water]/[surfactant]): 0.1; G/F-value ([glycerol]/[fatty acid]) 3.0	The degree of esterification at equilibrium state 62.7%	[47]
Sodium carbonate (Na_2CO_3)	a. *Step 1: reaction of ethyl caprate and 1,2-acetonide glycerol* Temperature 110°C; 24 h; molar ratio of ethyl caprate/1,2-acetonide glycerol, 1:8; 5% Na_2CO_3 (w/w); isolation product (1,2-asetonide-3-capryl glycerol) using n-hexane b. *Step 2: reaction of 1,2-acetonide-3-capryl glycerol and Amberlyst-15* Room temperature, 24 h, ethanol as a solvent, ratio weight 1:7 (Amberlyst-15/1,2-acetonide-3-capryl glycerol); isolation of crude 1-monocaprin with recrystallization in n-hexane, purification of 1-monocaprin using preparative thin-layer chromatography	a. Yield, 88.12%; purity, 87% (1,2-acetonide-3-capryl glycerol) b. Yield, 78.37%; purity, 100% (1-monocaprin)	[6]

Table 6.
Reaction conditions in the synthesis of 1-monocaprin.

the reaction conditions on the synthesis of monocaprin can be seen in **Table 6**. It is noticed that monocaprin can be produced with high yield and purity via two reaction steps. The first stage involves the transesterification reaction of ethyl caprate with 1,2-acetonide glycerol to produce intermediate 1,2-acetonide-3-capryl glycerol with a yield of 88.12%. The second step is deprotection reaction of 1,2-acetonide-3-capryl glycerol using a heterogeneous catalyst (Amberlyst-15) to produce 1-monocaprin with 100% purity and a yield of 78.37% after purification. A lipase enzyme *Candida antarctica* (CAL) is also reported to be used as the catalyst in the esterification of capric acid and glycerol, with conversion rate reaching 96.9%. Production of monocaprin in a reverse micelle system using isooctane (reaction medium) and bis(2-ethylhexyl) sodium sulfosuccinate (AOT, anionic surfactant) has marked the conversion rate of esterification reaction of capric acid and glycerol at equilibrium condition at 62.7%, which is achieved using *Porcine liver carboxylesterase* (PLE) as a catalyst.

5. Mechanism of monoglycerides as antifungal agents

Several publications related to the antifungal activity of monoglycerides such as monolaurin, monomiristine, and monocaprin have been explained in the previous section. The studies showed that monoglycerides with good amphiphilic properties were not only able to inhibit the fungal growth but also kill it, especially *C. albicans* (22) (9) and some food spoilage fungi species such as *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Penicillium citrinum* [45]. Publication data supported a

hypothesis that the saturated medium-chain monoglycerides such as monolaurin, monomiristine, and monocaprin have good antifungal activity. Based on their structure, each monoglyceride has an acyl group derived from lauric acid (C12: 0), myristic acid (C14: 0), and capric acid (C10: 0).

It is noted that the chemical structure of monoglyceride plays an important role in its antifungal activity by affecting the interaction with fungal organisms. Monoglycerides with lipophilic acyl groups and two hydrophilic hydroxyls (-OH) groups (**Figure 1**) are beneficial to interact with various chemical components that build fungal organisms.

Prasad et al. [4] have reported that the current development of antifungal agents is aimed at interactions with the fungal cell wall. There are two main targets of antifungal agents, firstly, by targeting the interactions with chemical components of fungal cell walls such as mannans, glucans, and chitins. The second target is aiming at the interactions with several enzymes that responsible for bioactivity and the biosynthesis pathway of ergosterol. Ergosterol is one of the main sterol components that build fungal cell membranes and regulate the fluidity, permeability, and structure of the membranes [48, 49].

One of the antifungal drugs that have been developed and are quite useful in invading fungal infections is polyene. There are three main types of fungal drugs from polyene compounds, i.e., Amphotericin B, Nystatin, and Natamycin [48]. These three fungal drugs are known as macrolides, which structurally are cyclic amphiphilic organic molecules. The amphiphilic aspects of macrolides are contributed by the unsaturated alkyl chain (around 14 C) as the lipophilic part that attached to the macrolactone ring and some hydroxyl (-OH) groups as the hydrophilic part.

The amphiphilic properties of some macrolide compounds (Amphotericin B, Nystatin, and Natamycin) from the polyene group are the main factors in their mechanism of action as the antifungal agents. Their amphiphilic structure allows these compounds to bind chemically to the components of lipid membrane, especially ergosterol, through van der Waals interactions. Interactions between these molecules will trigger the formation of pores on the cell membrane. Moreover, in the end, the pores will destabilize the cell membrane, damaging the balance of ions in the cell membrane and further resulting in the cell death [4, 48, 50].

Other interaction models can be predicted based on the chemical structure of macrolides as well as the chemical components of the fungal cell wall, such as mannans, glucans, and chitins. The hydrophilic part (hydroxyl groups) in macrolide compounds is can possibly interact with polar groups found in mannans, glucans, and chitins through hydrogen bonding. This interaction is also predicted to contribute to the fungal cell wall damage, so the cell lysis can occur resulting in cell death.

Monoglyceride compounds such as monolaurin, monomiristine, and monocaprin that have been proven to be antifungal agents are assumed to follow the inhibitory mechanism of macrolides (polyene) compounds. This assumption is very rational, considering that monoglycerides are having excellent amphiphilic properties (**Figures 1** and **2**). The acyl group of the lipophilic part of monoglycerides is expected to interact via van der Waals interaction with ergosterol in fungal cell walls and causes lysis and cell death. Meanwhile, the two hydroxyl groups in monoglycerides are responsible for the antifungal activity by forming hydrogen bonding with other polar components in the fungal cell wall (glucans, chitins) and assisting the cell membrane lysis process.

Comparing the number of hydroxyl groups in monoglycerides, macrolides compounds have more hydroxyl groups. Therefore, the hydrogen bonding interactions of monoglycerides with polar components in the fungal cell wall is expected to be

less effective than in macrolides. Based on the prediction of the mechanism of action and in vitro data of monoglycerides as antifungal agents, it can be concluded that monoglycerides, especially monolaurin, monomiristine, and monocaprin, have the potential to be developed as antifungal drugs. Thus, there will be new candidates for antifungal drugs from monoglyceride-based lipid.

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