

In Vitro Anti-Inflammatory Assay of Bitter Melon (*Momordica charantia* L.) Ethanol Extract

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Article info	Abstract
<p>History Submission: 23-10-2019 Review: 24-05-2020 Accepted: 26-08-2020</p> <p>*Email: rahmawati.rahmawati@umi.ac.id</p> <p>DOI: 10.33096/jffi.v7i3.655</p> <p>Keywords: Anti-inflammation; <i>Momordica charantia</i> L; Protein denaturation; UV-Vis spectrophotometry</p>	<p><i>Bitter melon contains flavonoids that have anti-inflammatory function. Inflammation can be caused by protein denaturation. This research tested the anti-inflammatory potential of ethanol extract of leaves and bitter melon (<i>Momordica charantia</i> L.) using a UV-Vis spectrophotometer and protein denaturation inhibition method. There were three experimental groups formed in this research including negative control group, positive control group, and test solutions. Diclofenac sodium was used in the positive control group at concentration series of 1, 2, 4, 8, and 16 ppm, obtaining a regression equation $Y = 3.546X + 2.163$ and $r = 0.9990$. Whilst, for bitter melon ethanol extract at a series of concentrations of 10, 20, 40, 80 and 160 ppm obtained a regression equation $Y = 0.243X + 11.74$ and $r = 0.9995$. The potential of diclofenac sodium as an anti-inflammatory agent was shown by IC_{50} of 13.490 $\mu\text{g} / \text{mL}$, while the ethanol extract of bitter melon fruit has an IC_{50} of 157.448 $\mu\text{g} / \text{mL}$. This result indicated that bitter melon ethanol extract had the potential as moderate anti-inflammatory agent.</i></p>

I. Introduction

Bitter melon (*Momordica charantia* L.) consists of high nutrients besides it is also efficacious traditional medicine. Bitter melon is known for its bitter taste. Bitter melon (*Momordica charantia* L.) contains flavonoids and saponins (Yuda dan Made, 2013), which and performs pharmacological activities as antipyretic and anti-inflammatory agents. This plant has been empirically used to treat asthma, burning sensation, abdominal pain, constipation, coughing, intestinal worms, inflammation, leprosy, skin diseases, ulcers and wounds (Garau et al, 2003). The flavonoid compounds can inhibit cyclooxygenase, providing anti-inflammatory effect as it is the first step on the pathway to eiksanoid similar to prostaglandins and thromboxane (Robinson, 1995).

Inflammation is a normal protective response to tissue injuries due to physical trauma, hazardous chemicals, or microbiological agents. Anti-inflammatory is a term for agents that work against or suppress the inflammatory process (Company, 2000). Inflammation can occur due to protein denaturation that stimulates pain mediators.

In vitro anti-inflammatory activity testing can be done by inhibiting the protein denaturation process using Bovine Serum Albumin (BSA) which will be denaturated when it is heated. This marks where albumin is damaged when heat is induced, allowing the body to recognize it as unfamiliar material (antigen) which will be fought by the body through inflammation.

In this research, anti-inflammatory activity of bitter melon fruit and leaves ethanol extracts was conducted in vitro by using a protein denaturation method.

II. Research Method

II.1 Equipment and Materials

Glassware (Pyrex), blender, micropipettes (Huawei), analytical scales (AND), pH meters (Jenco), Rotary Vacuum Evaporators, UV-Vis Spectrophotometer (Thermo Scientific e.201), vortex (Krisbow), and oven (Mettler Type UN-30).

Distilled water, samples (ethanol extract of leaves and bitter melon), glacial acetic acid, Bovine Serum Albumin (BSA) (pa. Merck), NaCl (pa. Merck), diclofenac sodium (pa. Merck), and tris base (pa. Merck).

II.2 Research Procedure

1. TBS (Tris Buffer Saline) Solution

A total of 0.87 g NaCl was dissolved in distilled water, added with 0.121 g of Tris Base. The pH was adjusted as glacial acetic acid was added to obtain a pH level between 6.2-6.5 and was added with distilled water until reaching a volume of 100 mL (Farida et al, 2018).

