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Research Article

Potential combined effect of *Spirulina platensis* and *Momordica charantia* fruits on attenuation of isoproterenol-induced myocardial infarction in rats: identification and prediction its mechanism

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Abstract

We established *in vivo* models of isoproterenol-induced myocardial infarction (MI) to determine the cardioprotective effect of *Spirulina platensis* (Spi), *Momordica Charantia* fruit (MC) and their combination. MI was induced in six groups of Male Wistar albino with isoproterenol, and cardioprotection was evaluated by measuring SGOT, SGPT, LDH, CK, CK-MB with commercially available test kits and analyzing histopathology. Online PASS was used to predict the mechanism of action of a marker compound in Spi and MC. Rat serum levels of LDH, SGOT, CK-MB, and CK were significantly reduced by the Spi and MC combination extract ($P < 0.05$). The combination extract at a dose of 50:50 mg/kg body weight preserve the integrity of the myocardial cell membrane. Identified compounds by LC-MS/MS in the ethanol extract of *Momordica charantia* are (3β,16α-Dihydroxy-lanosta-8,24-dien-21-oic acid), Eclalbasaponin II, Epianhydrobelachinal, Epianhydrobelachinal, Paeonenolide F, Ziyu glycoside II. Identified compounds by LC-MS/MS in *Spirulina platensis* extract are d-Lirioferine, Lycopodine, Yuanhunine, Candidate Mass C18H26N2O5 and Candidate Mass C36H38N4O5. In PASS analysis, Phycocyanobilin's cardioprotective mechanism of action is predicted to be kinase inhibitor, cytoprotectant, and platelet aggregation inhibitor, with Probable activities (Pa) of 0.06, 0.53, and 0.443, respectively. The Pa values for cholesterol antagonist, proliferative disease treatment, nitric oxide scavenger, and anti-inflammatory agents in Momordicoside A are respectively 0.841, 0.666, 0.59, and 0.55. These findings suggest that the cardioprotective activity of the combination of Spi and MC extracts at a dose of 50 mg/kg was synergistic.

Keywords

cardioprotective, isoproterenol, LC-MS/MS, *Momordica charantia*, *Spirulina platensis*

Introduction

Myocardial infarction (MI), also known as a heart attack, is a disease caused by prolonged ischemia that causes irreversible death or necrosis of cardiac muscle (Mythili and Malathi 2015). According to WHO data, heart attacks and strokes account for up to 85 percent of all cardiovascular disease-related fatalities. According to estimates, 17.9

million deaths worldwide from cardiovascular disease occurred in 2016, contributing for 31% of all fatalities (WHO 2022). In 2030, cardiovascular disease is projected to continue to be the leading cause of death, affecting 23.6 millions of people (Chadwick Jayaraj et al. 2019).

A heart attack causes more severe damage to the heart, which can result in a decline in cardiac function and death (CDC 2021). Currently, there is no drug that can prevent heart organ damage; therefore, the goal of this research is to discover alternative drugs that can prevent more severe heart damage (cardioprotective) so that heart function can be preserved and death from heart attack can be prevented.

As a beta – adrenergic agonist, isoproterenol (ISO) can induce infarction by inducing oxidative stress in the myocardium and necrosis similar to heart muscle infarction Additionally, it has been demonstrated that ISO generates free radicals and promotes lipid peroxidation, both of which are thought to contribute to the long-term deterioration of cardiac membranes. To prevent or even treat myocardial ischemia, novel medications are required because current treatments have a minimal impact on survival and recurring costs (Murugesan et al. 2011).

Spirulina platensis (Spi) are cyanobacteria containing a variety of compounds including proteins, lipids, polysaccharides, and pigments. Phycocyanobilin (PC) is the primary pigment responsible for Spirulina's green-blue color (Fernández-Rojas et al. 2014). An imbalance between the antioxidant system and free radical material will result in inflammation and a total number of diseases, including atherosclerosis, cancer, neurodegenerative diseases, and diabetes mellitus. Internal and external sources of free radicals can have a significant impact on the biological molecules of the human body. In addition to Phycocyanobilin, *Spirulina platensis* contains compounds that contribute to its antioxidant capacity, including chlorophyll, carotenoids, phenolic compounds, polysaccharides, fatty acids and vitamins (Asghari et al. 2016). In human skin fibroblast cell lines, Spirulina can enhance migration and proliferating (Liu et al. 2019). Spirulina extract also promotes re-epithelialization and enhance the vascularity of wound healing (Bahei-Eldin et al. 2017).

The Cucurbitaceae family, of which *Momordica charantia* (MC) is a member, has a wide geographical distribution, and this plant contains numerous phytochemicals including triterpenes, flavonoids, and saponins (Bhowmik 2010). Several reports of *M. charantia's* biological activity, including anti-inflammatory, anti-carcinogenic, hepatoprotective effect, anti-viral, anti-tumor, immunomodulating, anti-mutagenic, anti-ulcer, anti-lipolytic, and antifertility properties (Jia et al. 2017). Traditionally, MC has been used to treat conditions such as hyperglycemia, cancer disease, a variety of infection disorders (Bhowmik 2010). In this study, we evaluated the prospective cardioprotective effects of *Spirulina platensis* extract, bitter melon (*Momordica charantia*) fruit extract, and their combination.

