



**ANTIHYPERGLICEMIC EFFECT OF FLESHED AND SEEDED EXTRACT OF
*MOMORDICA CHARANTIA***

SITI AISYAH^{1*}, HAYAT SHOLIHIN¹, IQBAL MUSTHAPA¹, ATUN QOWIYAH²

¹Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Indonesia
University of Education, Jl. Setiabudi 229 Bandung

² Faculty of Pharmacy, University of Garut, Jl. Jati 42 Tarogong Garut

*email: sitiaisyah@upi.edu

ABSTRACT

The research is conducted in order to discover active fraction toward the reduction of blood glucose (antihyperglycemic) from fleshed and seed of *Momordica charantia* or pare. Extraction is carried out using methanol solvent proceeded with fractionation using organic solvent such as hexane, ethyl acetate, and n-butanol. Entire extracts and fractions are examined to obtain the information of blood glucose intensity reduction activities and the major compound group characteristics with Liebermann-Burchard methods and FT-IR spectroscopy. Based on the glucose tolerance test with in vivo way on Wistar rats it is discovered that hexane fraction of the fleshed is the most active and the fraction insertion causes the mouse with lower blood glucose intensity differs significantly from the positive control. Based on the characteristic test with color method Liebermann-Burchard and spectroscopy IR, active fraction hexane is presumed to contain the major compound such as unglycosides terpenes.

Keywords : *antihyperglycemic, Momordica charantia, glucose tolerance test.*

INTRODUCTION

Diabetes mellitus is the commonest endocrine disorder attacking more than 4% of people around the world. World Health Organization (WHO) assume that the disease will affect five times more people in the world (Grover, J.K., et al. 2002). The disease is indicated by the increase of blood glucose intensity. The intensity of blood glucose alone to normal people varies in a day. It is increasing after having meal and back to normal in 2 hours. The increasing of blood glucose after having meal or drink stimulate the pancreas to produce insulin that preventing the continued increase of blood glucose and even more decreasing it gradually. In the diabetes mellitus sufferer the blood glucose intensity is high and difficult to get back to normal the body is unable to release or use sufficient insulin. It causes the sufferer lose his/her weight. The serious matter of the disease is the proceeding complication it causes including arterosclerosis, retinopati, nefropati and heart coroner (Vats, V., 2004; Malik S., 2007).

The disease is divided into two types, namely insulin-dependent diabetes mellitus (T1DM, Type 1 Diabetes Mellitus) and insulin-independent diabetes mellitus (T2DM, Type 2 Diabetes Mellitus). Just about 90% of the diabetes mellitus sufferers are type T2DM (Giorgio et al., 2005). In dealing with the second type (T2DM), there are various oral medicines, such as metformin. However, in some cases, the existing medicines fail to turn back the blood glucose to normal level, or need higher doses. The increase of the dose surely causes the origination of some side effects. Consequently, there is a need to find the alternative or additional remedy in order to turn the blood glucose to the normal level such as by the use of traditional medicinal plants.

There are more than 400 plants are recognized to produce the effect of blood glucose intensity reduction (Ernst, 1997). One of them largely traditionally used to diabetes treatment is “paria” or “pare” (*Momordica charantia*). Antidiabetes studies of *M. charantia* have been carried out many times and they have proven that it is able to reduce blood glucose intensity to normal animal, aloksan induced diabetes animal, streptozotocin induced diabetes animal and genetical diabetes animal (Rao, BK., 2001; Grover and Yadav, 2004; Viridi, J., 2003). Furthermore, the ethanol extract of the fruit is also proven to decrease the blood glucose as good as glikenkamid effect in experiment using little mouse.

Instead of the antidiabetes study, the phitochemistry aspect of the plant has intensively been observed. Based on completed literature study, it contains compounds of glycoside, saponin, alkaloid, triterpen, protein, and steroi. (Raman and Lau, 1996). From those compounds, at least, there are 50 secondary metabolite compounds of triterpen aglikon and triterpen glycoside. (Buckingham, 2006). Some chemistry compounds such as momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, erythrodiol, galacturonat acid have been isolated successfully from the plant (Grover and Yadav, 2004).

Although the two studies, namely antidiabetes effect and phytochemistry, have been carried out frequently, the study of the correlation between them are rarely conducted. Based on the result of literature investigation, there is only one given study, resulting in the identification of some hypoglycemidal peptide. (Lotlikar and Rao, 1996; Khanna et al., 1981). In fact, *M. charantia* contains, instead of peptide, a number of secondary metabolite compounds of triterpana inheritor, as well as aglikon or glycoside (Buckingham, 2006). As the result, the research proposed attempts to answer a hypotheses that one of the secondary metabolite compounds or more could contribute to hypoglycemia effect of *M. charantia* fruit.

MATERIALS AND METHODS

Collection of plant material

Green, unripened fresh fruits of *Momordica charantia* were purchased from Kampung Pamahan, Jati Asih, Bekasi. Part of fleshed and seeded were sun dried to get *Momordica charantia* fleshed and seeded dried powder.

