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The effect of balinese mangosteen (*Garcinia mangostana* L.) wine on ethanol content in the blood serum of wistar rats

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Abstract
Balinese wine (brem) of mangosteen fruit flesh and peel contain ethanol which is formed during the fermentation process. The increase in ethanol content occurred due to the fermentation process which continued during the storage process, while the decrease was due to oxidation and evaporation processes. Ethanol content in the blood determine the speed of ethanol metabolism in the liver which produces acetaldehyde, increased NADH/ADH, and free radicals. Acetaldehyde deposits in the liver can cause liver damage. Flame ionization detector gas chromatography is an appropriate method for determining volatile ethanol content and has high accuracy when compared to other analytical methods. Based on the results of a study conducted by brem fruit flesh and peel of mangosteen had an alcohol content of 11.30 ± 0.04 and 11.34 ± 0.04 % (v/v). Ethanol content in the blood serum of Wistar rats increased with increasing doses of brem mangosteen fruit flesh and peel given to Wistar rats for 28 days.

Keywords: Alcohol content, liver disease, balinese mangosteen wine

Introduction

Balinese wine called brem is a fermented alcoholic drink which is currently not only made from glutinous rice, but it also made from various types of fruit such as mangosteen, grapes, and cocoa [1]. Brem fruit flesh and peel of mangosteen as local food in Sidatapa Village are considered capable of forming a tourism village which has superior tourism products [2].

The alcohol content in the form of ethanol in brem varies based on its treatment during the storage process. Furthermore, the continuous fermentation process can cause an increase in ethanol content, while a decrease in ethanol content occurs due to evaporation and oxidation processes. Ethanol oxidation reactions are formed due to aerobic conditions which occur during storage time [3]. According to Regulation of the Head of Food and Drug Supervisory Agency of the Republic Indonesia No. 14 of 2016 concerning safety and quality standards for alcoholic beverages, the ethanol content in good liquid brem is not less than 7% v/v and it is not more than 24% v/v while the methanol content is not more than 0.01% v/v.

Determination of the ethanol content in brem can be conducted by the gas chromatography method with a flame ionization detector (GC-FID) since it has high accuracy and it is volatile. Ethanol is a fat-soluble non-electrolyte liquid [4]. Ethanol easily circulates in the blood circulation and it is absorbed in the digestive tract. As much as 80% of the ethanol consumed will be distributed to the intestine while the remaining 20% is in the stomach. Furthermore, ethanol will undergo metabolism in the liver. Ethanol content in the blood determine the speed of metabolism in the liver which produces acetaldehyde, free radicals and an increase in NADH/ADH. In addition, acetaldehyde which accumulates in the liver can cause liver damage [5]. Therefore, it is necessary to conduct research regarding the ethanol content contained in brem made from fruit flesh and peel of mangosteen as well as the ethanol content in the blood after consumption of brem from fruit flesh and peel of mangosteen for 28 consecutive days which is tested on Wistar rats.

Materials and Methods

Ingredient and equipment

The materials used are brem fruit flesh and peel of mangosteen from Sidatapa Village, Banjar District, Buleleng Regency, Bali. Pro-analysis alcohol standards (p.a) are methanol, ethanol, and butanol, as well as Wistar rat blood serum. Meanwhile, the tools used include a microtube, syringe, centrifuge, a set of glassware and a set of gas chromatography tools for a flame ionization detector (GC Agilent Technologies 6890 N Network GC System) with an HP INNOWAX column 250 µm wide, 30 m long, and 0.15 µm diameter.

Treatment of Wistar rats

The test animal was a Wistar rat weighing 100-200 g, aged 3 months. Wistar rats were adapted for 7 days by giving drinking water ad libitum and standard rat food. The test animals were divided into 7 groups with 3 rats each as follows: P0 was the positive control group (33.31% of wine was given); PI, PII, and PIII were the treatment groups with the administration of mangosteen flesh brem orally at a dose of 0.5; 1.0, and 2.0 mL; and PIV, PV, PVI were the treatment groups with the administration of mangosteen peel brem orally at a dose of 0.5; 1.0, and 2.0 mL.

Mangosteen brem was administered orally by using a feeding tube for 28 days by administering mangosteen fruit flesh and peel brem once a day according to the treatment dose. On the 29th day, 3 cc of rat blood was collected from the retroorbital plexus by using a capillary tube in order to determine the effect of administering mangosteen fruit flesh brem and mangosteen peel brem on alcohol content in the blood of Wistar rats.