Materials and methods

Chemical

Ethanol 70% (Brataco), Isoproterenol (Sigma-Aldrich 5984-95-2), Paraffin (Merck K93120464), Hematoxylin (Sigma-Aldrich Co H9627-25G), Eosin Y (Sigma-Aldrich 230251), Entellan (Merck HX86597161), Kit Reagen CK-MB (Greiner Diagnostic GmbH No. 121 000), Kit Reagen LDH (Greiner Diagnostic GmbH No. 164 016), Kit Reagen SGOT (Greiner Diagnostic GmbH No. 141 000), Kit Reagen SGPT (Greiner Diagnostic GmbH No. 142 000).

Extraction of *Spirulina platensis* and *Momordica charantia*

Spirulina was acquired from the Center for Industrial Chemistry in Jakarta, while MC fruits were sourced from Manoko, Bandung. The collected plants are then processed in preparation to produce simplicia. The samples were rinsed with running water, drained, dried, and ground into powder before being sealed in a container. The obtained simplicia from MC fruits was extracted with 70% ethanol. In this study, extraction was performed via maceration using 70% ethanol as the solvent. The extraction will be repeated three times with a solvent to sample ratio of 1:10. To acquire a thick extract, the extract was filtered, and the solvent was evaporated using a rotavapor. In contrast, maceration of spirulina was performed using aqua bidestillata solvent, followed by freeze-drying to obtain a dry extract that could be used to assess its cardioprotective activity in vivo. The phytochemical examination of both extracts revealed the presence of their secondary metabolites.

Animal ethics

Ethical Concerns the Animal Research Ethics Committee of the School of Pharmacy at Institut Teknologi Bandung granted ethical approval for the investigation (No. 05/KEPHP-ITB/03-2021).

Experimental group

Male Wistar rodents obtained from the Institut Teknologi Bandung's Sekolah Ilmu dan Teknologi Hayati (SITH) laboratory. In the current study, 27 male Wistar rats weighing initially between 200 and 220 g were utilized. All the animals were confined in standard cages with four rats for 12 days. Prior to the experimental condition, animals were maintained at 222 °C, 45–55% constant humidity, and a twelve-hour light–dark cycle (07.00 with unrestricted access to meals and tap water). Wistar male rats were divided into 6 groups: (1) a negative control group administered Na-CMC carrier, (2) a positive control group administered Na-CMC carrier and induced isoproterenol, (3) a group administered ethanol extract of MC fruit at a dose of 100 mg/kg b.wt. and induced isoproterenol, (4) a group administered ethanol extract of MC fruit at a dose

of 300 mg/kg b.wt. and induced isoproterenol, (5) group administered spirulina extract at a dose of 100 mg/kg b.wt. and induced isoproterenol, (6) the group was administered a combination extract of MC fruit and spirulina with a total dose of 100 mg/kg b.wt. Selection of the dose using a low dose (100 mg/kg b.wt.) and a high dose (300 mg/kg b.wt.) and for the combination group using the lowest dose where the total dose of 100 mg/kg b.wt. is the total spirulina and bitter melon extract administered (1:1).

Cardioprotective effect test with isoproterenol induction

The Spirulina platensis, MC fruit, and combination test preparations were administered for 14 consecutive days. On days 13 and 14, intraperitoneal injections of isoproterenol solution were administered after dissolving 85 mg/kg isoproterenol powder in 0.9% NaCl. Based on research (Sharmila Queenthy et al. 2018), the induction of isoproterenol 85 mg/kg b. wt. twice with a 24-hour interval resulted in moderate to severe histological changes and significantly increased serum biomarker levels compared to control group. On day 14, following 24 hours of isoproterenol administration, blood was drawn, and animals were sacrificed.

Serum preparation and parameter measurement

On the 15th day after 24 hours of isoproterenol injection, intracardiac blood was extracted from rats. Serum levels of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Lactate Dehydrogenase (LDH), Creatine kinase (CK), and Creatine kinase myocardial band (CK-MB) were determined by centrifuging blood at 12,000 rpm for 10 minutes (Sukandar et al. 2019, p. kurniati). Levels were measured using a semi-automated clinical chemistry analyzer (Microlab 300) and commercial assay packages according to the manufacturer's instructions.

Histology evaluation

To evaluate the cardioprotective effect in rats, 10% neutral buffered formalin was used to fix heart samples. The samples were then embedded in paraffin blocks and sectioned to a thickness of 5 m. Hematoxylin and eosin (H&E) solution was used to stain slides (Argun et al. 2016) which is a strong antineoplastic agent, is limited due to its cardiotoxic side effects. Metformin is a drug with antihyperglycemic effects, and it has been shown to have a cardioprotective effect on left ventricular function in experimental animal models of myocardial ischemia. The present study investigated the cardioprotective effect of metformin in rats with doxorubicin cardiotoxicity.\n\nMethods:\nWistar albino rats were used in the study. Forty male, 10-week-old Wistar albino rats were randomly divided four groups. The control group rats were intraperitoneally administered saline solution twice a week, four doses in total. The doxorubicin group rats received doxorubicin (4 mg/kg, twice a week, cumulative dose: 16 mg/kg), and light microscopy was used to visualize the slides (Model: BX51; Olympus).

