ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 26 (1) (2023): 34–38

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Antioxidant, Cytotoxic, and Insulinotropic Activities of Several Leaves Extracts of Medicinal Plants

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https://doi.org/10.14710/jksa.26.1.34-38

Article Info

Article history:

Received: 31st October 2022
Revised: 11th January 2023
Accepted: 20th January 2023
Online: 31st January 2023

Keywords:
Total phenolic content; antioxidants; cytotoxicity; insulinotropic

Abstract

The prevalence of diabetes mellitus and cancer is increasing; thus, research into efficient treatments utilizing active compounds derived from medicinal plants has focused on these diseases. Through the agro maritime 4.0 approach, medicinal plants are explored in the archipelago of Indonesia, particularly on Tinjil Island, Banten Province. The medicinal plants identified on the island include *Morinda citrifolia*, *Terminalia catappa*, and *Gnetum gnemon*. Therefore, this study aimed to evaluate the in vitro of aqueous extracts of leaves of those three plant species. All aqueous extracts were analyzed for total phenolic content and further tested for antioxidant activity using the DPPH method (2,2-diphenyl-1-picrylhydrazyl), MTT cytotoxic activity (3-[4,5-dimethylthiazole-2-yl]-2- 5-diphenyl-tetrazolium-bromide) in MCF-7- (ATCC HTB 22) and Burkitt's Lymphoma Raji (ATCC CCL 86) cells, and insulinotropic activity in pancreatic BRIN BD11 cells. The results showed that the total phenolic content of *T. catappa* was significantly higher (9.21 ± 2.49 mg GAE/g extract sample) compared to *M. citrifolia* (3.00 ± 0.35 mg GAE/g) and *G. gnemon* (7.47 ± 0.33 mg GAE/g). Compared to the other two extracts, *T. catappa* extract has the best DPPH antioxidant activity of IC_{50} 7.44 ± 0.77 µg/mL ($p<0.05$). MTT cytotoxic activity in all samples did not inhibit the proliferation of Raji cells but did the proliferation of MCF-7 cells. The IC_{50} for the best cytotoxic activity was shown in *M. citrifolia* (8.06 µg/mL). *T. catappa* triggered insulin secretion at 62.5 µg/mL with the highest insulin concentration (54.55 mg/mL). The aqueous extract of *T. catappa* leaves shows potential as an antioxidant and insulinotropic agent, while *M. citrifolia* leaves have a cytotoxic effect with anticancer potential.

1. Introduction

Diabetes mellitus (DM) is one of the most widespread diseases in the world. The World Health Organization (WHO) reports that deaths from diabetes reached 1.6 million between 2000 and 2016. The International Diabetes Federation organization states that Indonesia is ranked 7th among the ten countries with the highest number of sufferers, which is 10.7 million people per year in 2020. The prevalence of diabetes increased from 6.9% in 2013 to 8.5% in 2018. Indonesia has the eighth-highest rate of cancer incidence in Southeast Asia but the 23rd-highest rate in Asia. Indonesia has experienced an increase in the prevalence of tumors/cancer, from 1.4 per 100 people in 2013 to 1.79 per 1000 people in 2018 [1].

The information above demonstrates that diabetes and cancer are serious issues that require attention. Exploring the most recent methods to prevent or treat the diseases mentioned above is of utmost importance due to the high expense of conventional medication. It is essential to develop medicines from natural products to prevent the side effects of synthetic drugs. Medicinal plants are one of the treatments derived from natural ingredients.

Through the agro maritime 4.0 approach, exploration was performed to obtain potential medicinal plants focused on Tinjil Island, Banten Province. The plant inventory on the island, which covers 600 ha, identified as many as 28 plant species, and 10 of them are food sources for long-tailed monkeys (*Macaca fascicularis*), such as *Dracaena elliptica*, *Antidesma montanum*, *Pandanus bidur*, *Eugenia* sp., *Ardisia humilis*, *Intsia amboinensis*, *Ficus* sp., *Morinda citrifolia*, *Terminalia catappa*, and *Gnetum gnemon* [2]. The three plants widely reported to have antioxidant, antidiabetic, and anticancer activities are *Morinda citrifolia*, *Terminalia catappa*, and *Gnetum gnemon*. However, there is no information regarding the mechanism of decreasing blood sugar by increasing insulin secretion (insulinotropic), cytotoxicity in Raji cells and MCF7.