Determination of alcohol content in samples of mangosteen flesh brem, mangosteen peel brem and blood serum of Wistar rats

A total of 3 cc of Wistar rat blood which had been taken from the retroorbital plexus, was centrifuged at 3000 rpm in order to obtain blood serum. Samples in the form of mangosteen fruit flesh and peel brem as well as blood serum of Wistar rats were diluted 10 times by using a 100 µL pipette, the sample was put into a microtube and then diluted to a volume of 1000 µL. Furthermore, the diluted sample was pipetted 100 µL into a microtube, added 100 µL of 500 mg/L butanol standard solution and diluted with distilled water in order to reach a volume of 1000 µL. The sample was injected as much as 1.0 µL on the gas chromatography of the flame ionization detector. The alcohol content of the sample can be known from the area formed at a certain retention time compared to the standard solution used.

Data analysis

Data on alcohol content in mangosteen fruit flesh and peel brem as well as blood serum of Wistar rats were statistically analyzed by using the SPSS version 25 program with the One Way Analysis of Variance (ANOVA) method with an α level of 0.05, followed by the Bonferroni method in order to determine differences in the effect between treatment group.

Results and Discussion

Based on the research which had been conducted, the ethanol content in the mangosteen flesh brem used in the study is 11.30 ± 0.04%. Meanwhile, the average ethanol content in mangosteen peel brem is 11.34 ± 0.04%.

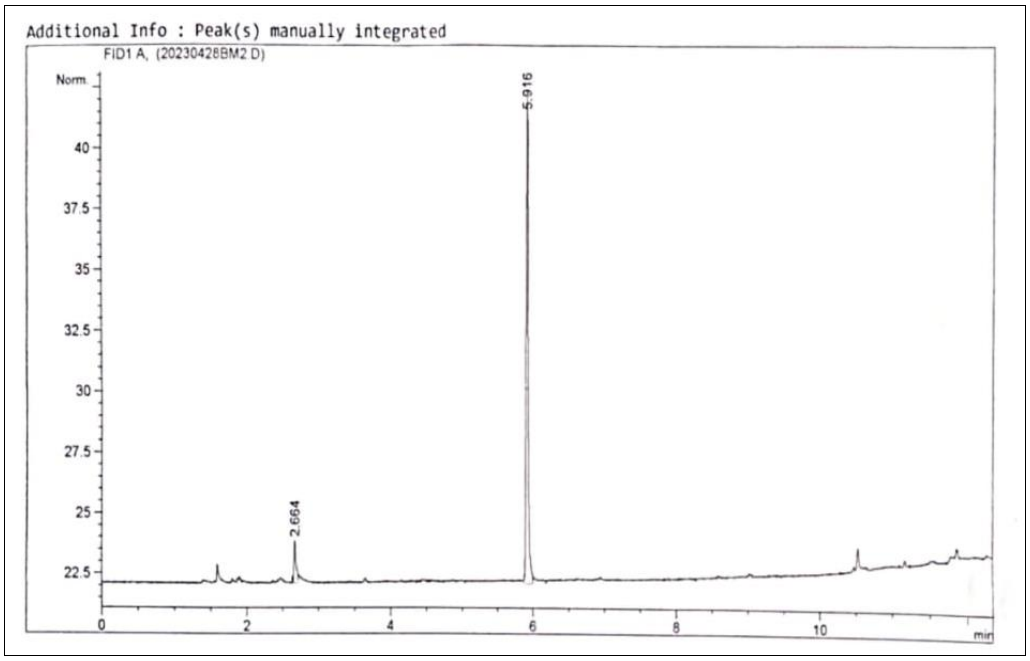

Fig 1: Chromatogram of Mangosteen Brem

From the chromatogram obtained, there are two peaks at retention times of 2.6 and 4.0 minutes. The retention time of 2.6 minutes is the retention time for ethanol type alcohol while the retention time of 4.0 minutes detects a peak from the standard butanol used in this study. Therefore, it shows that mangosteen flesh brem and mangosteen peel brem only contain ethanol type alcohol.

Mangosteen flesh brem and mangosteen peel brem have the same ethanol content of 11.3% since they contain the same amount of carbohydrates. Based on Nurkhasana (2013), at least one mangosteen fruit (196 grams) contains 35 grams of carbohydrates [6]. Meanwhile, according to Wihartopo (2010), each mangosteen fruit has peel which contains at least 35.6 grams of carbohydrates [7]. Carbohydrates in the mangosteen flesh and mangosteen peel are broken down into simple sugars through the hydrolysis of starch into glucose units. In addition, fermentation process occurs with the addition of Saccharomyces cerevisiae which has the enzyme zymase which accelerates the breakdown of glucose into ethanol and CO_2 [8].