In-silico PASS analysis

Prediction of Activity Spectra for Substances is a computer-based program used to assess the potential activity of an active compound (PASS). The program predicts the biological activities of chemical structures, including phytochemicals, based on structure-activity relationships with known chemical entities (Jamkhande et al. 2016).

Spirulina platensis (Phychocyanin) and Momordica charantia (Momordicoside A) were used to predict the value of Pi (Probability to be inactive) and Pa (Probability to be active) using PASS Online and canonicahl SMILES input. Considered bioactive if the Pa value > 0.5 http://www.pharmaexpert.ru/passonline (Filimonov et al. 2014).

Identification *Momordica charantia* and *Spirulina platensis* using LC-MS/MS.

General description: Name of the solvent A: 0.1%FA/WA, Name of the solvent B: acetonitrile+0.1FA, Solvent Selection A: A1, Solvent Selection B: B1, Seal Cleansing Interval: 5,000 minutes, Gradient start: At injection, Pre-Injector Volume: 0 µL, High Pressure Limit: 18000 psi, Low Pressure Limit: 0 psi.

Type of ionization: ESI, Polarity: Positive, Acquisition Start Time: 0.00 min, Acquisition End Time: 17.00 min, Start Mass: 100.00 m/z, End Mass: 1200.00 m/z, Scan Time: 0.100 s, Low CE: 6.00 eV, High CE Ramp Start: 10.00 eV, High CE Ramp End: 40.00 eV, Cone Mode: Method Settings, Cone Voltage: 30 V, Collision Mode: Specific, Collision Energy: 6.00 eV.

Statistical analysis

The statistical method One-way ANOVA test ($p < 0.05$) was used to analyze the experimental results' parameters using the SPSS and Graphpad Prism applications.

Results

Phytochemical screening of *Momordica charantia* fruits extract and *Spirulina platensis*

The result of the phytochemical screening showed that both extracts had flavonoid and tannin compounds (Table 1). Compounds discovered in each of the examined extracts may have cardioprotective properties. It has been reported that flavonoids contain antioxidants that can combat free radicals by Reactive Oxidative Stress (ROS). Flavonoids additionally inhibit the activity of cyclooxygenase, lipoxygenase, and nitric oxide enzymes (Di Majo et al. 2014). Tannin contains antioxidants that inhibit the activity of free radicals and lipid peroxide (Nahdi et al. 2018).

Table 1. Result of phytochemical screening of extract sample.

Phytochemical screening	Sample	
	Ethanol extracts of *Momordica charantia* fruits	extracts of *Spirulina platensis*
Flavonoid	+	+
Saponin	-	-
Tannin	+	+
Quinone	-	-
Alkaloid	+	-

Description: (+) = detected. (-) = not detected.

Effect of *Spirulina platensis* and *Momordica charantia* fruits extracts on cardiac injury marker

To examine the cardioprotective effect of 14-day administration of extracts, we measured a number of biomarkers in cardiac injury, specifically: SGPT, SGOT, LDH, CK-MB and CK. In this study, the combination of *Spirulina platensis* and MC fruits demonstrated the greatest cardioprotective effective. Effect of extract of *Spirulina platensis* and MC fruits and their combination on cardiac injury markers during isoproterenol-induced myocardial infarction in rat demonstrates a decrease in biomarker level (Fig. 1). 100 mg/kg of b.wt. of MC fruit extract and 300 mg/kg b. wt only attenuated the level of LDH significantly ($p < 0.05$) compared to the positive control. MC fruits extract at doses 100 mg/kg b. wt. and 300 mg/kg b.wt were not significantly different, indicating that increasing the dose did not increase activity. At a dose of 100 mg/kg, *Spirulina platensis* extract attenuated amount of LDH and CK significantly ($p < 0.05$) compared to the positive control. In comparison to the positive control, the combination of *Spirulina platensis* and MC fruits significantly decreased the levels of all cardiac injury markers (LDH, SGOT, CK-MB and CK) ($p < 0.05$) compared to positive control except SGPT level. The administration of isoproterenol in this study was able to increase all markers of cardiac injury indicating MI condition, with the exception of SGPT and CK, which were not significantly different when compared to negative controls; however, descriptively, we observed an increase when compared to negative controls.

Histology evaluation

In normal rat (negative control group), the myocardial cell membrane was clearly intact (Fig. 2). The integrity of the myocardial cell membrane was altered in ISO-treated rats. All extract-treated groups exhibited similar results as negative control rats, except for extract MC Fruits at a dose of 300 mg/kg.

