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Research Article

The Effect of Combined Extracts of Sappan Wood (*Caesalpinia sappan* L.) and Gotu Kola (*Centella asiatica* L.) in Improving Diabetic Condition in Rats

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ABSTRACT

This study aimed to determine the efficacy of combination of sappan (secang) wood and gotu kola extracts in reducing insulin resistance and Malondialdehyde (MDA) levels in diabetic rats induced by Streptozotocin (STZ) 65 mg/kg Body Weight (BW) and Nicotinamide (NA) 230 mg/kg BW. Forty-two male Sprague Dawley rats weighing ±200 g were divided into 7 groups: 1) control, 2) glibenclamide 0.45 mg/kg BW, 3) sappan wood extract (CS) 250 mg/kg BW, 4) gotu kola extract (CA) 500 mg/kg BW, 5) 1st combination of extracts of sappan wood and gotu kola (CSCA1) 125 mg/kg BW + 750 mg/kg BW, 6) 2nd combination (CSCA2) with 250 mg/kg BW + 500 mg/kg BW, and 7) 3rd combination (CSCA3) with 375 mg/kg BW + 250 mg/kg BW. The insulin resistance levels were measured using the HOMA-IR index based on fasting blood glucose and insulin. The Thiobarbituric Acid Reactive Substance (TBARs) method was used to measure MDA levels. All measurements were taken before treatment, 14 days after treatment, and 21 days after treatment. The group receiving CSCA3 showed significant reduction in insulin resistance (-3.32±0.05) and MDA levels (-2.04±0.37 nmol/ml) on Day 21 after treatment. The CSCA3 treatment did not show statistically different result compared to glibenclamide treatment ($p>0.05$). Hence, CSCA3 treatment was considered as the best proportion of sappan wood and gotu kola extracts mixture and the result is comparable to glibenclamide. This study shows that the combination of sappan wood and gotu kola extracts has the potential to be developed as a functional drink for people with diabetes.

INTRODUCTION

An increase in blood glucose levels caused by impaired insulin production, insulin resistance, or both, as well as carbohydrate, lipid, and protein metabolism abnormalities, is the most prevalent symptom for Diabetes Mellitus (DM) (World Health Organization 2019). A survey by the International Diabetes Federation revealed that Type 2 Diabetes Mellitus (T2DM) accounts for 90% of all cases of diabetes. Diabetes causes approximately 4.2 million deaths among individuals aged 20 to 79 years old in 2019. The global prevalence of diabetes is expected to be around 463 million people (9.3%) in 2019 and it will rise to 700.2 million people (10.9%) in 2045, which is a 51% increase (International Diabetes Federation 2019).

Diabetes causes macrovascular problems such as cardiovascular disease, peripheral vascular disease, and cerebrovascular disease leading to morbidity and mortality. Diabetes also frequently causes microvascular problems such as retinopathy, nephropathy, neuropathy, chronic disease, and diabetic ulcers (Silva *et al.* 2017). All of these health issues lower the quality of life and productivity of the human resources, therefore causing increasing burden of health care costs and lowering workforce productivity both for the patient and their caretaker in a country.

Insulin Resistance (IR) is one of the main pathogenesis of T2DM and it is frequently undiagnosed due to the lack of physical symptoms when it arises. A cohort study by Wang *et al.* (2020) found a stronger association of diabetes incidence among adults in China with insulin resistance than with pancreatic beta cell dysfunction. The body is unable to utilize the insulin in hyperglycemia condition caused by disruption of insulin receptors. The pancreas then compensates for the loss of insulin synthesis, resulting in hyperinsulinemia. T2DM development later results in pancreatic beta-cell dysfunction and decreased insulin production (Saisho 2015). Thus, IR is an important marker for T2DM diagnosis and treatment.

