The effect of incubation time on biotransformation of gurjun balsam oil by *Aspergillus niger* and its prediction activity of alpha-copaene

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Abstract

Background: Gurjun balsam oil is one of the essential oils from Indonesia which is isolated from the resin plant *Dipterocarpus turbinatus*. Gurjun balsam oil has a fragrant aroma and is used as a traditional medicine in the Indochina region. The main content of balsam gurjun oil is an alpha-copaene compound and several other sesquiterpenes ($C_{15}H_{24}$) class compounds. In this research, biotransformation of the compound content of gurjun balsam oil with *Aspergillus niger* was carried out.

Methods: The biotransformation process was carried out at room temperature with a speed of 130 rpm and a variation of the incubation time of 24, 48, 72, and 96 hours. The biotransformed products were analyzed by Gas Chromatography-Mass Spectrometer (GC-MS). Mechanism action of prediction was performed by Pass Online prediction and ADMET Prediction was performed pkCSM online

Results: The main products formed from the biotransformation of balsam gurjun oil were copaene (60.53%), beta-caryophyllene (22.76%), humulene (3.87%), and alpha-cadinene (12.83%). The optimum incubation time with the highest copaene product was 72 hours. According to data pass online prediction, it has probability active (Pa) antieczematic with value 0.74.

Conclusion: Biotransformation of gurjun balsam oil by *Aspergillus niger* does not produce new derivates compounds, but increase the yield of the alpha-copaene compound. Copaene in Gurjun oil has strong potential as antieczematic in skin problems.

Keywords: biotransformation, gurjun balsam oil, *Aspergillus niger*, alpha-copaene, antieczema

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Running Head : Biotransformation of gurjun balsam oil by *Aspergillus niger*

The effect of incubation time on biotransformation of gurjun balsam oil by *Aspergillus niger* and its prediction activity of alpha-copaene

Introduction

Gurjun balsam oil is one of the essential oils isolated from wood resins keruing tree (Dipterocarpus turbinatus). Dipterocarpus turbinatus is one of the flora that grows in Asia, especially Indonesia and India. Gurjun oil is used as a fixative, varnish, lithographic ink, torch, and boat caulk. In the tradition of the people of Indochina, gurjun oil is used as a medicine, namely for the disinfectant, diuretic, mild stimulant, and analgesic. Gurjun oil is one of Indonesia's non-oil and gas commodities with a high selling value of US $ 205.76 per liter in 2016 [1].

Gurjun balsam oil contains active compounds of the sesquiterpene (C15) group, including α-copaene, α-gurjunene, β-gurjunene, γ-gurjunene, humulene, and β-caryophyllene [2]. The α-copaene compound is the major compound in gurjun oil having one double bond in its structure (Fig. 1) [3]. In the α-copaene structure, there is one functional group point in the form of a double bond that can donate electrons to interact with the active site of other target compounds. The yield of compound α-copaene be indicators of the quality of gurjun balsam oil production of the Indonesian state. Research on the exploration of α-copaene compounds from gurjun balsam oil has not been widely reported. Wu et al. (2014) reported that the α-copaene in *Toona sinensis* essential oil has antibacterial activity against Staphylococcus aureus and anticancer bacteria [4]. The α-copaene is non-mutagenic and has cytotoxic activity against cancer cells [5].

Increasing the yield of α-copaene in gurjun oil can be done in various ways, one of which is biotransformation which is considered environmentally friendly and efficient. Biotransformation is the process of modifying chemical compounds into derivative compounds by an organism, one of which is by using fungi [6]. One of the fungi that are widely used for

the biotransformation of terpenoids is *Aspergillus niger* [7]. *A. niger* produces extracellular enzymes including amylase, protease, pectinase, lipase, cellulase, and chitinase [8]. *A. niger* can produce citric acid, gluconic acid, and gallic acid which can be a substrate for producing antioxidant compounds from food [9]. The presence of extracellular enzymes from *A. niger* can be used as a biotransformation agent.

A. niger activity can transform terpenoid group compounds into their derivatives by adding a hydroxyl group (-OH) on the active side. The existence of a carbon skeleton in the terpenoid group structure can be a source of substrate for *A. niger*. A review of research reported by Parshikov and Sutherland (2014) shows that *A. niger* can enzymatically transform various types of terpenoid compounds [7]. The metabolite products produced by *A. niger* have a high yield [10]. *A. niger* can convert the tricyclic aristolene sesquiterpene compound to aristolenoic acid (carboxylic acid group) [11]. Based on this review, A. niger is thought to be applied to transform the active compound content contained in balsam gurjun oil.