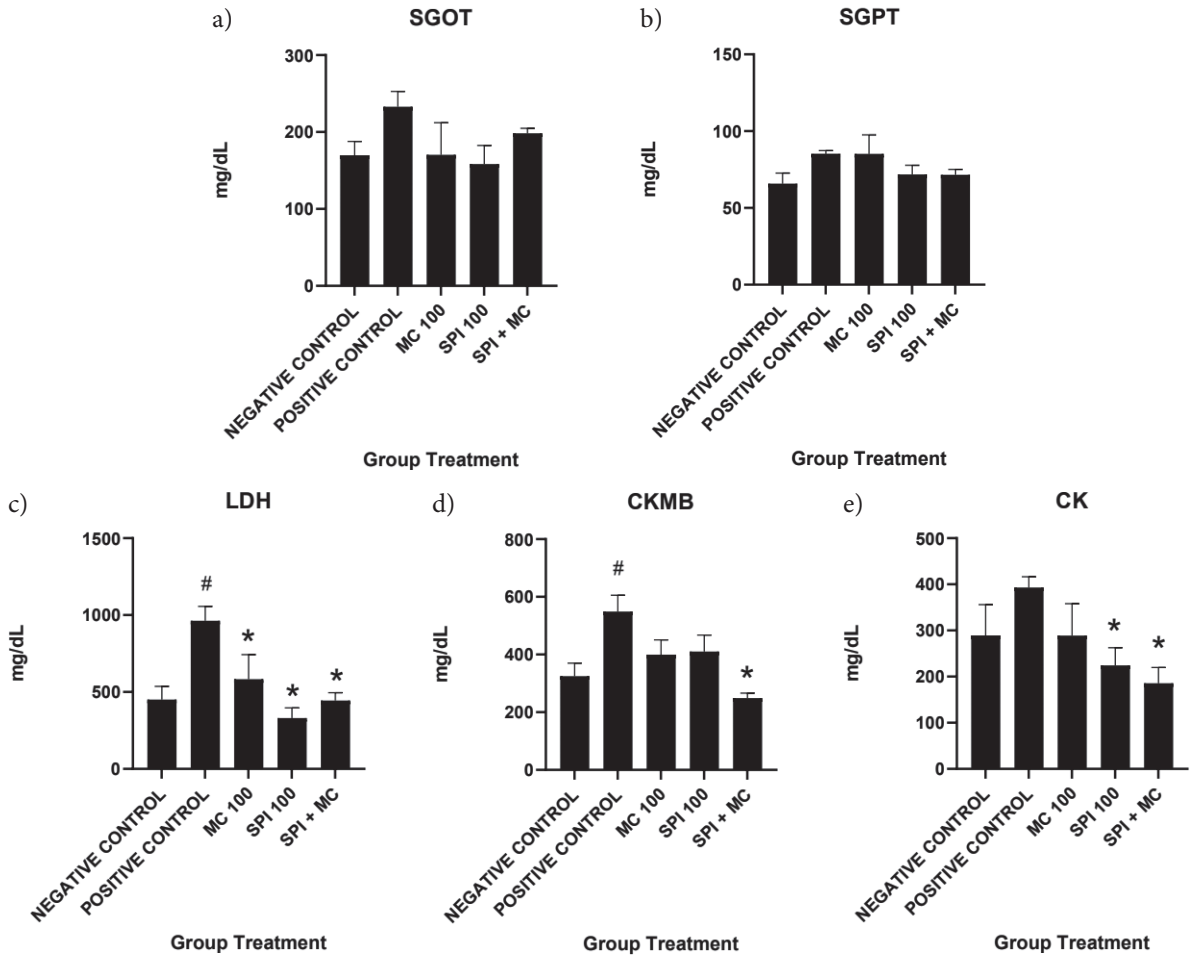


Figure 1. The levels of biomarkers affected by extract. Data is presented as mean (n = 4). Data analysis using one way-ANOVA test (# means $p < 0.05$ compared to the negative control and * indicates $p < 0.05$ compared to the positive control). **a.** SGPT level; **b.** SGOT level; **c.** CK level; **d.** CK-MB level, and **e.** LDH level.

