

## PROFILING AND GROUPING OF FLAVONOID COMPOUND USING LCMS FROM A COMBINATION OF WHITE TURMERIC EXTRACT AND HONEY AND ITS BIOACTIVITY TEST IN OBESE RATS

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### ABSTRACT

**Objective:** The aim of this study was to identify and classify flavonoid compounds from a combination of white turmeric (*Curcuma zedoaria*) extract and honey, which have the potential as anti-obesity and anti-diabetic type 2 agents in obese Wistar rats.

**Methods:** This study used 25 rats divided into 5 groups, namely the normal, the obese, the obese group plus 100 mg/kg bw white turmeric extract, the obese group plus 100 mg/kg bw honey, and the obese group plus the combination of white turmeric and honey. The treatment was carried out for 4 w and the parameters measured were body weight, free fatty acid, glucose, and serum triglycerides levels of rats. Identification of flavonoid compounds using Liquid Chromatography-Mass Spectrometry (LC-MS).

**Results:** The results showed that the combination of white turmeric extract and honey was able to reduce the weight of rats (Lee's obesity index), free fatty acid (FFA), glucose and triglycerides (TG) levels with significant differences ( $p < 0.05$ ) from the obese rats. The average decrease in glucose, triglyceride, free fatty acid, and Lee's obesity index levels was 66.12%, 74.31%, 42.57%, and 20.59%, respectively. The results of LC-MS analysis identified 33 flavonoid compounds from white turmeric and 15 flavonoids from honey, which are thought to play an active role in reducing weight, free fatty acids, glucose and triglycerides.

**Conclusion:** The results of this study stated that there were 48 flavonoid compounds identified from the combination of white turmeric extract (*Curcuma zedoaria*) and honey which have the potential as anti-obesity and anti-diabetic type 2 in obese wistar rats.

**Keywords:** White turmeric (*Curcuma zedoaria*), Honey, Glucose, Obesity, Triglycerides, Free fatty acids

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### INTRODUCTION

Obesity and type 2 diabetes mellitus are two metabolic conditions that are interrelated and are a global health challenge with increasing prevalence. Obesity contributes greatly to insulin resistance, which is the main trigger for the development of type 2 diabetes. According to the World Health Organization (WHO), more than 890 million adults are obese, and it is estimated that the number of people with type 2 diabetes will continue to increase significantly in the future. Efforts to control these two diseases generally involve pharmacological treatment, but long-term use is often accompanied by adverse side effects and limited effectiveness [1-4].

As awareness of the importance of natural and safe approaches in the management of chronic diseases increases, the use of natural ingredients such as medicinal plants and bee products has begun to gain widespread attention. The use of natural ingredients as an alternative or complement to conventional therapy continues to receive attention, especially because of the long-term side effects of some synthetic drugs. One group of bioactive compounds that has been shown to have various pharmacological benefits is flavonoids. Flavonoids are known to have antioxidant and anti-inflammatory activities and play a role in modulating glucose lipid metabolism [5-8].

One of the plants known to have pharmacological properties is white turmeric (*Curcuma zedoaria*). White turmeric contains bioactive compounds, including flavonoids, curcuminoids, and sesquiterpenes, which have been shown to have anti-inflammatory, antioxidant, hepatoprotective activities and potential as anti-obesity and anti-diabetic agents. These mechanisms include inhibition of the lipase enzyme, reduced fat accumulation, and increased insulin sensitivity [9-14].

Meanwhile, bee honey is known as a natural product rich in phenolic and flavonoid compounds such as quercetin, kaempferol, and apigenin. Honey also has antioxidant and antidiabetic properties through the mechanism of reducing oxidative stress, lowering blood glucose, and increasing glucose tolerance. The combination of white turmeric herbal extract and honey is believed to provide a synergistic effect in managing metabolic disorders, including obesity and type 2 diabetes [15-18].

Although the therapeutic potential of white turmeric and honey has been known separately, scientific studies on the combination of both are still limited, especially in the context of identifying flavonoids that play a role in anti-obesity and antidiabetic effects. Therefore, this study aims to identify flavonoid compounds using LC-MS from a combination of white turmeric extract and honey and evaluate its potential in reducing body weight, glucose, triglycerides, and free fatty acids levels in obese wistar rat animal models. By using obese wistar rat models as a representation of metabolic syndrome in humans, the results of this study are expected to provide a scientific basis for the development of effective and safe natural phytopharmaceutical candidates for the prevention and management of obesity and type 2 diabetes.

### MATERIALS AND METHODS

#### Materials of research

The materials used in this study were white turmeric rhizome (*Curcuma zedoaria* Rosc.) taken from Batubulan Gianyar, Bali, 96% methanol, distilled water, 25 female Wistar rats were taken from the animal laboratory of the Faculty of Veterinary Medicine, Udayana University, with the approval of the organization of Animal Ethics

Committees with Number: B/203/UN14.2.9/PT.01.04/2024, high-fat feed [3], bee honey from Kupang, standard feed CP 550, pp indicator.

## Methods

### Preparation of white turmeric rhizomes

White turmeric plants were taken from Batubulan, Gianyar, Bali, in May-June and determined by Eka Karya Bali LIPI. Preparation of white turmeric rhizome was carried out using the method [11].