Identification of the product of the compound transformation reaction in gurjun oil using the Gas Chromatography-Mass Spectrophotometer (GC-MS) instrument. The transformed derivative is detected from the mass spectrum obtained, while the derived compound content can be determined through the percent area value on the chromatogram. The purpose of this study was to determine the effect of incubation time by *A. niger* of gurjun balsam oil and to determine its potential as a medicinal ingredient by reviewing in silico analysis.

Materials and Methods

This research was carried out in May-August 2020 at the STIKES Microbiology Laboratory of Anwar Medika Hospital with a post-test only research design. Materials used include gurjun oil (CV. Darjeeling oil), *Aspergillus niger* isolate (ATCC 6275 from the Surabaya Center for Health Laboratory), n-hexane (Merck), Potato Dextrose Agar (Merck) media, aquades (PT. Brataco), and dextrose (Merck).

The compound content of gurjun oil was identified by GC-MS. A. niger isolate was cultured with Potato Dextrose Agar (PDA) media. *A. niger* culture was incubated at 30 ºC for 3 days. The incubation process adjusted to the *A. niger* growth curve which entered the log

phase on the third day [12]. The biotransformation process of gurjun oil used Potato Dextrose Broth (PDB) media and incubated at 30 ºC for 3 days with an agitation speed of 125 rpm. The biotransformation process is carried out by adding carp oil (0.1% v / v) to the PDB medium. The biotransformation process was carried out with time variations of 24, 48, 72, and 96 hours. The incubated biotransformation mixture suspension was extracted using n-hexane and the biotransformed compound isolate by evaporation. The observation of the biotransformation process in gurjun oil was identified using a thin layer chromatography method. The Retardation Factor (Rf) values at each variation of the incubation time were compared. The change in the Rf value indicates a transformation of the compounds in gurjun oil. The identification of biotransformed compounds was identified by GC-MS. Mechanism action of prediction was performed by Pass Online prediction and ADMET Prediction was performed pkCSM online [13].

Results

The gurjun balsam oil used as research material contains α-cubebene (1.54%), α-copaene (49.05%), beta-cubebene (3.4%), α-gurjunene (1.21%), β-caryophyllene (20.46%), humulene (4.57%), aromandendrene (1.8%), γ-muurolene (2.16%), 1H-Cyclopropa[a]naphthalene (2.77%), and α-cadinene (13.06%). The structure of the balsam gurjun oil content is presented in Table 1. Gurjun oil biotransformation process carried out with four variations of the incubation time is 24 hours, 48 hours, 72 hours, and 96 hours. Figure 2 shows the chromatogram of the biotransformation results based on the variation of the incubation time. The biotransformation of gurjun oil with A. niger produced four compounds, namely α-copaene, β-caryophyllene, humulene, and α-cadinene. Biotransformation products showed an increase in α-copaene yield from 0 hours to 72 hours of incubation time. Based on the fragmentation analysis on the mass spectrum of the GC-MS chromatogram, there are no new derivatives were formed from the biotransformation process of gurjun oil by *A. niger*.

The majority of the compounds in balsam gurjun oil are compounds of the sesquiterpene group. According to pass online prediction, the probability active (Pa) value of copaene has to greater than 0.7, which means to have strong potential as a mechanism

activity that was meant (Table 2). The copaene in gurjun balsam oil for applying topical administration, according to data pkCSM online, it has low skin permeability absorption with the value of log Kp is -2.194.

Discussion

The initial level of copaene in balsam gurjun oil has met international quality standards, namely 49.04%. The use of balsam gurjun oil as a medicinal raw material has not been widely reported due to the limited pharmacophore groups in copaene as the main ingredient. Biotransformation efforts were made to transform the compound content of balsam gurjun oil. The biotransformation of gurjun balsam oil by *A. niger* begins during the logarithmic phase (the third day of the incubation period of *A. niger*) because in this phase cell division occurs and its activity becomes more active.