Plants generally contain secondary metabolites, such as saponins, triterpenoids, flavonoids, tannins, alkaloids, phenols, and fatty acids, metabolites with medicinal properties [3]. The phytochemical content of water and ethanol extracts of *Gnetum gnemon* leaves contains flavonoids, saponins, tannins, alkaloids, and steroids [4]. Metabolites that have been identified in *Terminalia catappa* leaves are polyphenolic compounds such as 1-degalloyl-eugeniin, 2,3-(4,4',5,5',6,6'-hexahydroxy-diphenoyl)-glucose, tannins (punicalin, punicalagins, chebulagic acid, geraniin, granatin-b, terflavin-a, terflavin-b, tergallagin, and tercatain), and flavonoids (quercetin, kaempferol, gallic acid, gentisic acid, and rutin) [5, 6]. The content of noni leaf metabolites showed positive results for the alkaloid, flavonoid, triterpenoid, and saponin but negative results for the tannin [7].

This study aimed to evaluate the potential antioxidant, cytotoxic, and insulinotropic activity of *M. citrifolia*, *T. catappa*, and *G. gnemon* leaf extracts. The results of this study are expected to complement the in vitro activity information of the three plants, which have the potential as antioxidants, anticancer, and antidiabetics.

2. Methods

2.1. Cell Culture and Growth Media

The cell cultures used in this study were MCF-7 breast cancer adenocarcinoma (ATCC HTB 22) and Burkitt's Lymphoma Raji (ATCC CCL 86). The growth media for the two cell types above were RPMI 1640 cells (Gibco, USA), which were supplemented with 10% FBS (Sigma, USA) and penicillin-streptomycin (Gibco, USA). The cytotoxic assay was performed using the MTT method (Sigma, USA). Cells were incubated using a CO_2 incubator (Thermo Fisher Scientific, Singapore). The insulinoma assay was performed using BRIN-BD11 glucose-responsive clonal insulin-producing conserved cells (donation from Prof. Andrè Herchuelz, Brussels, Belgium). ELISA method (Mercodia, Sweden) was utilized to measure insulin concentrations.

2.2. Sample Collection and Leaf Extraction

Leaf samples of *M. citrifolia*, *T. catappa*, and *G. gnemon* were collected in March-April (rainy season) from the forests of Tinjil Island, Banten Province, in the Indian Ocean. The leaves were collected from the ends of the twigs in the third to fifth order, cleaned with running water, air-dried, made into powder, and sieved to a size of 60 mesh. Water extraction by maceration (1:10) was conducted for 3 × 24 hours and dried by spray drying.

2.3. Total Phenolic Content

The total phenolic content was determined using the Folin Ciocalteu method, referring to Kruawan and Kangsadalampai [8], with modifications to the three sample extracts. A total of 10 µL of extract, 160 µL of distilled water, 10 µL of 10% Folin Ciocalteu reagent, and 20 µL of 7.5% $NaHCO_3$ solution were put into the microplate and incubated for 30 minutes at room temperature. Absorbance was measured at a wavelength of 765 nm. Calibration curves were made with standard solutions, which received the same treatment as the extracts. The standard compound used was gallic acid. The phenolic content of each extract was determined with three repetitions and expressed in mg gallic acid/g extract (mg GAE/dry extract).

2.4. DPPH Free Radical Scavenging Activity

The free radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) was determined as described by Salazar-Aranda *et al.* [9] with some modifications. A total of 10 mg of the extract was redissolved in 1 mL of DMSO. Then 100 µL of the sample extract solution was added to each well of the 96-well microplate and mixed with 100 µL of 125 µM DPPH solution (dissolved in ethanol, 1 mg/mL). Each extract was prepared with different concentrations (0, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL). Plates were incubated for 30 minutes in a dark room at room temperature. Absorbance was measured using a microplate reader (Epoch-Biotek, Winooski, USA) at 515 nm using ethanol as a blank. Vitamin C as a positive control was given the same treatment as the extract. Data are presented as IC_{50} (inhibitory concentration 50). Percent inhibition was calculated using Equation (1).

$$\text{DPPH scavenging effect (\%)} = \left\{\frac{A_1 - A_2}{A_1}\right\} \times 100 \qquad (1)$$

where, A_1 is the absorbance of the control reaction (solution containing all reagents without adding sample), and A_2 is the absorbance of the sample.

2.5. MTT Assay

Cytotoxic assays were performed on Raji and MCF7 cells using the MTT method to measure formazan crystals formed from living cells. The formazan produced will be directly proportional to the number of living cells resulting from the reaction of mitochondria and tetrazolium salts [10]. Cells were grown using a 96-well culture plate at a rate of 5000 cells/well at 37℃, 5% CO_2 for 18–20 hours in a growth medium. Samples prepared in several concentrations were added to each well and incubated under the same conditions at 37℃, 5% CO_2 for 48 hours. MTT solution was added as much as 50 µg at each age and then incubated for 4 hours. Media was discarded, and 100 µL of HCl 1 N in isopropanol (Merck, USA) was added to each well. The

absorbance of the formed formazan crystals was measured at 562 nm using a microplate reader (I-max, BioRad USA). Data were presented as percentage inhibition for each cell culture with sample concentrations from 0, 6.25, 12.5, 25, 50, 100, 200, 400, and 800 µg/mL.