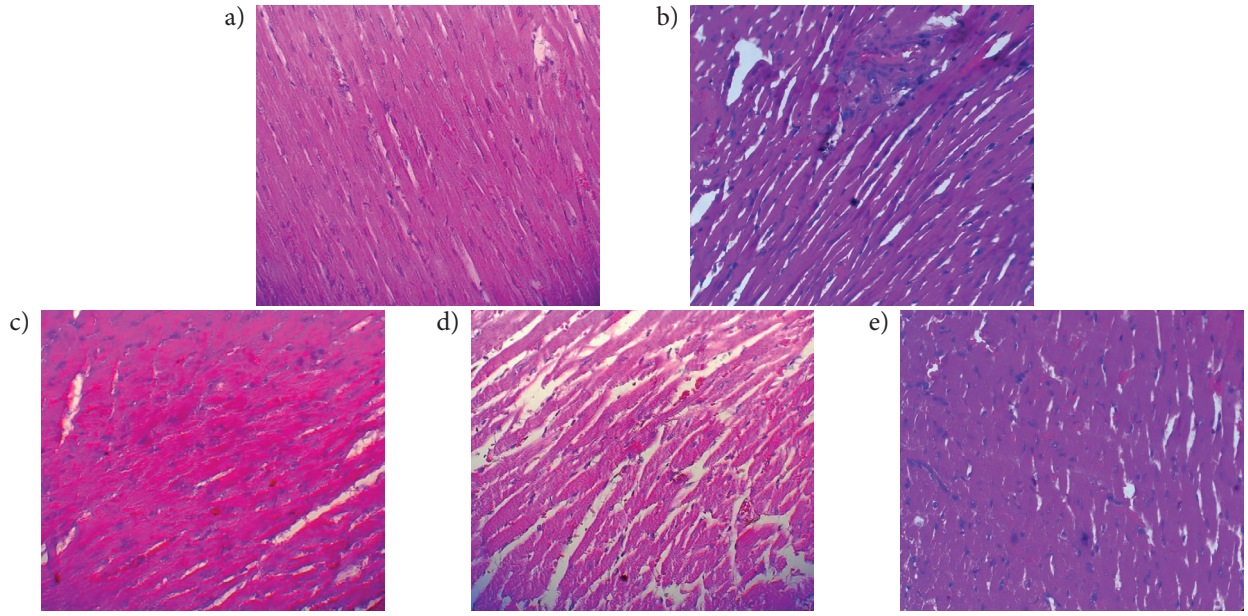


Figure 2. Histopathological observation of the heart using H&E staining. **a.** negative control; **b.** positive control; **c.** MC Fruit ethanol extract 100 mg/kg b.w.; **d.** Spirulina platensis extract 100 mg/kg b.wt., and **e.** Combination of MC Fruit 50 mg/kg b.wt and Spirulina platensis 50 mg/kg b.wt. Leukocyte infiltration was observed in the myocardium cells of the positive control group, whereas the negative control group had adequate integrity (arrow).

Cardioprotective prediction mechanism in phycocyanobilin and momordicoside A

Using an online PASS software, the biological activity spectra of Phycocyanobilin and Momordicine were evaluated. The actives were classified according to the Pa and Pi parameters, and the derived results (Table 2 and Table 3) were interpreted. At Table 2, predicted mechanism of action in Phycocyanobilin related to cardioprotective mechanism includes Kinase inhibitor, Cytoprotectant, Platelet aggregation inhibitor with Probable activity (Pa) values of 0,6; 0,535; 0,443, respectively. Furthermore, the predicted mechanisms of action in Momordicoside A that are associated with the cardioprotective mechanism are Cholesterol antagonist, Proliferative diseases treatment, Nitric oxide scavenger, and Antiinflammatory with Probable activity (Pa) values of 0.841, 0.666, 0.599, and 0.551, respectively (Table 3).

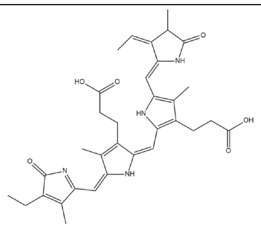
Identification of *Momordica charantia* and *Spirulina platensis* extract with LC-MS/MS

The results of the LC-MS/MS analysis can be used to describe the differences in the compound content of the ethanol extract of *Momordica charantia* and the water extract of *Spirulina platensis*. Peak chromatograms of compounds with varying molecular weights characterize the contents of these distinctions. LC-MS/MS (Liquid Chromatography Mass Spectrometry) analysis of the ethanol extract of *Momordica charantia* (Fig. 3 and Table 4) and *Spirulina platensis* extract (Fig. 4 and Table 5) revealed the presence of 5 compounds in each sample.

Discussion

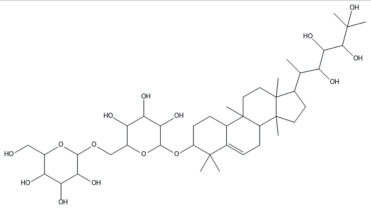
Myocardial infarction is strongly associated with mortality and morbidity (Chan and Ng 2010). MI refers to the loss of myocardial cells caused by prolonged ischemia. Reduced cellular glycogen and loosened myofibrils can occur within 10 to 15 minutes of the onset of ischemia (Thygesen et al. 2018). Cardioprotection incorporates all techniques and strategies that aid in maintaining heart healthy by reducing or preventing myocardial damage (Kubler and Haass 1996). Rise/fall of cardiac troponin detection can be used to asses MI condition and one of the following: 1) ischemia–related symptoms, 2) new pathological Q waves, 3) electrocardiogram alterations of new ischemia (Chan and Ng 2010).

Table 2. Activity of Phycocyanobilin in PASS analysis.

Structure	Pa	Activity
	0,878	Platelet derived growth factor receptor kinase inhibitor
	0,6	**Kinase inhibitor**
	0,583	HIF1A expression inhibitor
	0,535	**Cytoprotectant**
	0,443	**Platelet aggregation inhibitor**
	0,414	CYP2C19 inducer

* Bold sign indicates activity related to cardioprotective mechanism.

Table 3. Activity of Momordicoside A in PASS analysis.

