Study of composition chemical compounds methanol extract of Tambelo (Bactronophorus thoracites) Mimika Regency

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Abstract. This study aims to analyze bioactive compounds found in Tambelo maceration extracts in Mimika Regency. In this study, HPLC amino acid and GC-MS analysis were used. The results of the analysis show that Tambelo extract contains 16 types of amino acids and eight types of fatty acids. Amino acids and fatty acids function as energy sources, regulation of metabolism. Some types of antioxidant amino acids can reduce or counteract the negative effects of oxidant hydrogen ions by donating one electron. Based on the results of the study it can be concluded that the highest composition of HPLC amino acid analysis results is L-alanine (3.04%) while the highest composition results of GC-MS analysis are palmitic fatty acids (12.67%) included in the group of saturated fatty acids.

1. Introduction

Some marine organisms are able to produce chemical compounds to defend themselves from attack by predators. Many of the chemical compounds produced have the potential to inhibit bacterial growth and actively inhibit cancer cell growth and another bioactivity [1]. Marine organisms that have been used as natural medicinal ingredients include sponges, seaweed, sea worms, sea cucumbers, and mollusks. One of the mollusks that have been used besides as food is also used as natural medicine is Tambelo. Tambelo is one type of marine invertebrate that is included in the mollusk phylum. Tambelo is known as shipworm and lives on mangrove trees that have decayed. Nateewathana explains the physical characteristics of Tambelo, including soft, white and elongated like a worm, on the head, there is a file or scar that is useful for punching holes in wood [2]. Holes in wood are usually made perpendicular to wood fibers or turned parallel to the direction of wood fibers. The wall of the hole in the wood is coated with a substance containing lime.

The Kamoro tribe community in Papua Mimika Regency calls Tambelo "koo" and believes that Tambelo can cure diseases including lumbago, rheumatism, coughing, flu, malaria, increasing breast milk production, appetite, and male vitality [3]. In addition, bacteria isolated from Tambelo gills Neo teredo reynei (Teredinidae) can inhibit Gram-positive and Gram-negative bacteria (Sphingomonas, Stenotrophomonas maltophilia, Bacillus cereus, and Staphylococcus sciuri). As well as in a study it is
reported that the protease enzyme isolated from bacteria found in the glands of Tambelo Psiloteredo healdi (Teredinidae) is a detergent for cleaning floors, plates, and glass lenses, so that these organisms have potential [4,5]. At present the study of the composition of bioactive compounds from Tambelo found in Mimika regency is not widely known, therefore the study aims to analyze the bioactive compounds contained in Tambelo.

2. Methodology

2.1. Time and place
This research was conducted from January 2016 to April 2017. The Tambelo sample was taken from Mimika Regency, Papua. The process of methanol maceration extract was carried out in the SITH ITB Animal Physiology laboratory and PAU ITB. HPLC analysis of amino acids was carried out at the UGM Chemistry Laboratory and GC-MS analysis was carried out at the National Police Headquarters PUSLABFOR.

2.2. Materials and instrument
Tambelo (Bactronophorus thoracites), methanol (MeOH) solvent, aquadest, 30% KOH solution. Coolbox for preparation of Tambelo (Bactronophorus thoracites), macerator, rotary evaporator, mortar, freezer, centrifugation, measuring cup (5 ml, 100 ml, and 1L).

2.3. Sample Preparation
Tambelo is obtained from the Bruguera sp. mangrove tree trunk and Avicenna sp. which has decayed in the mangrove area of Timika Pomako, Papua. Tambelo was taken and then stored in a coolbox containing ice cubes for further extraction of methanol.

2.4. Process of extracting Tambelo samples
1500 g of fresh Tambelo are taken and cleaned by removing the teeth and pallet so that only the commonly consumed part is obtained, the abdomen. After cleaning the weight decreases to 900 g. Tambelo which has been cleaned then in freeze-dry with a temperature of -50 ° C. After freeze-drying the Tambelo then was crushed with mortar then weighed. Tambelo has a weight loss of 400 g or about 20% of the weight before freeze-drying. 400 g of Tambelo were macerated using methanol (MeOH) solvent with a ratio of 1: 5 (400: 2000ml) at room temperature for 3x24 hours with filtration carried out every 1x24 hours. The resulting filtrate is combined and concentrated with a rotary evaporator. Concentrated extracts were obtained by GC-MS analysis and HPLC analysis of amino acids to determine chemical composition. The amino acid composisition of each material calculated with the following formula [6]:

\[
\text{Amino Acid (\%) = \frac{\text{Sample Peak Area}}{\text{Standard Peak Area}} \times C \times FB \times BM \times 100}{\text{Sample Weight (\mu g)}}
\]