### White turmeric rhizome extraction

One kg of fine powder (100 mesh) was placed in a 3-liter beaker, then 2.5 l of methanol solvent was added (1: 2.5) and left to soak for 24 h at 25 °C. The maceration process was repeated 3 x 24 h, then filtered. The filtrate was combined, concentrated with an Evaporator and continued with LCMS identification and then *in vivo* biological activity tests were carried out using Wistar rats, according to the method [11, 19].

### Combination treatment of white turmeric extract and honey on obese rats

The minimum number of rats used in this study was 25 rats that were adapted for one week so that they could adjust to the new environment. During the adaptation stage, body weight was measured and standard feed was given uniformly. Twenty five of rats were divided into 5 groups and placed in cages with room temperature according to the treatment group. A total of 5 rats as a normal control group were given standard feed, while the other 20 rats were given high-fat feed until obese (IOL>0.3). All the experimental works with the animal were carried out after obtaining approval from the organization of Animal Ethics Committees with Number: B/203/UN14.2.9/PT.01.04/2024.

$$\text{Indeks Obesity Lee (IOL)} = \frac{\sqrt{\text{Berat badan (gram)} \times 10}}{\text{Panjang Nasoanal (mm)}}$$

The division of groups based on treatment is as follows according to the method of [6]:

#### Group I (normal control)

Female Wistar rats in this group were given standard feed. Every week the weight and nasoanal length of the rats were measured for 4 w.

#### Group II (control obese rats with high-fat diet)

Female Wistar rats in this group were fed high-fat diet and drinking water ad libitum. Every week the weight and nasoanal length of the rats were measured for 4 w.

#### Group III (obese rat group with 100 mg/kg bw white turmeric extract)

Obese female Wistar rats in this group were fed high-fat feed and ad libitum drinking water and were given white turmeric extract at a dose of 100 mg/kg by means of a probe. Every week the weight and nasoanal length of the rats were measured for 4 w.

#### Group IV (obese rat group with 100 mg/kg bw honey)

Female obese Wistar rats in this group were fed high-fat feed and ad libitum drinking water and given honey at a dose of 100 mg/kg by means of a probe. Every week the weight and nasoanal length of the rats were measured for 4 w.

#### Group V (Group of obese rats with a combination of white turmeric and honey) 100 mg/kg bw.

Female obese Wistar rats in this group were given high-fat feed and ad libitum drinking water and were given a combination of white turmeric extract and honey at a dose of 100 mg/kg by means of a probe. Every week the weight and nasoanal length of the rats were measured for 4 w.

After 4 w of treatment, the rats blood was taken from the eye orbital, then centrifuged at 1000 rpm. The serum obtained is then used in

determining glucose, free fatty acids, and triglyceride levels. In accordance with the method of [20].

### Determining blood glucose in rat

Glucose levels were assessed using a glucometer, where a drop of blood was applied to the test strip and allowed to process until the value appeared on the device. In mice, fasting blood glucose typically ranges between 50–125 mg/dl, and values exceeding 125 mg/dl indicate a diabetic state.

### Determining serum triglyceride in rat

Serum triglycerides were quantified using the ERBA XL-100 chemistry analyzer with Sekisui Medical Triglyceride Kit reagents (Lot No. R1: 105R10; R2: 105R10). For the control assay, 260 µl\*\* of Reagent R1 and 130 µl\*\* of Reagent R2 were dispensed into the analyzer according to the designated reagent tray sequence. A 3.0 µl\*\* aliquot of control and test sample was then introduced, with initial absorbance monitored between 600–700 nm. Subsequent sample analysis was carried out by adding 3.0 µl\*\* of serum, and the absorbance was recorded at 546 nm to determine triglyceride concentrations.

### Analysis of serum free fatty acid in rat

Free fatty acids were quantified through alkalimetric titration following lipid extraction by the Folch method. In this procedure, 200 µl\*\* of serum was mixed with 10 ml of a methanol–chloroform solution (1:2, v/v) to extract lipids, after which water was added to induce phase separation. The chloroform fraction, containing the lipid components, was isolated, and phenolphthalein indicator (2–3 drops) was introduced. The sample was then titrated with 0.01 N NaOH until a pale pink endpoint was reached. The NaOH volume consumed was recorded and used to calculate the free fatty acid concentration in mmol/l.

### Analysis of white turmeric rhizome extract and honey with LCMS method

Analysis of white turmeric and honey extracts was performed using Liquid Chromatography-Mass Spectrometry (LC-MS) using a Shimadzu LCMS-8040 IC-MS instrument. 1 µl\*\* of the solution was transferred into a 2 ml vial and injected into the LC-MS-8040 system. The mobile phase was methanol, acetonitrile, and water in a ratio of 40:15:45 (v/v/v), respectively. The mobile phase flow rate was 0.5 ml/min, with an analysis time of 60 min, a capillary temperature of 350 °C, and a gas atomizer of 60 ml/h, and a voltage source of 5.0 V. The stationary phase used was a Shimadzu Shim Pack FC-ODS column (2 mm x 150 mm, 3 µm) with positive ionization mode. The full scan m/z range was 10–1000 [21].