Biotransformation products showed an increase in α-copaene yield from 0 hours to 72 hours of incubation time. Based on the fragmentation analysis on the mass spectrum of the GC-MS chromatogram, there are no new derivatives were formed from the biotransformation process of gurjun oil by *A. niger*. The incubation times of biotransformation 24 hours, 48 hours, and 72 hours occurred in the exponential phase of A. niger. In the exponential phase, A. niger cells divide maximally due to the availability of many nutrients so that cell activity increases [12]. The extracellular enzymes from A. niger are produced in the exponential phase, this is evidenced by the high yield of copaene (60.53%) at the 72 hour incubation time.

At the 96-hour incubation period, copaene yield decreased from 60.53% to 58.44%. The 96-hour incubation time has entered the stationary phase of the *A. niger* growth curve. In the stationary phase, the number of living *A. niger* cells is the same as the number of cells that have died [8]. At the 96-hour incubation time, there was an increase in β-caryophyllene and α-cadinene, this is presumably because in the stationary phase A. niger produces more diverse secondary metabolites.

The extracellular enzymes of A. niger cause the transformation of minor compounds in balsam gurjun oil including α-cubebene, α-gurjunene, aromandendrene, γ-muurolene, and 1H-Cyclopropa- [a]-naphthalene into α-copaene compounds. This assumption is strengthened

by a study that the carbon skeleton of minor compounds in gurjun balsam oil is a substrate for *A. niger* [14]. At the incubation time of 48 hours, the β-cubebene compound transformed into α-copaene (Figure 3). The presumed reaction mechanism that occurs is a rearrangement reaction that causes a change in the carbon framework [15]. The study of the biotransformation reaction mechanism needs to be carried out molecular analysis with other instruments.

The copaene in gurjun balsam oil, according to data pass online prediction, it has Pa (probability active) antiaczematic with value 0.74, it means copaene have strong potential as antieczematic in skin problem. Eczematic is a condition wherein patches of skin become inflamed, itchy, cracked, and rough. The copaene in gurjun oil is mighty able to moisturize the skin and better treat skin with eczema problems [16]. The copaene in for applying topical administration, according to data pkCSM online, it has low skin permeability absorption with the value of log Kp is -2.194. Copaene has a local effect, provides a moisturizing and occlusive effect to the skin and does not need to pass through the circulating blood system. Skin permeability absorption is a significant consideration for many compounds of interest for the development of the transdermal drug delivery system. The pkCSM predictor was built using 211 compounds whose in vitro human skin permeability has been measured. A compound is considered to have a relatively good skin permeability if it has log Kp >-2.5 [13].

The copaene in Gurjun oil has been predicted non-mutagenic and non-skin sensitization in the topical application based on data AMES toxicity. AMES toxicity test is a widely employed method to assess a compound’s mutagenic potential using bacteria. A positive test indicates that the compound is mutagenic and therefore may act as a carcinogen. The pkCSM model was built on the result of over 8000 compounds AMES test [13].

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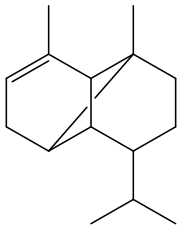


Figure 1: The structure of the α-copaene (https://pubchem.ncbi.nlm.nih.gov/)