Based on the category of liquor listed in the Regulation of the Minister of Health of the Republic of Indonesia No. 86/Menkes/Per/IV/77, the ethanol content is 11.30 and 11.34% and it does not contain methanol. Mangosteen flesh brem and mangosteen peel brem are classified as group B liquor since it has ethanol content between 5-20%. Group B liquor can cause loss of consciousness if consumed in large quantities. In addition, continuous consumption can cause blurred vision, convulsions, and inflammation of the liver [9].

According to Regulation of the Head of Indonesian National Agency of Drug and Food Control No. 14 of 2016 concerning Safety and Quality Standards for Alcoholic Beverages, the ethanol content in fermented drinks is traditionally good that is at contents of 7-24%, and it has a methanol content of no more than 0.01% (v/v) [10]. In addition, the results of the study shows that mangosteen flesh brem and mangosteen peel brem met the safety and quality standards of alcoholic beverages according to the Head of Indonesian National Agency of Drug and Food Control No. 14 of 2016 so that it is suitable for consumption and selling.

Based on the results of the research which had been conducted, the ethanol levels in the blood serum of Wistar rats are shown in Table 1.

Table 1: Ethanol Content in Blood Serum of Wistar Rats

Sample code	Treatment	Ethanol Content (%v/v)
P_0	Wine 33,31%; 1,0 mL	0,34 ± 0,06
P_I	Mangosteen flesh brem 11,30%; 0,5 mL	0,31 ± 0,01
P_{II}	Mangosteen flesh brem 11,30%; 1,0 mL	0,57 ± 0,09
P_{III}	Mangosteen flesh brem 11,30%; 2,0 mL	0,84 ± 0,09
P_{IV}	Mangosteen peel brem 11,34%; 0,5 mL	0,34 ± 0,05
P_V	Mangosteen peel brem 11,34%; 1,0 mL	0,39 ± 0,06
P_{VI}	Mangosteen peel brem 11,34%; 2,0 mL	0,71 ± 0,04

Note: Average ethanol content ± standard deviation; notation (*) in each treatment indicates a significant difference with the positive control (P <0.05)

These results show that the blood serum ethanol contents of Wistar rats induced by mangosteen flesh and mangosteen peel for 28 days increased with increasing doses of mangosteen brem given.

The statistical test was conducted by using a one-way ANOVA test with a 95% confidence level. From the results of data analysis with SPSS 25, it shows that the data is normally distributed and homogeneous (Sig > 0.05). Furthermore, the one-way ANOVA test shows a significance value of not more than 0.05 so that it means that there are significant differences between each group in the ethanol content of mangosteen flesh brem and mangosteen peel brem. Follow-up tests were conducted by using the Post Hoc test with the Bonferroni method. From the results obtained, it shows that each treatment group has the same notation so that there is no significant difference in each treatment.

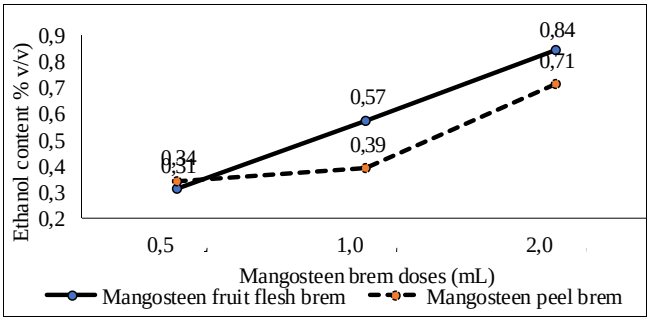

Fig 2: Blood Serum Ethanol Contents of Wistar Rats Fed Mangosteen Fruit Flesh and Peel Brem

When there is an increase in ethanol consumption, excess ethanol will be excreted from the liver as an organ of alcohol metabolism and circulates in the blood vessels so that ethanol can be detected at certain levels in the blood. Moreover, research which had been conducted by Marchitti, *et al* (2010) stated that the greater the volume of ethanol given to the test animals, namely Wistar rats, the faster the absorption and metabolism of ethanol occurs in the rats' bodies [11]. This results in the complete reaction of ethanol to acetaldehyde. Acetaldehyde is a product of ethanol metabolism which is toxic to the body. High content of ethanol cause more acetaldehyde to be produced by the body so that the possibility of liver damage will be higher which is characterized by damage to liver cells as the center of alcohol metabolism.

Conclusion

The ethanol content in the mangosteen flesh brem is 11.30% (v/v) while in the mangosteen peel brem is 11.34% (v/v). Both of them still meet the quality standard requirements for fermented alcoholic beverages from the Regulation of the Head of Indonesian National Agency of Drug and Food Control No. 14 of 2016. The value of ethanol content in the blood serum of Wistar rats induced by mangosteen flesh brem and mangosteen peel brem increased with increasing doses of brem given for 28 consecutive days.

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