### Data analysis

The research data were analyzed statistically using the Analysis of Variance (ANOVA) method with the SPSS software (version 30 for Windows), applying a 95% confidence level. The statistical analysis was conducted in the following stages:

a) The Shapiro-Wilk test was used to assess the normality of the data (n = 30). A p-value greater than 0.05 indicated that the data were normally distributed.

b) Levene's Test was used to evaluate the homogeneity of variances between groups. Homogeneity was confirmed when the p-value exceeded 0.05

c) Continued with Post Hoc tests, namely the Tukey test if homogeneous and the Tamhan test if not homogeneous [6].

## RESULTS

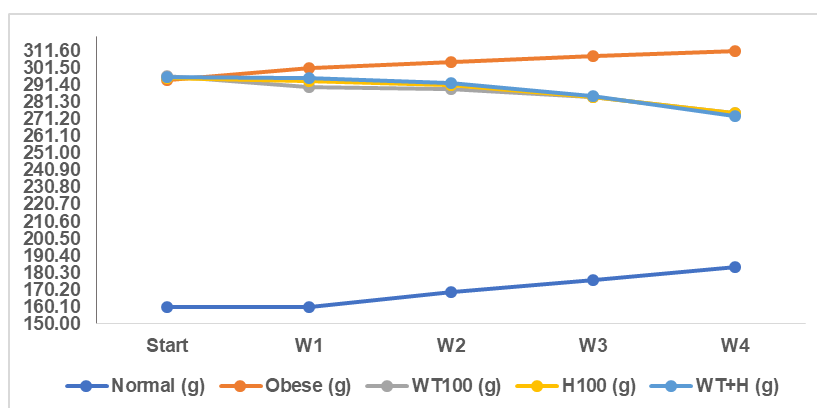
### The effect of combination of white turmeric rhizome extract (*Curcuma zedoaria* Rosc.) and honey on body weight of rats

The treatment on the test animals was given for 4 w. The development of rat body weight during the treatment is presented in table 1 and fig. 1.

**Table 1: Average weight development of rats**

Group	Normal (g)	Obese (g)	WT100 (g)	H100 (g)	WT+H (g)
Start	159.99±0.83 <sup>b,c,d,e</sup>	294.72±1.17 <sup>a</sup>	296.59±1.21 <sup>a</sup>	295.71±1.77 <sup>a</sup>	296.50±0.65 <sup>a</sup>
W1	159.99±0.37 <sup>b,c,d,e</sup>	301.63±0.51 <sup>a,c,d,e</sup>	290.20±0.50 <sup>a,b</sup>	293.01±1.13 <sup>a,b</sup>	295.52±0.65 <sup>a,b</sup>
W2	169.07±0.92 <sup>b,c,d,e</sup>	305.21±0.52 <sup>a,c,d,e</sup>	289.06±0.55 <sup>a,b</sup>	291.78±1.37 <sup>a,b</sup>	292.86±0.64 <sup>a,b</sup>
W3	175.99±1.43 <sup>b,c,d,e</sup>	308.89±0.41 <sup>a,c,d,e</sup>	284.60±0.47 <sup>a,b</sup>	284.27±0.56 <sup>a,b</sup>	285.00±0.31 <sup>a,b</sup>
W4	183.88±1.46 <sup>b,c,d,e</sup>	311.60±0.24 <sup>a,c,d,e</sup>	274.79±0.38 <sup>a,b</sup>	274.70±0.87 <sup>a,b</sup>	273.48±0.61 <sup>a,b</sup>

Mean±SD (n = 5) followed by different letters (superscript notations) in the same row indicates significant difference p<0.05 between group, obese = high-fat diet, WT 100 = white turmeric 100 mg/kg, H100 = honey 100 mg/kg, WT+H = combination of white turmeric and honey, W = week, a = normal, b = obese, c = WT 100, d = H 100, e = WT+H



**Fig. 1: Average weight development of rats**

The data in table 1 and fig. 1 show that the weight development of rats during 4 w of treatment increased in the normal and obese groups every week, while in the treatment group with white turmeric extract, honey and a combination of white turmeric extract and honey, there was a decrease in weight every week, with a significantly different (p<0.05).

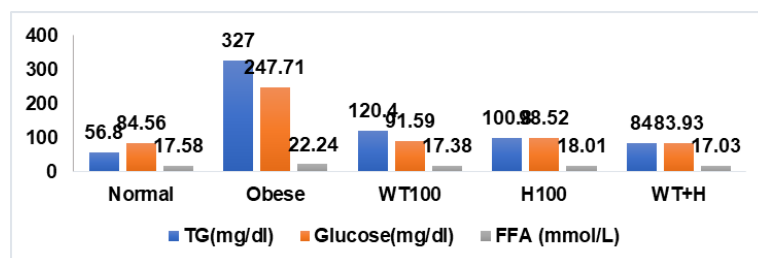
**Effect of combination of white turmeric extract and honey on *in vivo* test parameters of rat blood serum**

The average levels of glucose, triglycerides, free fatty acids and index obesity Lee ratio during 4 w of treatment in rats can be seen in table 2 and fig. 2.