Structure	Pa	Activity
	0,908	Hepatoprotectant
	0,841	**Cholesterol antagonist**
	0,778	Caspase 8 stimulant
	0,72	Apoptosis agonist
	0,666	**Proliferative diseases treatment**
	0,599	**Nitric oxide scavenger**
	0,558	**Antiinflammatory**

* Bold sign indicates activity related to cardioprotective mechanism.

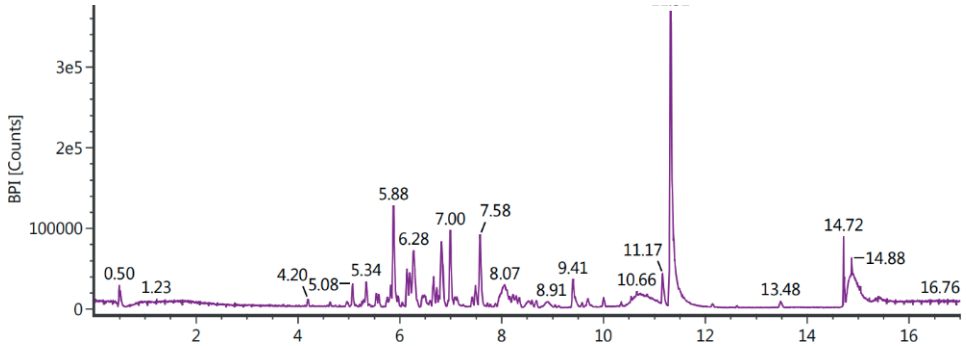


Figure 3. Chromatogram of *Momordica charantia* ethanol extract.

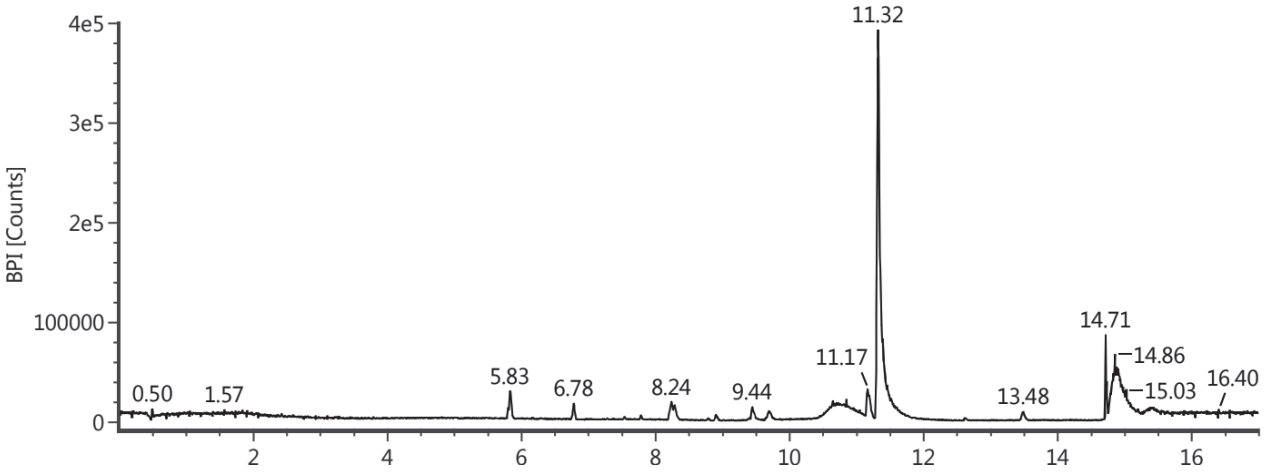


Figure 4. Chromatogram of *Spirulina platensis* extract.

Table 4. Identified compounds by LC-MS/MS in *Momordica charantia* ethanol extract.

Identified components	Observed m/z	Neutral mass (Da)	Retention time (Rt) in min
3β,16α-Dihydroxy-lanosta-8,24-dien-21-oic acid	495.3447	472.355	7
3β,16α-Dihydroxy-lanosta-8,24-dien-21-oic acid	495.346	472.355	7.58
Eclalbasaponin II	657.403	634.4	5.88
Epianhydrobelachinal	469.331	468.32	7.49
Paeonenolide F	511.341	510.33	6.15
Ziyu glycoside II	641.403	618.41	6.83
Total compounds	**5**		

Table 5. Identified compounds by LC-MS/MS in *Spirulina platensis* extract.

Identified components	Observed m/z	Neutral mass (Da)	Retention time (Rt) in minutes
d-Lirioferine (Lirioferine)	342.17	341.162	2.91
Lycopodine	248.201	247.193	7.18
Yuanhunine	356.185	355.178	3.37
Candidate Mass C18H26N2O5	351.192	350.184	2.13
Candidate Mass C36H38N4O5	607.292	606.284	11
Total compound	**5**		

Both the production of free radicals and the induction of lipid peroxidation by isoproterenol are likely to contribute to the long-term deterioration of cardiac membranes (Murugesan et al. 2011). After isoproterenol-induced myocardial infarction, elevated troponin I and urea, leukocyte and neutrophil, SGOT concentration, decreased catalase enzyme activity, and increased glutathione levels were all observed in the myocardium (Lobo Filho et al. 2011).