The development of T2DM is also affected by oxidative stress. Hyperglycemia causes an increase in free radicals, particularly the type of Reactive Oxygen Species (ROS) in all body tissues. The presence of high free radicals in the body can cause lipid peroxidation in cell membranes, resulting in the formation of Malondialdehyde (MDA). MDA is a carcinogenic secondary product that is more persistent than other aldehydes, so it is the ideal indicator for oxidative stress on lipids (Ayala *et al.* 2014).

Metformin and sulfonylureas (glibenclamide, glycuidone, glicazide, and glimepiride) are two oral anti-diabetic medications that T2DM patients in Indonesia regularly take. However, they often suffer from side effects of these medications such as nausea and hypoglycemia (Putra *et al.* 2017). So, there is a need to develop innovative treatments for T2DM that have fewer side effects, are less toxic, and inexpensive.

High level antioxidant activity has been found to have a therapeutic impact for T2DM. Antioxidants serve in the body's defense system, trapping oxidants, producing inflammatory mediators, repairing damaged molecules, and beginning and enhancing endogenous antioxidant production (Adwas *et al.* 2019). Antioxidants are required to improve insulin sensitivity and oxidative stress conditions.

Sappan wood (*Caesalpinia sappan* L.) and gotu kola (*Centella asiatica* L.) are known as natural ingredients proven to be effective in treating T2DM due to their strong antioxidant capacity. Both of the plants have been used as functional drinks for people with T2DM (Badan Pengawasan Obat dan Makanan 2016; Fitriyanti *et al.* 2020). Many people combine plants or other ingredients that have a synergistic effect or are more potent than a single ingredient in traditional drink recipes to reduce side effects. However, there has been no research reported on the effect of combination of sappan wood and gotu kola as two natural components on DM. Therefore, to fill the gap of knowledge the research team analyzed the effect of combined extracts of sappan wood and gotu kola on fasting blood glucose, insulin levels, insulin resistance, and MDA levels in diabetic rats induced with STZ and NA. The researchers expected that this study could show the potentials of combination of sappan wood and gotu kola as a functional drink in improving T2DM conditions as compared to functional drink without the combination of both extracts.

METHODS

Design, location, and time

The study design was a randomized control group pretest-posttest design. The material origin and extraction process located in Materia Medika Batu, Malang City, Indonesia. The research on experimental animal was carried out at The House of Experimental Rats, Center for Food and Nutrition Studies, UGM, DIY Yogyakarta. The research was carried out from June to July 2021 with ethical clearance by The Research Ethics Committee of Faculty Medicine, Universitas Sebelas Maret No 36/ UN27.06.6.1/ KEP/EC/2021 and ID: 01/02/04/38.

Materials and tools

The materials for the treatment were sappan wood and gotu kola extracted with 96% ethanol as solvent. Modeling of hyperglycemia was done by inducing Streptozotocin (STZ) dissolved in citrate buffer and Nicotinamide (NA) dissolved in saline. Fasting Blood Glucose (FBG) was measured by the Glucose GOD FS Kit (DiaSys, Germany). Insulin levels was measured by mouse insulin ELISA Kit (FineTest, China). H_3PO_4, TBA, methanol, distilled water, and 1,1,3,3-Tetraethoxypropane (TEP) were used for measurement of MDA levels.

The tools used in this study were rotary evaporator, reciprocating shaker, syringe, oral gavage, microhematocrit, waterbath, cooling bath, centrifuge, microplate reader, cuvette and Uv-Vis spectrophotometer.

Procedures

Extraction of sappan wood and gotu kola. Sappan wood and gotu kola were washed and dried before being mashed and processed into simplicia. The extraction method used was maceration. Sappan wood was diluted with 96% ethanol as solvent in a ratio of 1:9 for 120 hours, while gotu kola was diluted with a ratio of 1:5 for 96 hours and stirred at room temperature. The amount of solvent was adjusted to the simplicia and must be completely submerged. Then, each was filtered with filter paper. The filtrate was evaporated at speed of 100 rpm and temperature of 60°C. The evaporation of sappan wood and gotu kola took 4 hours and 2 hours, respectively. The evaporation was stopped when the solvent was no longer dripping and the extract has thickened. The result of extracts was thick liquid with a yield of 10.71% from 700 g of sappan wood simplicia, while the extract of gotu kola leaves obtained a yield of 15.71% from 700 g of gotu kola simplicia.