**Table 2: Average levels of *in vivo* test results of rat blood serum**

Group	Normal	Obese	WT100	H100	WT+H
TG (mg/dl)	56.80±1.92 <sup>b,c,d,e</sup>	327.00±1.58 <sup>a,c,d,e</sup>	120.40±1.14 <sup>a,b,d,e</sup>	100.80±1.30 <sup>a,b,c,e</sup>	84.00±2.91 <sup>a,b,c,d</sup>
Glucose (mg/dl)	84.56±1.36 <sup>b,c,d</sup>	247.71±2.05 <sup>a,c,d,e</sup>	91.59±2.85 <sup>a,b,d,e</sup>	98.52±1.23 <sup>a,b,c,e</sup>	83.93±0.77 <sup>b,c,d</sup>
Free fatty acids (mmol/l)	17.58±0.42 <sup>b</sup>	22.24±0.58 <sup>a,c,d,e</sup>	17.38±0.56 <sup>b</sup>	18.01±0.41 <sup>b</sup>	17.03±0.25 <sup>b</sup>
IOL	0.26±0.01 <sup>b</sup>	0.34±0.03 <sup>a,c,d,e</sup>	0.27±0.02 <sup>b</sup>	0.27±0.02 <sup>b</sup>	0.27±0.02 <sup>b</sup>

Mean±SD (n = 5) followed by different letters (superscript notations) in the same row indicates significantly different p<0.05; Obese = high-fat diet, WT100 = white turmeric extract 100 mg/kg, H100 = honey 100 mg/kg, and WT+H = combination of white turmeric extract and honey. IOL = Index Obesity Lee. TG = Triglyceride, a = normal, b = obese, c = WT100, d = H100, e = WT+H.



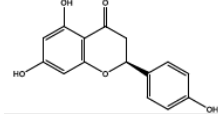
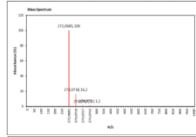
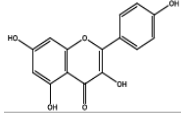
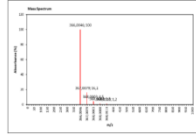
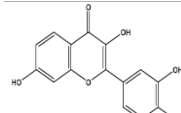
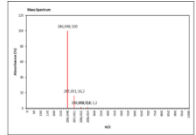
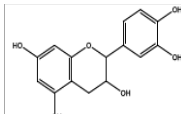
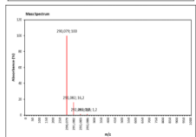
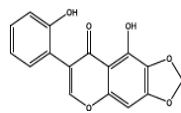
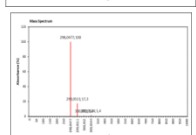
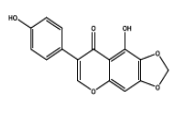
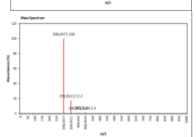
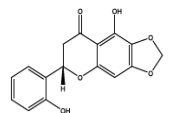
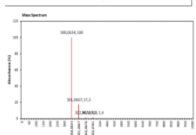
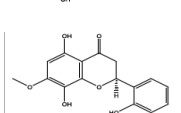
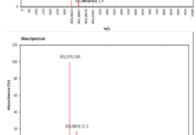
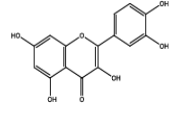
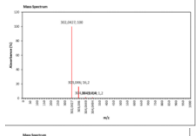
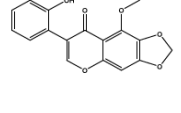
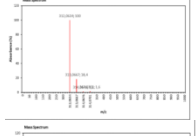
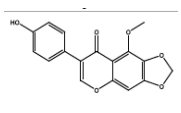
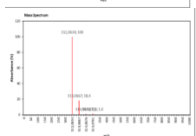
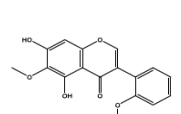
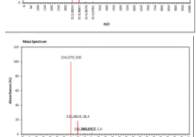
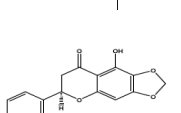
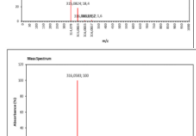
**Fig. 2: Average levels of glucose, free fatty acids, TG in rat blood serum**