2. BSA (Bovine Serum Albumin) 0.2% Solution

A total of 0.2 g of BSA was put into a 100 mL volumetric flask, then added with a TBS (Tris Buffer Saline) solution up to a volume of 100 mL (Farida et al, 2018).



3. Extraction

As much as 100 g of dried bitter melon as raw material was put into maceration container added with 96% ethanol solvent up to 1 cm above the sample surface. The solvent was stirred until it was evenly distributed. The extraction was carried out for 3x24 hours before the filtrate was filtered and the residue was re-macerated. The filtrate was then evaporated using a rotary vacuum evaporator until a thick extract was obtained (Amelia, 2013).

4. Negative Control Solution (Ethanol 96%)

A total of 50 μ L 96% ethanol solvent was added to the volumetric flask, then added with 0.2% BSA solution until reaching a volume of 5 mL (Muliati, 2014).

5. Positif Control Solution (Diclofenac Sodium)

A total of 125 mg of standard diclofenac sodium is then dissolved with 96% ethanol in a 25 mL volumetric flask and added with ethanol up to a volume of 25 mL and the solution had a concentration of 5000 ppm as the parent solution. From this 5000ppm parent solution, a series of positive control solution concentrations was made to 100, 200, 400, 800 and 1600 ppm using a pipette of 0.1; 0.2; 0.4; 0.8 and 1.6 mL sizes before ethanol 96% was added to reach a volume of 5 mL (Muliati, 2014).

6. Test Solution

A total of 200 mg of bitter melon (*Momordica charantia* L.) ethanol extract was dissolved into 96% ethanol solvent in a 10 mL volumetric flask, obtaining a parent solution with a concentration of 20000 ppm. The stock solution was then diluted and made into series concentrations of 1000, 2000, 4000, 8000 and 16000 ppm (Muliati, 2014).

7. Anti-Inflammatory Activity Test

As much as 50 μ L of each concentration of the solution (negative control solution, positive control and test solution) were pipetted and added with 0.2% BSA solution until the volume reached 5 mL. The solution was incubated at room temperature for 30 minutes, heated for 45 minutes at 100°C in an oven, then cooled for 25 minutes at room temperature. After that, the solution was vortexed for 1 minute and absorbance measurements were performed using a UV-Visible spectrophotometer at a wavelength of 660 nm (Muliati, 2014; Ramadhani, 2019).

III. Results and Discussion

Bitter melon was used as a sample because it contained flavonoids which have the potential as anti-inflammatory agent (Robinson, 1995). During the maceration of bitter melon (*Momordica charantia* L.), a total 8.64962 grams of bitter melon extract was obtained with extraction yield of 8.64%.

The diclofenac sodium standard is one of the non-steroidal anti-inflammatory drugs (NSAIDs). Diclofenac sodium is used as a comparison for its similar mechanism in preventing protein denaturation at a pathological pH of 6.2-6.5.12. In addition, sodium diclofenac can inhibit the enzyme silkooksigenase (COX). Bitter melon contains flavonoids which can also inhibit the cyclooxygenase enzyme. The results of measurements of anti-inflammatory activity and calibration curves of standard diclofenac sodium are presented in Table 1 and Figure 1.

Table 1. The Results of Anti-Inflammatory Activity Test using the Positive Control Solution (diclofenac sodium standard)

Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀ (μ g/mL)
1	0.940	4.858	
2	0.899	9.008	
4	0.822	16.801	13.490
8	0.673	31.882	
16	0.413	58.198	

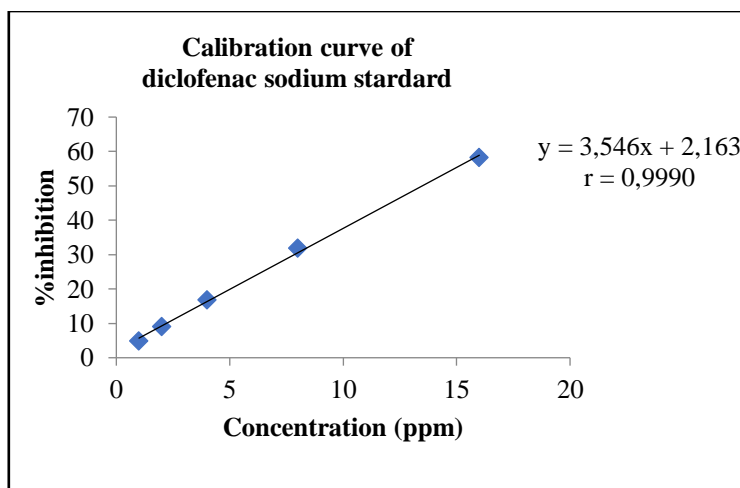


Figure 1. The calibration curve of the positive control solution (diclofenac sodium standard)