Animal study. Total number of samples in this study was 42 male white rats (*Rattus novergicus*), Sprague Dawley strain. The rats were 8−10 weeks old and weighed ±200 g. The rats were acclimatized for 7 days under standard animal housing conditions (with the temperature was controlled at 25±2°C and maintained with 12 light-dark cycle) and were given standard AD II feed of 10−20 g and *ad libitum* water. T2DM modeling for all rats was done by intraperitoneal injection of 230 mg/kg BW of NA dissolved in 2 mg/200 g BW of saline. After 15 minutes, 65 mg/kg BW of STZ dissolved in 2 mg/200 g BW of citrate buffer was also injected (Muhlishoh *et al.* 2019). Hyperglycemic conditions (>150 mg/dl) were obtained within 72 hours (Ghasemi *et al*. 2014). Then, randomization was done where the rats were divided into 7 groups; each group was given different treatments: distilled water (Control), Glibenclamide 0.45 mg/kg BW (Glibenclamide), sappan wood extract (CS) 250 mg/kg BW, gotu kola extract (CA) 500 mg/kg BW, combination of sappan wood and gotu kola extracts 1 (CSCA1) 125 mg/kg BW + 750 mg/kg BW, combination 2 (CSCA2) 250 mg/kg BW + 500 mg/kg BW, and combination 3 (CSCA3) 375 mg/kg BW + 250 mg/kg BW. The doses of CA (500 mg/kg BW) and CS (250 mg/kg BW) in this study were based on previous studies by Fitrianda *et al.* (2017) and Sakir and Kim (2019) that showed a significant and same effect. The combination doses based on trial. The treatment was administered by using oral gavage for rats for 21 days. The Animal handling during treatment was the same as during the acclimatization phase.

Blood was drawn through the eye vein (orbital sinus) using the retro-orbital plexus method 4 times: before injecting STZ and NA, before treatment (0 day), 14 days and 21 days after the treatment. Microhaematocrit was scrapped on the medial canthus (under the eyeball towards the foramen poticus) and rotated 4 times to injure the plexus. Then, the blood was centrifuged at 1,000 rpm ±10 minutes at 40°C to obtain the supernatant/serum.

Fasting blood glucose (FBG) levels measurement. FBG levels were measured by the GOD-PAP (Enzymatic Calorimetric Test of Glucose Oxidase Phenol 4-Aminophenazone). The method applied was according to Subiyono *et al.* (2016).

Insulin levels measurement. Insulin levels were checked by reacting serum with monoclonal anti-mouse insulin (antibodies) that had been coated in microplate wells and the reagents provided in the mouse insulin ELISA kit. The procedure for analysis was performed following the protocol specified for the kit (FineTest, China).

Insulin resistance measurement. The assessment of insulin resistance was based on the FBG levels and insulin levels. Assessment of insulin resistance used a simple method using the HOMA-IR calculation formula (Fitriyanto *et al.* 2020).

$$\text{HOMA} - \text{IR} = \frac{\text{fasting blood sugar level (mg/dl) x fasting insulin level } (\mu g/l)}{405}$$

Malondialdehyde (MDA) levels measurement. MDA levels were measured with serum as a sample, standard, and blank using the Thiobarbituric Acid Reactive Substance (TBARs) method according to the method used by (Zainuddin *et al.* 2019).

Data analysis

All data obtained were presented as mean and standard deviation. Analysis was done by using SPSS (IBM, version 23) with one-way ANOVA statistical test, followed by post-hoc Tukey HSD test and Games-Howell test with a significant value of $p<0.05$.