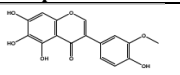
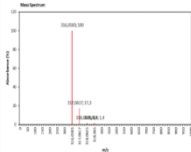
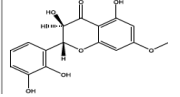
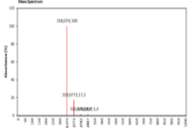
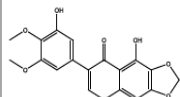
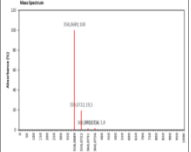
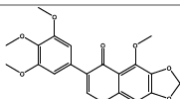
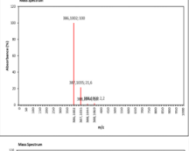
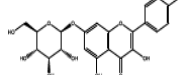
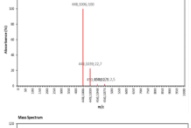
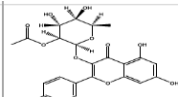
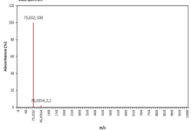
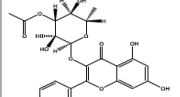
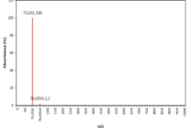
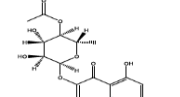
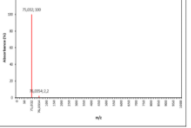
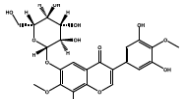
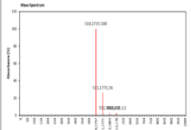
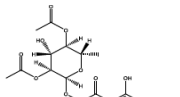
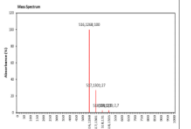
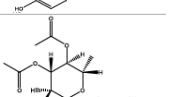
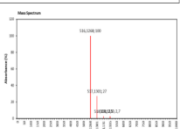
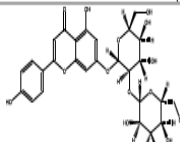
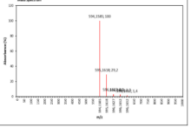
**Identification and analysis of flavonoids from a combination of white turmeric extract and honey using LC-MS**

The results of the identification and analysis of flavonoids from the combination of white turmeric extract and honey using LC-MS can be seen in table 3 and 4.

Based on the chromatograms in table 3 and 4, it shows that the combination of white turmeric extract and honey identified 48 compounds and 6 classes of flavonoid (flavonol, flavanone, isoflavone, flavone, flavanol, and anthocyanin) with different retention times. Chemical formula, Molecular weight, compound name, class, the structures, and mass spectrum of flavonoids can be seen in table 3 and 4.

Table 3: LC-MS chromatogram of white turmeric extract

RT(min)	Chemical formula and molecular weight (g/mol)	Name and classes of flavonoid compound	Structure of flavonoid compound	Mass spectrum
9.732	Chemical formula: C <sub>15</sub> H <sub>12</sub> O <sub>5</sub> Molecular weight: 272	Naringenin (Flavanone)		
10.322	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 286	Kaempferol (Flavonol)		
10.325	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 286	Fisetin (Flavonol)		
10.502	Chemical formula: C <sub>15</sub> H <sub>14</sub> O <sub>6</sub> Molecular weight: 290	Catechin (Flavanol)		
10.523	Chemical formula: C <sub>16</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 298	2',5-dihydroxy-6,7-methylenedioxyisoflavone (Isoflavone)		
10.526	Chemical formula: C <sub>16</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 298	5,4'-dihydroxy-6,7-methylenedioxyisoflavone (Isoflavone)		
11.040	Chemical formula: C <sub>16</sub> H <sub>12</sub> O <sub>6</sub> Molecular weight: 300	5,2'-dihydroxy-6,7-methylenedioxyflavanone (Isoflavone)		
11.407	Chemical formula: C <sub>16</sub> H <sub>14</sub> O <sub>6</sub> Molecular weight: 302	5,8,2'-trihydroxy-7-methoxyflavanone (Flavanone)		
11.427	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>7</sub> Molecular weight: 302	Quercetin (Flavonol)		
11.561	Chemical formula: C <sub>17</sub> H <sub>12</sub> O <sub>6</sub> Molecular weight: 312	2'-hydroxy-5-methoxy-6,7-methylenedioxyisoflavone (Isoflavone)		
11.564	Chemical formula: C <sub>17</sub> H <sub>12</sub> O <sub>6</sub> Molecular weight: 312	5-methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone (Isoflavone)		
11.606	Chemical formula: C <sub>17</sub> H <sub>14</sub> O <sub>6</sub> Molecular weight: 314	5,7-dihydroxy-2',6-dimethoxyisoflavone (Isoflavone)		
11.61	Chemical formula: C <sub>16</sub> H <sub>12</sub> O <sub>7</sub> Molecular weight: 316	5,2',3'-trihydroxy-6,7-methylenedioxyflavanone (Flavanone)		