The absorbance level was measured using 5 series of standard solutions which results showed a decrease in absorbance value and an increase in the value of inhibition percentage. This outcome is normal since the concentration was inversely proportional to the absorbance value and directly proportional to the value of inhibition percentage. The results of the regression test between the can be seen in Table 2 and Figure 2.

concentration and inhibition percentage produced a linear equation of $y = 2.163 + 3.546x$. The IC_{50} value was then measured, obtaining a value of $13.490 \mu\text{g} / \text{mL}$.

Subsequently, samples of bitter melon extract were analyzed for anti-inflammatory activity using protein denaturation method. The results of the measurement.

Table 2. The results of anti-inflammatory test of bitter melon ethanol extract (*Momordica charantia* L.)

Concentration (ppm)	Absorbance	% Inhibition	IC_{50} ($\mu\text{g}/\text{mL}$)
10	0.905	14.218	157.448
20	0.873	17.251	
40	0.834	20.947	
80	0.729	30.900	
160	0.517	50.995	

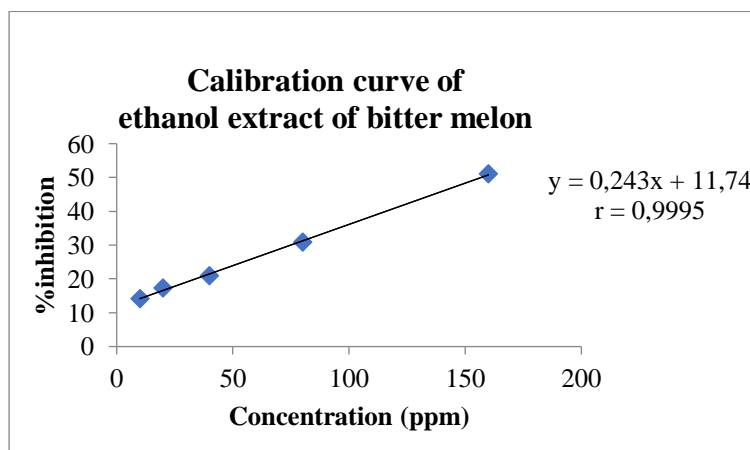


Figure 2. The calibration curve of ethanol extract from bitter melon (*Momordica charantia* L.)

The measurmeent of absorbance level using 5 series of sample solution showed a decrease in absorbance value and an increase in the value of inhibition percentage. The regression test between the concentration and inhibition percentage produced a linear equation $y = 11.74 + 0.243x$. The

IC_{50} value was then measured, obtaining a value of $157.484 \mu\text{g} / \text{mL}$ (moderate level).

The anti-inflammatory potential of the sample is reflected by the IC_{50} value. IC_{50} value shows the concentration of the test sample that can inhibit protein denaturation by 50% (Farida et al, 2014). Anti-inflammatory activity is categorized

highly active if the IC₅₀ value is less than 50 µg / mL, active when 50-100 µg / mL, moderate if 101-250 µg / mL, weak if 251-500 µg / mL, and categorized inactive if the IC₅₀ value is greater than 500 µg / mL (Jun et al, 2003).

IV. Conclusions

The ethanol extract of bitter melon (*Momordica charantia* L.) analyzed in vitro condition has been found to have moderate anti-inflammatory activity with an IC₅₀ value of 157.444 µg / mL.

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Buah Pare (*Momordica charantia* L.) dan Pengaruhnya terhadap Penurunan Kadar Glukosa Darah Tikus Putih Jantan (*Rattus novergicus*) yang di Induksi Aloksan, *Buletin Veteriner*, Udayana.