RESULTS AND DISCUSSION

The results of this study indicate that there is a significant effect of the combination treatment of sappan wood extract and gotu kola in decreasing fasting blood glucose levels, and insulin resistance based on HOMA-IR index, and MDA levels as well as an increase in insulin levels in T2DM rats.

Fasting blood glucose levels. Table 1 shows that fasting blood glucose levels in all groups before STZ NA induction were normal. Fasting Blood Glucose (FBG) levels experienced a significant increase in all groups after STZ-NA induction (before each group was treated). This proves the success after 72 hours of STZ (65 mg/kg bw) and NA (230 mg/kg bw) induction. STZ and NA cause delayed onset of diabetes through β cell damage and immunologic reactions. STZ is a diabetagonic agent that causes damage to pancreatic β cells while NA aims to protect the cytotoxic effects of STZ (Szkudelski *et al.* 2013). NA is a derivative of vitamin B3 (niacin) which functions to increase the concentration of NAD+ or partially inhibit PARP-1 (Kishore *et al.* 2017). The data between groups before or and on day 0 after STZ-NA induction did not differ significantly, indicating the success of randomization and the results were considered homogeneous.

The results showed that all treatments groups experienced a significant decrease in FBG levels after 14 and 21 days of treatment, except for the control group. After 21 days of treatment, there was a much greater decrease in FBG levels closely to normal FBG levels (before STZ-NA induction) than the decrease at 14 days after treatment. Blood glucose levels did not decrease in the control group because STZ inhibited the Krebs cycle so that ATP production in the mitochondria was limited and continuously reduced pancreatic β cell nucleotides as reported by (Szkudelski *et al.* 2013).

The combination treatment showed that the CSCA1 treatment was not significantly different from the CA treatment. CSCA2 treatment was also not significantly different from CS in reducing FBG levels after 14 days of treatment. However, after 21 days of CSCA2 treatment the FBG levels was significantly reduced compared to CS, CA, or CSCA1. While CSCA1 treatment still showed no difference with CA, even though it was given for 21 days. This shows that the proportion of CSCA1 is not better in reducing FBG levels than the treatment without the combination (CA or CS). CSCA3 treatment showed the most reduction in FBG levels among other treatments. CSCA3 treatment for 21 days decreased the FBG levels (69.73%) more than 14

Table 1. The effect of combined extracts of sappan wood and gotu kola on fasting blood glucose levels

Group	Mean±SD (mg/dL)			
	Before STZ-NA	Day 0	Day 14	Day 21
Control	70.81±1.04	267.92±3.33	271.01±3.91^{d}	273.39±3.51^{e}
Glibenclamide	72.46±2.24	267.26±5.01	103.89±3.73^{a}	84.04±4.29^{a}
CS	70.22±2.62	265.22±4.36	137.03±3.44^{b}	114.76±3.79^{c}
CA	70.22±2.88	267.86±5.30	152.39±4.09^{c}	127.50±3.63^{d}
CSCA1	69.23±2.84	264.98±4.17	146.82±3.69^{c}	122.44±2.74^{d}
CSCA2	70.42±3.24	264.80±3.88	135.60±3.76^{b}	101.36±2.63^{b}
CSCA3	70.81±2.54	264.98±4.78	103.82±3.10^{a}	82.73±2.06^{a}
p	0.529	0.698	0.000	0.000

CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Tukey's post-hoc test; SD: Standard Deviation

days of treatment (61.69%) in the control group. CSCA3 treatment was not significantly different from Glibenclamide after 14 and 21 days of treatment. This shows that CSCA3 treatment is the best combination proportion with the a comparable ability as Glibenclamide in reducing FBG levels in T2DM rats.