RT(min)	Chemical formula and molecular weight (g/mol)	Name and classes of flavonoid compound	Structure of flavonoid compound	Mass spectrum
11.615	Chemical formula: C <sub>16</sub> H <sub>12</sub> O <sub>7</sub> Molecular weight: 316	5,6,7,4'-tetrahydroxy-3'-methoxyisoflavone (Isoflavone)		
11.82	Chemical formula: C <sub>16</sub> H <sub>14</sub> O <sub>7</sub> Molecular weight: 318	3,5,2',3'-tetrahydroxy-7-methoxyflavanone (Flavanone)		
012.645	Chemical formula: C <sub>16</sub> H <sub>12</sub> O <sub>7</sub> Molecular weight: 316	5,3'-dihydroxy-4',5'-dimethoxy-6,7-methylenedioxyisoflavone (Isoflavone)		
12.991	Chemical formula: C <sub>20</sub> H <sub>18</sub> O <sub>8</sub> Molecular weight: 386	5,3',4',5'-tetramethoxy-6,7-methylenedioxyisoflavone (Isoflavone)		
22.624	Chemical formula: C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> Molecular weight: 448	Kaempferol-7-O-glucoside (Flavonol)		
24.768	Chemical formula: C <sub>23</sub> H <sub>22</sub> O <sub>11</sub> Molecular weight: 474	Kaempferol 3-(2''-acetylramnoside) (Flavonol)		
24.77	Chemical formula: C <sub>23</sub> H <sub>22</sub> O <sub>11</sub> Molecular weight: 474	Kaempferol 3-(3''-acetylramnoside) (Flavonol)		
24.773	Chemical formula: C <sub>23</sub> H <sub>22</sub> O <sub>11</sub> Molecular weight: 474	Kaempferol 3-(4''-acetylramnoside) (Flavonol)		
26.304	Chemical formula: C <sub>23</sub> H <sub>24</sub> O <sub>13</sub> Molecular weight: 508	8,3',5'-trihydroxy-7,4'-dimethoxyisoflavone 6-O-glucoside (Isoflavone)		
28.206	Chemical formula: C <sub>25</sub> H <sub>24</sub> O <sub>12</sub> Molecular weight: 517	Kaempferol 3-(2'',4''-diacetylramnoside) (Flavonol)		
28.208	Chemical formula: C <sub>25</sub> H <sub>24</sub> O <sub>12</sub> Molecular weight: 517	Kaempferol 3-(3'',4''-diacetylramnoside) (Flavonol)		
33.618	Chemical formula: C <sub>27</sub> H <sub>30</sub> O <sub>15</sub> Molecular weight: 595	Apigenin 7-sophoroside (Flavone)		

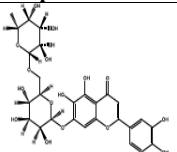
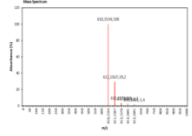
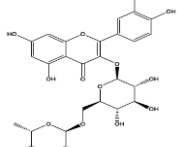
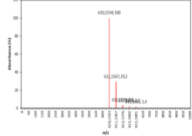
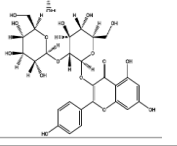
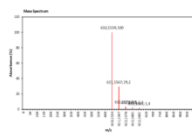
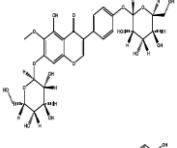
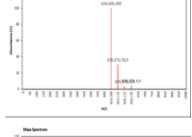
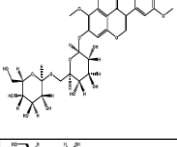
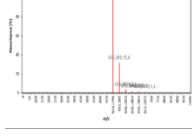
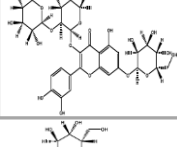
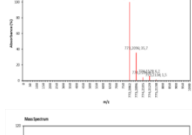
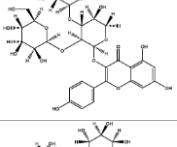
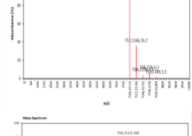
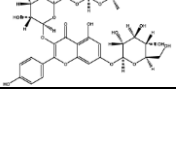
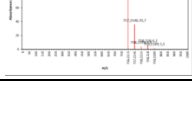
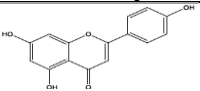
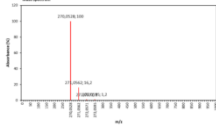
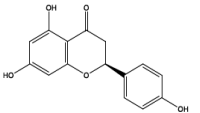
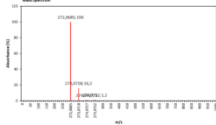
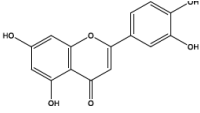
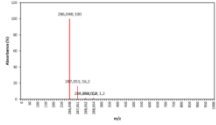
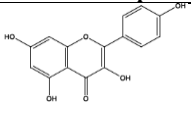
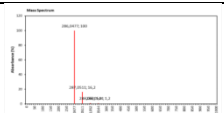
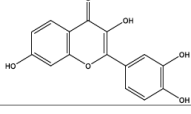
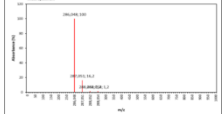
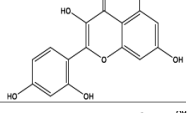
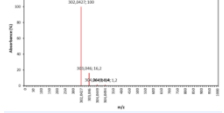
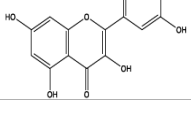
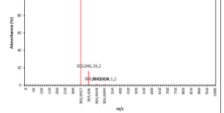
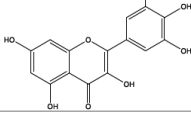
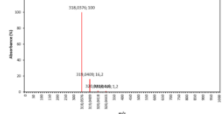
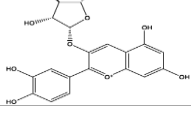
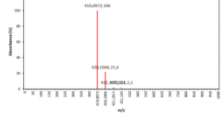
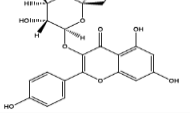
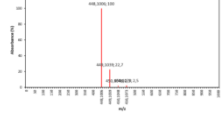
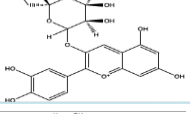
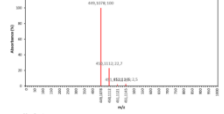
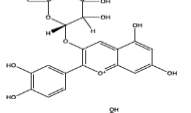
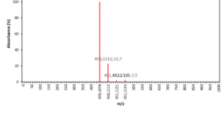
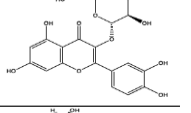
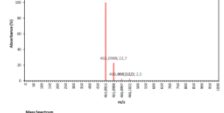
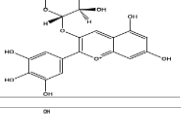
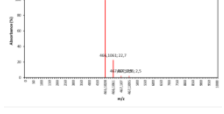
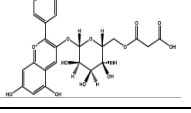
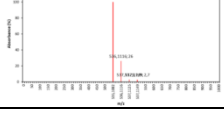
RT(min)	Chemical formula and molecular weight (g/mol)	Name and classes of flavonoid compound	Structure of flavonoid compound	Mass spectrum
35.513	Chemical formula: C <sub>27</sub> H <sub>30</sub> O <sub>16</sub> Molecular weight: 611	6-hydroxyluteolin 7-rutinoside (Flavone)		
35.517	Chemical formula: C <sub>27</sub> H <sub>30</sub> O <sub>16</sub> Molecular weight: 611	Rutin (Flavonol)		
35.518	Chemical formula: C <sub>27</sub> H <sub>30</sub> O <sub>16</sub> Molecular weight: 611	Kaempferol 3-O-sophoroside (Flavonol)		
36.831	Chemical formula: C <sub>28</sub> H <sub>32</sub> O <sub>16</sub> Molecular weight: 625	5,7,4'-trihydroxy-6-methoxyisoflavone 7-O-glucoside-4'-O-glucoside (Isoflavone)		
36.833	Chemical formula: C <sub>29</sub> H <sub>34</sub> O <sub>17</sub> Molecular weight: 655	5,7,4'-trihydroxy-6,3'-dimethoxyisoflavone 7-O-(6''-glucosylglucoside) (Isoflavone)		
46.014	Chemical formula: C <sub>33</sub> H <sub>40</sub> O <sub>21</sub> Molecular weight: 773	Quercetin 3-glucosyl-(1→2)-rhamnoside-7-glucoside/ (Flavonol)		
46.565	Chemical formula: C <sub>33</sub> H <sub>40</sub> O <sub>20</sub> Molecular weight: 757	Kaempferol 3-glucosyl-(1→2)-[glucosyl-(1→3)-rhamnoside] (Flavonol)		
46.567	Chemical formula: C <sub>33</sub> H <sub>40</sub> O <sub>20</sub> Molecular weight: 757	Kaempferol 3-rutinoside-7-glucoside (Flavonol)		