Preparation of extracts

The active principle/s of *M. charantia* fleshed and seeded were extracted into three different solvents, hexane, ethyl acetate, and n-butanol. *M.charantia* fleshed and seeded powder was soaked in methanol solvents in plastic jars for 1 day at room temperature and the solvent was filtered. This was repeated three times until the extract gave no coloration. The extracts were distilled and concentrated under reduced pressure in the Buchi, rotavapour R-114 and finally freeze dried. These extracts were extracted into three different solvents, hexane, ethyl acetate, and n-butanol. These extract was used for further studies.

Experimental animals

Females Wistar rats weighing 150–180 g were purchased from Sekolah Ilmu dan Teknologi Hayati ITB. The rats were housed in air-conditioned animal house. Each rat was kept in a separate cage.

The method of glucose tolerance

The method of glucose tolerance was used in the test of antihyperglycemic effects. Diabetes was induced in Wistar rats which is orally fed glucose by gastric intubation. The extract powders were orally fed at a dosage of 0.25 g/kg body weight and glibenclamide at a dosage of 0.45 mg/kg body weight. In every batch the rats were divided into 12 groups and each group consisted of 3 rats.

Group 1—Diabetic untreated rats

Group 2— Diabetic rats treated with 0.25 g/kg b.w. of fleshed methanol extract.

Group 3— Diabetic rats treated with 0.25 g/kg b.w. of fleshed hexane extract.

Group 4— Diabetic rats treated with 0.25 g/kg b.w. of fleshed ethyl acetate extract.

Group 5— Diabetic rats treated with 0.25 g/kg b.w. of fleshed n-butanol extract.

Group 6— Diabetic rats treated with 0.75 g/kg b.w. of fleshed methanol-water extract.

Group 7— Diabetic rats treated with 0.25 g/kg b.w. of seeded methanol extract.

Group 8— Diabetic rats treated with 0.25 g/kg b.w. of seeded hexane extract.

Group 9— Diabetic rats treated with 0.25 g/kg b.w. of seeded ethyl acetate extract.

Group 10— Diabetic rats treated with 0.25 g/kg b.w. of seeded n-butanol extract.

Group 11— Diabetic rats treated with 0.75 g/kg b.w. of seeded methanol-water extract.

Group 12—Diabetic rats treated with 0.45 mg/kg b.w. of Glibenclamide.

After an overnight fast, the plant extract suspended in *tragakan* was fed by gastric intubation, using a force feeding needle. Group 1 rats were fed *tragakan* alone. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 30, 60, 90 and 120 minutes after feeding glucose. The results were compared with those of the 12th group of rats which was treated glibenclamide (OHA). Blood glucose levels were determined by using Optimum Omega® Glucometer.

Statistical analysis

Data are expressed as mean S.D.

Characterisation of mayor compounds in each extract

Each extract was measured by Liebermann-Burchad methods and FT-IR spectroscopy

RESULTS AND DISCUSSIONS

The fruit of *M. charantia* (11 Kg) was separated from flesh (8 Kg) and seeds (3 Kg). Then they were dried to get powder of flesh (770 g, 9.6%) and seed powder (605 g, 20.16%). The powder were then extracted by methanol (3x5L). The solvent from both of extracts were evaporated by rotary evaporator and yielded fleshed extract (28 g, 3.6%) and seeded extract (26 g, 4.3%). The concentrated of fleshed extract (11,4 g) was diluted by methanol then extracted in succession with hexane to yield (0.8 g, 7%), ethyl acetate to yield (4.9 g, 43%), n-butanol to yield (5.2 g, 45.6%), and the rest fraction of methanol-water (0.5 g, 4.4%). As well as the flesh part, the seeded extract (29.2 g) was diluted by methanol, then extracted in succession with hexane, to yield (0.2 g, 0.7%), ethyl acetate to yield (3.2 g, 11%), n-butanol to yield (3.4 g, 11.6%), and the rest fraction of methanol-water (22.4 g, 4.4%).

Test of glucose tolerance was carried out in vitro to white Wistar rats. The methode of glucose tolerance was used in the test of antihyperglykemic effects which was meassured every 30 minutes. First meassurement was carried out after 30 minutes induction of test preparations and comparison. This activity was indivated as zero time. On the tests of induction of the extract fractions showed that the rate of glucose concentration in the blood of animals were 80-90 mg/dl on the first minute. It was higher than comparison group which stated at 74 mg/dl. It means that hypoglicemia was caused by introduction of gibenklamide. 30 minutes induction, all of animal blood glucose concentrations had increased. Then after the following 60 minutes some groups were still high, therefore most of them had decreased. Accordingly, the glucose concentration all of groups had decreased during 90 minutes. Meanwhile the data of the rate glucose concentrations before and after treatment with fleshed extract then compared to both of positif control and comparison group can be shown on the figure 1. Base on statistical count resulted that induction of fleshed extract of *M charantia* on minute of 90 and 120 lead to inhibit increasing of glucose concentration. Introduction this fraction caused glucose concentration of rat blood was lower than positif control group ($p < 0,05$).