This decrease in FBG levels occurs because the bioactive compound of the two ingredients that are thought to be effective as antidiabetic substances. It is caused by the positive effect of antioxidants such as flavonoids in both ingredients. Flavonoids are the most popular compounds for antioxidants because of their ability to breakdown free radicals and modulate signals to several cells. Flavonoids work by activating the Phosphoinositide 3-kinase (PI3K/AKT) pathway, inhibiting gluconeogenesis, and stimulating glycogen synthesis. Flavonoids work by activating the synthesis and translocation of Glucose Transporter Type 4 (GLUT4), increasing hexokinase activity in the liver, reducing the occurrence of pancreatic β cell apoptosis, activating PPARϒ expression to improve glucose uptake, activating the AMPK pathway, inhibiting tyrosine kinase activity, and activating NF-kB (Al-Ishaq *et al.* 2019).

Gotu kola also has main compounds that have potential as antioxidants, especially its asiaticoside and asiatic acid. Thipkaew *et al.* (2012) reported that asiaticoside has an antioxidant effect on neuropathy in diabetic rats. Another study reported that asiatic acid also works as an antidiabetic agent through increased glycolysis by restoring the activity of enzymes such as hexokinase, Glucose-6-Phosphate Dehydrogenase (G6PDH), and pyruvate kinase and found a decrease in glycogen in the liver in STZ-induced diabetic rats (Ramachandran & Saravanan 2013).

Insulin levels. All treatment groups experienced a significant increase in insulin secretion both at 14 and 21 days after treatment, except in the control group (Table 2). Without any treatment, the function of pancreatic β-cells in the control group is disrupted so that they are unable to produce enough insulin to compensate for insulin resistance (Saisho 2015).

The combination treatment showed that CSCA1 was not significantly different from CA after 14 and 21 days of treatment. CSCA2 treatment was also not significantly different from CS in terms of increasing insulin level. This showed that CSCA1 treatment was not better than CA and CSCA2 treatment was not better than CS treatment. On the other hand, CSCA3 treatment was able to increase insulin levels the most after 14 days (22.34%) and 21 days

Table 2. The effect of combined extracts of sappan wood and gotu kola on insulin levels

Group	Mean±SD (pg/dL)		
	Day 0	Day 14	Day 21
Control	421.74±6.89	417.38±6.09^{a}	413.92±5.66a
Glibenclamide	426.47±5.50	515.38±5.91^{e}	547.01±5.74^{d}
CS	425.56±8.21	484.65±8.12^{cd}	514.65±8.40^{c}
CA	428.47±7.75	476.29±7.68^{bc}	499.01±8.92^{b}
CSCA1	421.20±4.91	469.01±5.84^{b}	488.83±7.12^{b}
CSCA2	421.20±4.40	492.11±6.38^{d}	524.65±4.29^{c}
CSCA3	424.65±6.04	510.65±6.42^{e}	542.65±5.70^{d}
p	0.323	0.000	0.000

CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Tukey's post-hoc test; SD: Standard Deviation

of treatment (31.09%) compared to the control group. The result form CSCA3 treatment was not significantly different from Glibenclamide treatment. This means CSCA3 treatment is the best proportion of combination from both extracts and it has comparable result to Glibenclamide in increasing insulin levels.

Impaired Insulin Receptor Substrate (IRS) production, decreased the GLUT-4 translocation, and glucose oxidation cause a decrease in insulin levels, thus glucose unable to enter cells and remains in circulation (hyperglycemia) (Lee 2006 in Nurhidajah & Nurrahman 2017). Increased insulin levels are thought to be because of a positive effect on insulin sensitivity, thereby the increasing secretion of insulin after treatment. Compounds that play a role in insulin secretion, especially through the Calcium (Ca) pathway, are flavonoids (Al-Ishaq *et al.* 2019). The increase in Ca ions in the cytoplasm of pancreatic β-cells will cause insulin secretion.