Table 4: LCMS chromatogram of honey

RT(min)	Chemical formula and molecular weight (g/mol)	Name of the compound resulting from analysis	Structure and classes of flavonoid compound	Mass spectrum
9.365	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>5</sub> Molecular weight: 270	Apigenin (Flavone)		
9.732	Chemical formula: C <sub>15</sub> H <sub>12</sub> O <sub>5</sub> Molecular weight: 272	Naringenin (Flavanone)		
10.266	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 286	Luteolin (Flavone)		

RT(min)	Chemical formula and molecular weight (g/mol)	Name of the compound resulting from analysis	Structure and classes of flavonoid compound	Mass spectrum
10.322	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 286	Kaempferol (Flavonol)		
10.325	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>6</sub> Molecular weight: 286	Fisetin (Flavonol)		
11.405	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>7</sub> Molecular weight: 302	Morin (Flavonol)		
11.427	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>7</sub> Molecular weight: 302	Quercetin (Flavonol)		
11.514	Chemical formula: C <sub>15</sub> H <sub>10</sub> O <sub>8</sub> Molecular weight: 318	Myricetin (Flavonol)		
14.466	Chemical formula: C <sub>20</sub> H <sub>19</sub> O <sub>10</sub> Molecular weight: 419	Cyanidin-3-arabinoside (Anthocyanin)		
22.173	Chemical formula: C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> Molecular weight: 448	Astragalin (Flavonol)		
22.747	Chemical formula: C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> Molecular weight: 449	Cyanidin-3-O-glucoside (Anthocyanin)		
22.754	Chemical formula: C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> Molecular weight: 449	Chrysin (Flavone)		
24.018	Chemical formula: C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> Molecular weight: 464	Isoquercitrin (Flavonol)		
24.121	Chemical formula: C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> Molecular weight: 465	Delphinidin-3-glucoside (Anthocyanin)		
31.012	Chemical formula: C <sub>24</sub> H <sub>23</sub> O <sub>14</sub> Molecular weight: 535	Cyanidin-3-(6'-malonylglucoside) (Anthocyanin)		

## DISCUSSION

The statistical analysis data in table 1 and fig. 1 show that the weight development of rats in the normal and obese groups experienced weight gain every week, while in the treatment group of the combination of white turmeric extract and 100 mg/kg honey, experienced weight loss every week. This indicates that intake of a

combination of white turmeric extract and honey are able to suppress weight gain in rats and also the IOL decreased with a significant difference ( $p < 0.05$ ) from obese rats group.