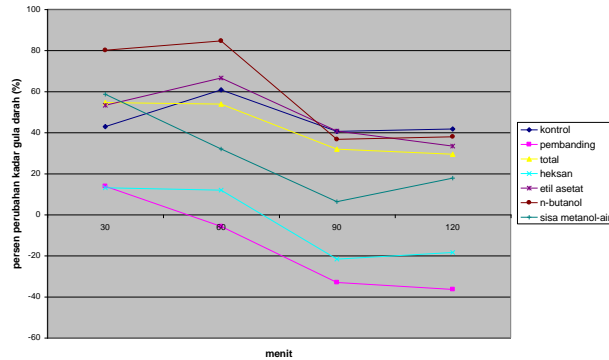


Figure 1. Percentage of glucose concentration change before and after treatment with fleshed extract of *M. charantia*

On the tests of induction of the seeded extract fractions showed that the rate of glucose concentration in the blood of animals were 80-110 mg/dl on the first minute. It was higher than comparison group which stated at 74 mg/dl. After 30 minutes induction, all of animal blood glucose concentrations had increased. Then after the following 60 minutes some groups were still high, therefore most of them had decreased. Accordingly, the glucose concentration all of groups had decreased during 90 minutes except on the total methanol and hexane fractions. Meanwhile the data of the percentage change of rate glucose concentrations before and after treatment with seeded extract compared to both of positif control and comparison group can be shown on the figure 2. Base on statistical count resulted that induction of methanol seeded extract of *M charantia* on minute of 60 and hexane fraction on minute of 90 lead to inhibit increasing of glucose concentration. Introduction this fraction caused glucose concentration of rat blood was lower than positif control group ($p < 0,05$).

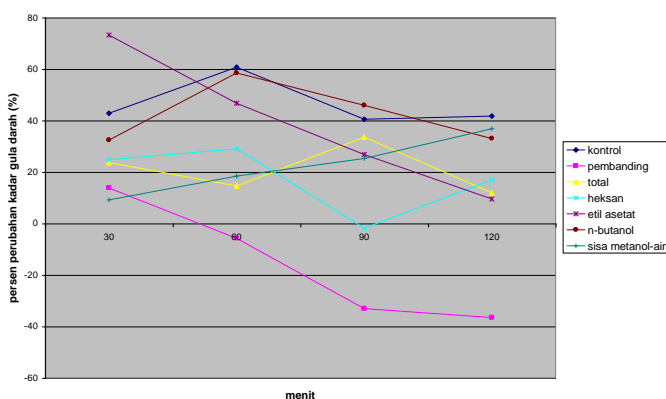


Figure 2. Percentage of glucose concentration change before and after treatment with seeded extract of *M. charantia*

Based on the experiment data, showed that antihyperglykemic effect of flashed and seeded extract on dosage of 250mg/Kg b.w. was lower than comparison group (gibenklamid). However, the hexane fractions had decreased glucose concentration significantly.

Based on identification of phytochemistry showed that all of flashed and seeded extract fraction (except of fleshed methanol-water residue) contain terpenoid groups. However, this test cannot distinguish between terpenes itself and glycosides terpenes. Meanwhile on the flashed extract was also found flavonoid groups. The phytochemistry test proved that flesh and seed of *M. charantia* is a source of terpenoid which also been indicated by its IR spectrum.

Its spectrum IR showed two models of spectrum. Firstly was shown in hexane fraction which has strong absorbance at wave number of 2923.9 cm^{-1} and 2854.5 cm^{-1} correspond to stretching of aliphatic C-H bondings. This data was also supported with strong absorbance at 1465.8 dan 1377.1 cm^{-1} due to bending of aliphatic C-H bondings. As well maximum absorbance at 1735,8 cm^{-1} indicated of presence of carbonyl group as an ester. Based on the spectrum can be resulted that both of flashed and seeded hexane fractions was predicted contain major group of unglycosides terpenes. The second IR spectrum model was presented by flashed and seeded polar fractions. The flashed ethyl acetate fraction spectrum absorbed wave number at 3402.2 cm^{-1} correspond into hydroxyl groups which predicted come from -OH sugar binding into terpenoid structures. The presence of terpenoid structures was shown by strong absorbance at 2935.5 cm^{-1} correspond to aliphatic C-H stretching and C-H bendings at 1384.8 and 1222.8 cm^{-1} . The presence of carbonyl group was also indicated by absorbance at 1778.2 and 1720.4 cm^{-1} . Based on this spectrum can be predicted that fraction of ethyl acetate and other polar contain glycosides terpenes as the major compounds. Related on sugar tolerance and phytochemistry tests from each fractions can be identified that compounds active which lead to decrease glucose concentration on rat blood is unglycosides terpenes.

CONCLUSION

Based on sugar tolerance and phytochemistry tests into ten fractions of extract *M. charantia* can be identified that hexane fraction is the most active part. It contain active compounds which lead to decrease glucose concentration on rat blood. Moreover correspond to IR spectrum and colored Lieberman Buchard method, this active fraction contain unglycosides terpenes compound as an active ones.

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