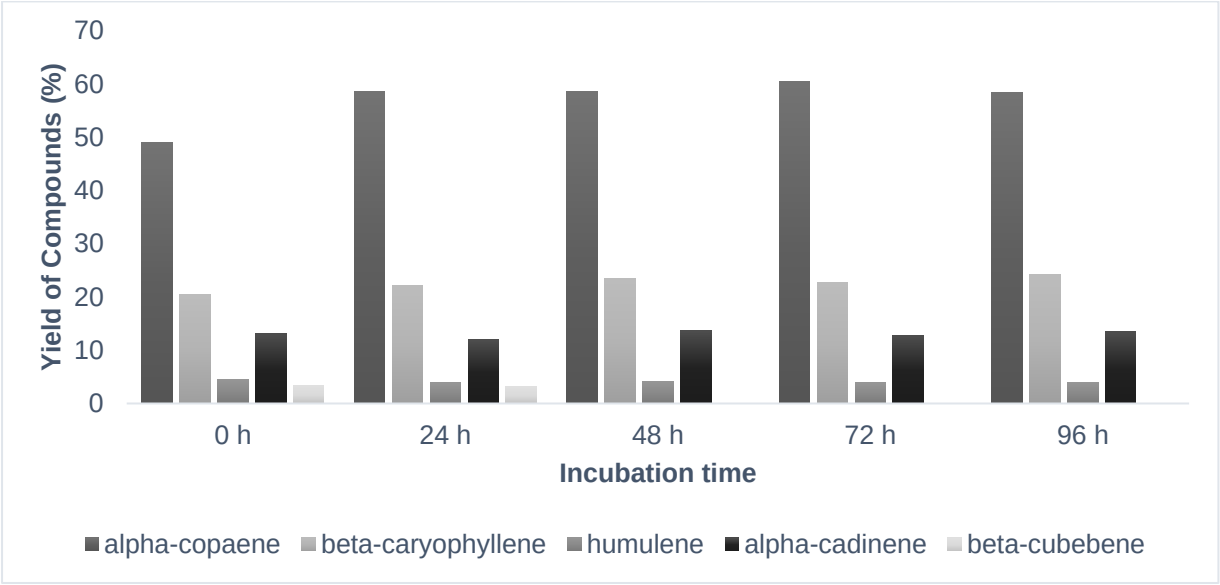


Figure 2: Yield (%) of the main metabolites produced from biotransformation gurjun balsam oil by *A. niger*.

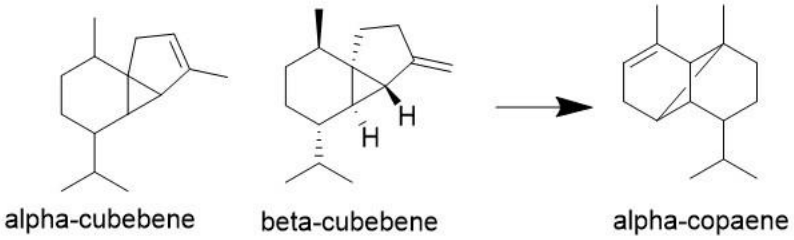
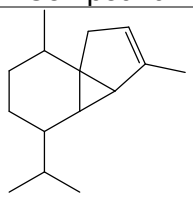
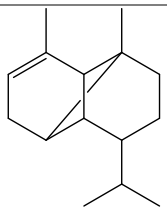
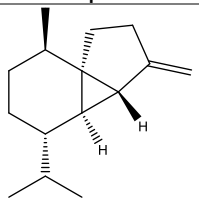
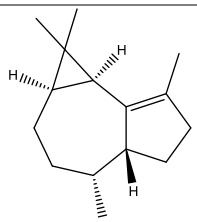
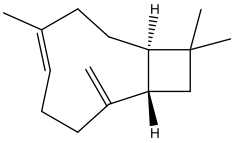
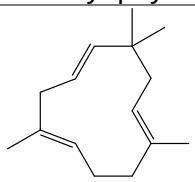
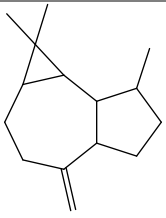
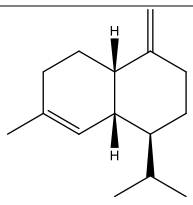


Figure 3: Tranformation alpha-cubebene and beta-cubebene to alpha-copaene

Table 1: The content of compounds in gurjun balsam oil

Peak	Compound	SI	Time retention (min)	Yield (%)
1	 α-cubebene	99	11,705	1,537
2	 α-copaene	99	12,089	49,049
3	 Beta-cubebene	98	12,252	3,400
4	 Alpha-gurjunene	99	12,529	1,207
5	 Beta-Caryophyllene	99	12,669	20,460
6	 Humulene	97	13,096	4,576
7	 aromandendrene	99	13,191	1,801
8	 γ-muurolene	99	13,355	2,156

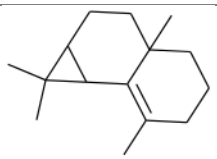
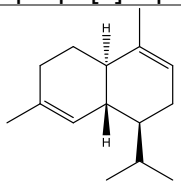
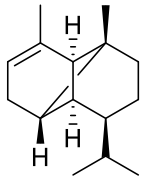
9	 1H-Cyclopropa[a]naphthalene	98	13,632	2,766
10	 Alpha-cadinene	95	13,942	13,06

Table 2: ADMET prediction of copaene in gurjun balsam oil

Compound	probability active as antieczematic	skin permeability absorption (log Kp)	AMES toxicity	skin sensitization
 Copaene	0.74	-2.194	Negative	No