In addition to symptoms of ischemia, abnormal cardiac biomarkers serve as supporting evidence for diagnosing MI (Thygesen et al. 2018). In our study, the assessment of cardioprotective characterization was seen from changes in biomarker levels in MI conditions. Biomarker levels including

SGPT, SGOT, CK, CK-MB and LDH were anticipated to decrease after 14 days of pretreatment with extracts. We found that the administration of ISO has no effect on the level of SGPT. 100 mg/kg body weight and 300 mg/kg body weight of MC fruit extract only reduces LDH levels. In the past, the AST (SGOT) and LDH enzyme were originally employed to diagnose MI. However, because these enzymes lacked the optimal cardiac indicators, they were never utilized.

However, because they are limited and simple to assess in hospitals, along with the patient's medical history, they can provide doctors with a general understanding. CK-MB and CK enzyme measurements are indeed useful in the diagnosis of AMI (Aydin et al. 2019)increase the quality of life of patients, and decrease health expenditure in many countries. In this study, the advantages and disadvantages of the enzymatic and nonenzymatic biomarkers used in the diagnosis of patients with AMI are given in historical sequence, and some candidate biomarkers – hFABP, GPBB, S100, PAPP-A, RP, TNF, IL6, IL18, CD40 ligand, MPO, MMP9, cell-adhesion molecules, oxidized LDL, glutathione, homocysteine, fibrinogen, and D-dimer procalcitonin – with a possible role in the diagnosis of AMI are discussed.\n\nMethods\nThe present study was carried out using meta-analyses, reviews of clinical trials, evidence-based medicine, and guidelines indexed in PubMed and Web of Science.\n\nResults\nThese numerous AMI biomarkers guide clinical applications (diagnostic methods, risk stratification, and treatment. Statistical tests revealed no significant difference between the two concentrations of MC fruit extracts in their ability to reduce LDH levels, indicating that increasing the dose had no effect.

The organs with the highest concentration of LDH include the kidney, skeletal muscle, liver, and heart. Within six to twelve hours of the onset of chest pain, it rises, peaks within one to three days, and returns to normal within eight to fourteen days. Infarct size is correlated with total CK and CK-MB levels, which are important prognostic markers. This reaction is catalyzed by the enzyme CK, which contributes to the conversion of creatine and ATP into creatine phosphate and ADP. Approximately 20% of myocardium contains CK in the MB form (CK-MB), making the measurement of CKMB very useful for supporting data for the diagnosis of AMI (Aydin et al. 2019)increase the quality of life of patients, and decrease health expenditure in many countries. In this study, the advantages and disadvantages of the enzymatic and nonenzymatic biomarkers used in the diagnosis of patients with AMI are given in historical sequence, and some candidate biomarkers – hFABP, GPBB, S100, PAPP-A, RP, TNF, IL6, IL18, CD40 ligand, MPO, MMP9, cell-adhesion molecules, oxidized LDL, glutathione, homocysteine, fibrinogen, and D-dimer procalcitonin – with a possible role in the diagnosis of AMI are discussed.\n\nMethods\nThe present study was carried out using meta-analyses, reviews of clinical trials, evidence-based medicine, and guidelines indexed in PubMed and Web of Science.\n\nResults\nThese numerous AMI biomarkers guide clinical applications (diagnostic methods, risk stratification, and treatment.

Single extract of *Spirulina platensis* reduces CK and LDH levels, whereas single extract of MC fruit reduces only LDH levels. The evaluation of MC fruit extract at a dose of 300 mg/kg b. wt. revealed no significant difference compared to MC fruit extract at 100 mg/bw (data not shown). According to our findings regarding the cardioprotective effects of *Spirulina platensis*, MC fruits, and their combination extracts, the combination extracts of *Spirulina platensis* and MC fruits provided the greatest cardioprotective effect in rats with isoproterenol-induced MI. SGOT, CK, CK-MB, and LDH levels can be reduced by the combination of *Spirulina platensis* and MC fruit extracts. Natural product combinations are frequently advocated for medical objectives on the grounds that their "synergistic" interactions render them more potent than pure substances (Caesar and Cech 2019) approximately 18% of the U.S. population uses natural products (including plant-based or botanical preparations. Cardioprotective agents function as a pretreatment to reduce the incidence of necrosis or safeguard the heart in the event of a myocardial infarction (MI) (Chan and Ng 2010). Two extracts of Spirulina platensis and MC fruits may have a synergistic effect, particularly as cardioprotective, when combined.

The term "cardioprotective medications" refers to substances that protect the heart from the harmful effects of acute myocardial ischemia. Therefore, they must first prevent sudden cardiac mortality due to local myocardial ischemia; then, if possible, they must minimize the size of the developing infarct; and finally, they must reduce the likelihood of persistent infarct or infarct extension (Szekeres, 1987)such as sudden coronary death (SCD. Histological examination revealed that the myocardium cells in the negative group had excellent integrity, whereas in the positive control group administered isoproterenol, the myocardium cells exhibited leukocyte infiltration. In terms of cell integrity, integrity of the cell nucleus, and minimal leukocyte infiltration, the dose of 100 mg/kg b.w. of MC in the test group and the combination group produced the greatest results.