Brazilin is the main ingredient in sappan wood, member of flavonoid group and works specifically in inhibiting protein kinase C and insulin receptor serine kinase which plays a role in the regulation of insulin signaling. Brazilin induces GLUT4 from intracellular storage areas to plasma membrane via PI3K activation without affecting protein and GLUT4 synthesis (Nirmal *et al.* 2015). Asiatic acid has also been shown to increase insulin secretion by enhancing the PI3KT/Akt signaling pathway. Moreover, these compounds also improve glucose response by increasing muscle protein GLUT-4, IRS, IRS-1, and IRS-2 as reported by Ramachandran and Saravanan (2013 & 2015).

Insulin resistance. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is an insulin resistance biomarker that uses fasting plasma glucose and insulin concentration to determine insulin sensitivity. The HOMA-IR is simple, efficient, and validated for evaluating insulin resistance against DM. The study results showed that all groups, except the control group, experienced a significant decrease in HOMA-IR after 14 and 21 days of treatment (Table 3). The results in the control group are not different from the result in a study conducted by Wang *et al.* (2020) which reported that there was no significant difference in the HOMA-IR index in patients with duration of T2DM of less than 1 year and patients with duration of T2DM of 30 years.

The combination treatment showed that the CSCA1 and CSCA2 treatments were not significantly different from the CA and CS treatments after 14 days and 21 days of treatment. This shows that the proportion of the combination

Table 3. The effect of combined extracts of sappan wood and gotu kola on HOMA-IR index

Group	Mean±SD (nmol/mL)		
	Day 0	Day 14	Day 21
Control	8.37±0.23	8.38±0.24^{d}	8.38 ± 0.21d
Glibenclamide	8.44±0.24	3.96±0.14^{a}	3.40±0.16^{a}
CS	8.36±0.24	4.92±0.19^{b}	4.37±0.20^{bc}
CA	8.50±0.30	5.37±0.21^{c}	4.71±0.18^{c}
CSCA1	8.26±0.17	5.10±0.12^{bc}	4.43±0.08^{c}
CSCA2	8.26±0.11	4.94±0.08^{b}	3.94±0.12^{b}
CSCA3	8.33± 0.10	3.92±0.08^{a}	3.32±0.05^{a}
p	0.423	0.000	0.000

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Tukey's (day 14) and Games Howell (day 21) post-hoc test; SD: Standard Deviation

of CSCA1 and CSCA2 is not better in reducing the HOMA-IR index than the treatment without the combination (CA and CS treatment). The treatment that showed the ability to reduce the HOMA-IR index the most was CSCA3. This treatment was not significantly different from Glibenclamide treatment after 14 and 21 days. CSCA3 treatment, compared to the control group, was able to reduce HOMA-IR as much as 61.69% after 14 days of treatment and 69.73% after 21 days of treatment.

High HOMA-IR index indicates disruption of the uptake and use of glucose by the body cells, resulting in an increase in blood glucose levels. In this study, the increase in insulin ability was thought to be caused by the repair response of target cells (muscle, adipose, and liver) to activate the use of glucose in cells as indicated by resistance values based on the HOMA-IR index which decreased after treatment. CSCA3 treatment showed the most optimal decrease in insulin resistance values and was not significantly different from Glibenclamide.

Malondialdehyde levels. The MDA levels of all groups showed a significant decrease after 14 days and 21 days of treatment, except the control group (Table 4). The control group after 14 days of treatment did not show a significant difference, in fact, there was a significant increase after 21 days of control treatment. This is thought to be due to the occurrence of excessive metabolic stress as a result of the development of T2DM conditions through several metabolic pathways; polyols, hexosamines, Advanced Glycation End Products (AGEs), and Protein Kinase Activation (PKC). Moreover, an increase in MDA levels proves that STZ-NA induction increases the ROS levels. This happens through a shift in the balance of redox reactions due to changes in carbohydrate and lipid metabolism which in turn will increase the formation of ROS from glycation reactions and lipid oxidation. Thereby reducing the antioxidant defense system (Halliwell & Gutteridge 2015). STZ is a source of free radicals, damaging DNA and causing cell death. A study reported a significant increase in MDA levels and decreased endogenous antioxidant enzyme activity after STZ induction (Husna *et al.* 2019).