Information:
- C = Standard amino acid concentration (2.5 µg)
- FB = Dilution factor (133.1 mL)
- BM = Molecular weight of each amino acid

3. Results and discussion

3.1. Results of analysis of HPLC amino acids
Based on the results of HPLC analysis of amino acids from the Tambelo extract, it is known that there are 16 types of amino acids consisting of eight types of essential amino acids and eight types of amino acids without essential. The results of the HPLC amino acid analysis of Tambelo methanol extract are shown in table 2.
In this study, alanine is thought to play a role in the regulation of glucose use and shows the effect of red bloodstream and then passed into muscle cells to be used as an energy source during physical activity. Alanine contained in muscle cells is converted to pyruvate and NH3. Pyruvate is converted into glucose into the bloodstream and then passed into the liver, alanine is converted to pyruvate and NH3. Pyruvate is converted into glucose into the bloodstream and then passed into muscle cells to be used as an energy source during physical activity.

HPLC (High-Performance Liquid Chromatography) is one method that is often used to analyze amino acids. HPLC is one of the techniques used to separate mixtures of compounds in both chemical and biochemical chemistry. This analysis aims to identify, measure and even purify individual components of the mixture. The results of the amino acid HPLC analysis of Tambelo extract are known to contain eight types of essential amino acids and eight types of non-essential amino acids. The results of HPLC analysis of amino acids from Tambelo extract were found to be alanine as the highest amino acid (Table 1). Alanine is a non-essential amino acid and is the most widely used amino acid and is involved in the metabolism of tryptophan and vitamin pyridoxine acts as the main energy source for muscles and helps in the metabolism of sugars and organic acids. Alanine serves as an important energy source for the muscles and central nervous system, strengthening the immune system, helps metabolize sugars and organic acids and shows the effect of reducing cholesterol in animals [4]. Amino acids in amphoteric solutions can react with acid-base depending on the environment. The functions of amino acids include compilers of proteins, including enzymes, the basic framework of a number of important compounds in metabolism (especially vitamins, hormones, and nucleic acids), and important metal binders which are needed in enzymatic reactions (cofactors) [6]. The results of HPLC analysis of amino acids from Tambelo extract revealed that alanine is an amino acid with the highest percentage. Alanine is a non-essential amino acid and is the most widely used amino acid and is involved in the metabolism of tryptophan and vitamin pyridoxine acts as the main energy source for muscles and helps in the metabolism of sugars and organic acids. Alanine serves as an important energy source for the muscles and central nervous system, strengthens the immune system, helps metabolize sugars and organic acids and shows the effect of reducing cholesterol in animals [7]. Some studies suggest that the amino acids most commonly found in marine mollusks are glutamic acid, aspartic acid, glycine, alanine, and taurine. The high amino acid alanine which is a non-essential amino acid serves to help the body convert glucose into energy and eliminate excess toxins [8].

In this study, alanine is thought to play a role in the regulation of glucose used as an energy source when carried out physical activity. Alanine contained in muscle cells is passed into the blood and liver. In the liver, alanine is converted to pyruvate and NH3. Pyruvate is converted into glucose into the bloodstream and then passed into muscle cells to be used as an energy source during physical activity.