Using in silico PASS analysis prediction, we then attempted to determine the mechanism underlying the cardioprotective effect of *Spirulina platensis* and MC fruits extract. Prediction of the mechanism of action based on the main constituents of *Spirulina platensis* (Phycocyanobilin) and MC fruits extract (Momordicoside A). Potential cardioprotective activities in Phycocyanobilin include kinase inhibitor, cytoprotectant, and platelet aggregation inhibitor. Among Momordicoside A's putative cardioprotective activities are cholesterol antagonist, treatment for proliferative diseases, nitric oxide scavenger, and anti-inflammatory.

Myocardial infarction is associated with an inflammatory response, which is required for healing and scar formation (Frangogiannis et al. 2002). Myoglobin (Mb) is a key intracellular nitric oxide (NO) scavenger that regulates the antioxidant effect in coronary flow and cardiac contractility, according recently published experimental evidence. Low Mb level and increased iNOS activity have been observed in patients with heart failure. Therefore, pathophysiology of prevalent disease states may be influenced by the loss of Mb and resulting decrease in O_2 supply and NO scavenging capacity in failing hearts (Merx et al. 2005). Commonly, the formation of fibrous scar tissue in the infarct zone results in chronic problems and functional deficiencies. Fibroblast

stimulation and proliferation are essential for maintaining heart integrity and function shortly after cardiac injury (Shen et al. 2015). In addition to its heart-protective anti-atherogenic properties, a growing body of evidence from clinical and experimental investigations indicated that HDL (also known as a cholesterol antagonist) has a variety of positive health impacts. HDL can protect the myocardium by acting as an energy source, thereby supporting anti-inflammatory and antioxidant mechanisms, as well as NO-mediated vasodilators (Nagao et al. 2018).

The primary component of *Spirulina platensis*, Phycocyanobilin, has potential cardioprotective properties such as kinase inhibitor, cytoprotectant, and platelet aggregation inhibitor. Study from (Patel et al. 2016) show that Ibuprofen has a cardioprotective effect on rats with isoproterenol-induced myocardial infarction by inhibiting RhoA/Rho kinase, indicating that kinase inhibitor can be considered in the development of cardioprotective agents. As reported, cytoprotective drugs such as urocortin (Davidson et al. 2009), 7-oxoPGI2 (Szekeres 1987) such as sudden coronary death (SCD, and trimetazidine have cardioprotective effects (Tallarico et al. 2003). Most cytoprotective drugs function by stabilizing cell membranes. They protect cellular integrity from the harmful effects of toxic substances or metabolites by preventing or reducing harmful effects such as electrolyte changes, cell swelling, lysosomal enzyme release, and free radical release. The mechanism for cell membrane stabilization contributes to cytoprotection (Szekeres 1987).

During the early hours following myocardial ischemia, damaged cardiac cells can release adenosine, opioids, and bradykinin, which enhance myocardial survival by activating the G protein signaling pathway (Liu et al. 2011). According to prediction in PASS analysis, Phycocyanobilin has the potential to inhibit platelet aggregation which can be advantageous. Only active thrombolytic treatment, angioplasty, or surgical revascularization can restore blood flow in the case of developed thrombi. If there is sufficient time and the injury is not life-threatening, ischemia-induced spontaneous development and expansion of coronary anastomoses may be able to reduce the infarct size (Szekeres 1987).

Limitations

Based on the results of the LC-MS/MS analysis, five compounds have been identified, but neither in vitro nor in vivo testing has been conducted. Therefore, it cannot be determined whether these compounds are cardioprotective. Based on marker compounds found in *Spirulina platensis* and *Momordica charantia* extracts, we conducted a PASS analysis test to make a prediction.

Conclusions

In conclusion, we were able to demonstrate that combination of *Spirulina platensis* and *Momordica charantia* fruits extract has cardioprotective effect with attenuated cardiac injury biomarker in rats with isoproterenol-induced myocardial infarction. Histological evaluation reveals that the 100 mg/kg bw MC test group and the combination group maintain cell integrity, nucleus integrity, and minimal leukocyte infiltration. Phycocyanobilin in *Spirulina platensis* and Momordicoside A in *Momordica charantia* fruits possess a cardioprotective mechanism as a kinase inhibitor, cytoprotectant, platelet aggregation inhibitor, cholesterol antagonist, treatment for proliferative diseases, nitric oxide scavenger, and anti-inflammatory. The administration of a solitary extract did not have a satisfactory cardioprotective effect, but administration as a combination yielded the best results. However, the dose increase had no effect on the activity of the substance.

Ethical issue

Ethical Concerns the Animal Research Ethics Committee of the School of Pharmacy at Institut Teknologi Bandung granted ethical approval for the investigation (No. 05/KEPHP-ITB/03-2021).

Author´s note

The authors declare publication of this article does not involve any conflicts of interest. Authors confirm that the paper was original.

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