The results showed that the CSCA1 and CSCA2 treatments were not significantly different from the treatment without the combination, namely, CS treatment after 14 days of treatment. However after 21 days of treatment, CSCA2 significantly reduced MDA levels compared to CS and CSCA1. These results indicate that the proportion of CSCA1 combination is not better at reducing MDA levels than the treatment without the combination, namely, CS. The CSCA3

Table 4. The effect of combined extracts of sappan wood and gotu kola on MDA levels

Group	Mean±SD (nmol/mL)		
	Day 0	Day 14	Day 21
Control	9.61±0.30	9.84±0.28^{e}	10.06±0.27^{e}
Glibenclamide	9.28±0.34	3.63±0.44^{a}	2.32±0.24^{a}
CS	9.04±0.43	5.48±0.53^{bc}	3.91±0.16^{c}
CA	8.51±0.38	6.84±0.23^{d}	4.70±0.20^{d}
CSCA1	8.88±0.25	6.36 ±0.13^{c}	3.90±0.18^{c}
CSCA2	9.26±0.53	5.18± 0.15^{b}	3.25±0.46^{b}
CSCA3	9.49±0.30	4.45±0.22^{a}	2.04±0.37^{a}
p	0.082	0.003	0.000

MDA: Malondialdehyde; CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Games Howell (day 14) and Tukey's (day 21) post-hoc test; SD: Standard Deviation

treatment showed the most reduction in MDA levels among the other treatments and showed comparable results to Glibenclamide treatment at both 14 and 21 days after treatment. This indicates that the proportion of CSCA3 treatment is the best combination of sappan wood extract and gotu kola for lowering MDA levels, and is similar to Glibenclamide. CSCA3 treatment for 21 days suppressed MDA levels (60.38 %) more than the control group's and (53.22 %) more at 14 days treatment.

Bioactive components of CSCA3 treatment are thought to be more effective in lowering the production of free radicals like ROS and other oxidants, inhibiting lipid peroxidation, and improving pancreatic cell injury in diabetic rats than other treatments. Brazilin has anti-inflammatory properties that prevent the formation of NO and iNOS (Nirmal *et al.* 2015). According to the study in metabolic syndrome rats by Pakdeechote *et al.* (2014), increased bioavailability of asiatic acid helped reduce ROS and other proinflammatory cytokines. Furthermore, it is claimed to be able to inhibit lipid peroxidation and a number of proinflammatory cytokines due to an increase in FFA in T2DM patients. Lower MDA levels indicate inhibition of lipid peroxidation. Muchtaromah *et al.* (2016) found that antioxidant overload increased MDA levels in diabetic rats, suggesting that an effective dose and duration of administration is required.

CONCLUSION

The combination of sappan "secang" wood extract and gotu kola had a significant effect in reducing insulin resistance and MDA levels in the rat models. The CSCA3 treatment, which was a combination of sappan wood extract 375 mg/kg BW, and gotu kola extract 250 mg/kg BW, was selected as the optimal dose to improve the condition of diabetic rats. Administration of CSCA3 for 14 days resulted in significant decrease in insulin resistance with decrease of HOMA-IR index by 61.69% and MDA levels by 53.22% as compared to the control group. More prominent decrease occurred on Day 21 with a decline of 69.73% for HOMA-IR and 60.38% for MDA level as compared to the control group. This study shows that combination of sappan wood and gotu kola has a potential to decrease oxidative stress and increase insulin secretion as well as insulin sensitivity in T2DM better than the administration of each extract alone without combination.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest with the parties involved in this research.

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