The weight loss in obese rats was caused by the presence of flavonoid compounds contained in the combination of white turmeric extract and honey, which were able to suppress weight

gain in obese rats. Flavonoids can inhibit the activity of pancreatic lipase, an enzyme responsible for fat digestion. Inhibition of fat can reduce absorption from food, resulting in weight loss. Flavonoids can also increase fat metabolism by stimulating the activity of enzymes involved in fat oxidation process, such as adenosine monophosphate-activated protein kinase (AMPK), which helps burn fat. Several flavonoids have been reported to reduce the activity of enzymes in the formation of new fat, such as the acetyl CoA Carboxylase (ACC) which plays a role in body fat [2, 22-24]. The results of the analysis of the combination of white turmeric and honey with LC-MS showed that there were 33 flavonoid compounds identified from the white turmeric rhizome extract (table 3) and 15 flavonoid compounds from honey (table 4). Flavonoids found in the combination of white turmeric rhizome extract and honey are bioactive compounds that can play a role in reduce weight in obese rats.

The data in table 2 and fig. 2 show that the obese group has higher levels of glucose, free fatty acids, and TG with a significant difference ( $p < 0.05$ ) than the normal group. This is due to the impact of obesity given a high-fat diet. In obesity, there will be an increase in free fatty acids in the blood, which will be followed by an increase in the uptake of free fatty acids by muscle tissue. Muscles in normal conditions will use glucose to produce energy. Thus, fatty acid oxidation in muscles increases, this will inhibit glucose uptake by muscles so that hyperglycemia occurs. The same condition occurs in the liver, where the liver will accommodate most of the free fatty acids and become material for gluconeogenesis, with increased gluconeogenesis, fasting blood glucose will increase and hyperglycemia occurs. This hyperglycemic condition will result in insulin resistance in the liver [5]. The data in table 2 and fig. 2 show that there is an effect of intake of combination of white turmeric and honey, which is able to reduce the average IOL, glucose, free fatty acids, and TG in the blood serum of obese rats with a significantly different ( $p < 0.05$ ) from the obese group. The average decrease in glucose, triglyceride, free fatty acid, and Lee's obesity index levels was 66.12%, 74.31%, 42.57%, and 20.59%, respectively.

The decrease in blood glucose, triglyceride, and free fatty acid levels in Wistar rats is thought to be due to the presence of active flavonoid compounds and have hypoglycemic and antioxidant properties. Hyperglycemia will cause increased oxidative stress and decreased endogenous antioxidants. Intake of natural antioxidants is one of the protections against diabetes progression by inhibiting peroxide reactions that damage pancreatic beta cells [13-17]. One of the secondary metabolites from the combination of white turmeric extract and honey, which plays an active role as an antiobesity and antidiabetic type 2 in obese rats is a flavonoid compounds. There are 33 flavonoid compounds from white turmeric rhizome extract and 15 flavonoid compounds from bee honey which are suspected to play an active role as anti-obesity and anti-diabetes type 2. The results of this study are supported by several researchers who reported that flavonoids have activity as antiobesity and antidiabetes [25-28].

Dzomba, 2014 reported that Flavonoids in the saphoricoside compound which is an antioxidant compound are thought to act as pancreatic lipase inhibitors and reduce appetite [23]. The results of this study are also supported by research by [29] reported that dragon fruit extract (*Hylocereus costaricensis*) containing flavonoids has antiobesity activity.

Quercetin and kaempferol are a class of flavonoids (Flavonol) that show antidiabetic effects in obese animal models through multi-target mechanisms, namely as antioxidants (Nrf2/HO-1), anti-inflammatory ( $\downarrow$ NF- $\kappa$ B,  $\downarrow$ NLRP3/STING), AMPK activation, increased insulin signaling (PI3K/Akt  $\rightarrow$  GLUT4), inhibition of gluconeogenesis (PEPCK/G6Pase),  $\beta$ -cell protection and modulation of microbiota and lipid metabolism [30-32]. Flavonoids (quercetin, kaempferol and its derivatives, apigenin, rutin, Naringenin, luteolin, catechins) in addition to increasing the activity of antioxidant in the body and also able to regenerate damaged pancreatic beta cells so that insulin deficiency can be prevented [30-43].

## CONCLUSION

There are 48 flavonoid compounds identified from the combination of white turmeric rhizome extract and honey, namely 33 from white turmeric rhizome and 15 from bee honey, which are thought to play an active role as antiobesity and antidiabetes type 2 in obese rats.

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The contribution of each author to the preparation of the manuscript is as follows: Ni Wayan Bogoriani: Conceptualization, Methodology, Writing – Original Draft.

I Gusti Ayu Putu Eka Pratiwi: Data Collection, Analysis, Writing – Review and Editing.

Ni Made Suaniti: Data collection from LCMS analysis.

Komang Tria Noviana Dewi: Data collection from glucose, triglyceride, free fatty acid analysis, writing

## CONFLICT OF INTERESTS

The authors do not have any conflict of interest

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