### Table 1. Results of Analysis of HPLC Amino Acid Tambelo methanol extract

| Mean       | Result from HPLC (ppm) | After calculation (%) |
|------------|-------------------------|-----------------------|
|            | Rep1 | Rep1 | Mean | Rep1 | Rep1 | Mean |
| L-aspartic acid | 6.227 | 7.276 | 6.7515 | 0.12 | 0.15 | 0.14 |
| L-Glutamic acid | 26.188 | 25.886 | 26.037 | 0.52 | 0.52 | 0.52 |
| L-Aspargine | NDD | NDD | NDD | <0.04ug/ml | <0.04ug/ml | <0.04ug/ml |
| L-Histidine | 2.977 | 3.137 | 3.057 | 0.06 | 0.06 | 0.06 |
| L-Arginine | 8.914 | 9.837 | 9.3755 | 0.18 | 0.20 | 0.19 |
| L-Glutamine | NDD | NDD | NDD | <0.05ug/ml | <0.05ug/ml | <0.05ug/ml |
| L-Glycine+L-Arginine | 32.342 | 33.112 | 32.727 | 0.65 | 0.66 | 0.65 |
| L-Threonine | 22.072 | 22.0185 | 22.0185 | 0.44 | 0.44 | 0.44 |
| L-Alanine | 15.048 | 15.321 | 15.1845 | 3.01 | 3.06 | 3.04 |
| L-Tyrosine | NDD | NDD | NDD | <0.05ug/ml | <0.05ug/ml | <0.05ug/ml |
| L-Thryptophan+L-Methionin | NDD | NDD | NDD | <0.13ug/ml | <0.13ug/ml | <0.13ug/ml |
| L-Valine | 17.851 | 16.128 | 16.9895 | 0.36 | 0.32 | 0.34 |
| L-Phenylalanine | 3.894 | 4.293 | 4.0935 | 0.08 | 0.09 | 0.08 |
| L-Isolucine | 12.423 | 13.007 | 12.715 | 0.25 | 0.26 | 0.25 |
| L-Leucine | 12.431 | 11.37 | 11.9005 | 0.25 | 0.23 | 0.24 |
| L-Lysine | 10.809 | 11.935 | 11.372 | 0.22 | 0.24 | 0.23 |

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This cycle is called the glucose-alanine cycle. The solvent used for extracting the sample in this study was 100% methanol. Methanol is a type of polar compound solvent. According to [3] methanol is the most effective solvent in the extraction process because it produces the highest yield compared to other types of solvents.

3.2. GC-MS analysis results
Tambelo from Mimika Regency contains eight types of fatty acids, which consist of four saturated fatty acids and four unsaturated fatty acids. The results of fatty acid content are shown in table 2.

![Figure 1. Results of GC-MS analysis of Tambelo Methanol Extract](image)

**Table 2. Results of GC-MS analysis of Tambelo methanol extract**

| No | Fatty acid              | (%)  |
|----|-------------------------|------|
| A  | Saturated Fatty Acid    |      |
| 1  | Palmitate               | 12.67|
| 2  | Miristat                | 4.5  |
| 3  | Arakidat                | 0.19 |
| 4  | Pentadecanoate          | 0.63 |
| B  | Unsaturated Fatty Acid  |      |
| 5  | Oleic                   | 6.8  |
| 6  | Linoleat                | 0.15 |
| 7  | Linoleate               | 0.24 |
| 8  | Aracidonate             | 0.33 |

GC-MS analysis is a method of separating organic compounds using two methods of compound analysis, namely gas chromatography (GC) to quantify the amount of compound quantitatively and mass spectrometry (MS) to analyze the molecular structure of the analyte. Table 2 shows the total fatty acid content of 25.51%. Palmitic fatty acids are the highest saturated fatty acids of 12.67%. While the highest
unsaturated fatty acid is Oleat at 6.8%. This is different from the research conducted by Leiwakabessy (2011) which explained that Tambelo extract analyzed there were 15 types of fatty acids [4]. This was influenced by the type of solvent used in the extraction process in this research was n-hexane which is a non-polar solvent and environment from Tambelo origin [6]. High oleic acid content can help in the growth process, besides, it can maintain skin health, especially in preventing the occurrence of skin inflammation/dermatitis [9].

4. Conclusion
The highest essential amino acid content in Tambelo from Mimika regency is L-Threonine (0.44%), L-Valine (0.34%) and, L-Isoleucine (0.25%) and the lowest is histidine (0.06%). The highest non-essential amino acid was L-Alanine (3.04%) followed by a mixture of L-glycine and L-Arganin (0.65%) and the lowest L-Aspartate 0.14%. The total fatty acid content of Tambelo is 25.51%. The highest saturated fatty acid is palmitic acid (12.67%). The highest monounsaturated fatty acids are oleic acid (6.8%) and arachidonic acid (0.33%).

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