2.6. Insulinoma

BRIN BD-11 insulinoma cells were grown in 24-well tissue culture plates at 300,000 cells/well concentrations. Incubation was performed for 20–24 hours at 37℃, 5% CO_2. KRB solutions were prepared (KRB I contained 1.11 mM glucose, and KRB III contained 16.7 mM glucose). pHs of 7–7.4 were measured by flowing the solution with 5% CO_2 and 95% O_2 for 15 minutes. BSA was added after this process. The plant extracts were dissolved in KRB I solution according to each concentration and prepared by serial dilution. Each concentration was prepared for three repetitions. Various concentrations of extract solutions were prepared to determine the optimal dose for testing with diabetes drugs. The cell media grown in the tissue culture plate wells was discarded, then washed with KRB I solution three times. Preincubation was done by adding KRB I after the last wash and incubating at 37℃, 5% CO_2, for 40 minutes. After removing the KRB I media, each extract concentration was added to each well. Media control was prepared by pouring KRB I and III solutions without extract into each well. Incubation was performed at 37℃, 5% CO_2. The insulinotropic phase was performed for 60 minutes with RBC III; for the fast phase, insulinotropic was incubated for 20 minutes in KRB I. The supernatant from the incubation was harvested, and the insulin concentration was measured using the Elisa Rat Insulin Kit (Mercodia, Sweden).

2.7. Data Analysis

The results shown were expressed as the mean ± standard deviation (SD) of three repetitions (total phenolics) and two repetitions (IC_{50} antioxidant activity and cytotoxicity) measurements. These parameters used analysis of variance (ANOVA) followed by Duncan's test to compare significant values at the 5% level ($p < 0.05$). Data on insulin concentration and IC_{50} of cancer cells were analyzed descriptively.

3. Results and Discussion

3.1. Total phenolic and DPPH radical scavenging activity

The measured total phenolic content and antioxidant activity are shown in Table 1. The highest phenolic content was *T. catappa* ($p<0.05$), followed by *G. gnemon*, then *M. citrifolia*. The total phenolic content of *T. catappa* was (9.21 ± 2.49 mg/GAE/g). This value is comparable to the DPPH radical scavenging activity, which has the highest IC_{50} (7.44 ± 0.77 µg/mL) ($p<0.05$) compared to the other two extracts.

M. citrifolia leaves contain a broad spectrum of secondary metabolites such as flavonoids, alkaloids, tannins, triterpenoids, and sterols [11]. The phytochemical metabolites of *T. catappa* are phenols, flavonoids, triterpenoids, and tannins [5]. However, studies on *G. gnemon* are still limited. A strong positive correlation exists between total phenolic content and DPPH free radical scavenging activity [12] to indicate antioxidant activity [13]. This was proven in the leaf extract of *T. catappa*, followed by *G. gnemon* and *M. citrifolia*. The three extracts did not show strong antioxidant activity compared with the positive control. High antioxidant capacity can enhance mitochondrial oxidation-reduction reactions and increase tissue oxygenation, ultimately preventing and repairing degenerated cells [14].

Table 1. Total phenolic content and IC_{50} value of DPPH radical scavenging activity of aqueous extracts of *M. citrifolia*, *T. catappa*, and *G. gnemon* leaves

Leaf extract	Total phenolic (mg GAE/g dry sample)	DPPH radical scavenging activity (IC_{50}, µg/mL)
M. citrifolia	3.00 ± 0.35^a	31.22 ± 0.42^c
G. gnemon	7.47 ± 0.33^b	23.96 ± 1.02^b
T. catappa	9.21 ± 2.49^c	7.44 ± 0.77^a
Vitamin C	-	3.39 ± 0.03

Note: different superscript numbers indicate significant differences based on ANOVA and Duncan.

3.2. Cytotoxic Assay

The results of the MTT assay of the three extracts on Raji cells showed no inhibition of cell growth, which means they did not have a cytotoxic effect. The results of testing the three sample extracts on MCF-7 cells showed inhibition of cell growth (IC_{50}), presented in Table 2.

Table 2. IC_{50} value of cytotoxic assay on MCF-7

Leaf Extract	MCF-7 (IC_{50}, µg/mL)
M. citrifolia	8.06
G. gnemon	137.16
T. catappa	22.07

The morphology of the treated cells can be seen in Figure 1. The cells experienced growth inhibition and showed different morphologies at 800 µg/mL concentration, detached from where they grow.

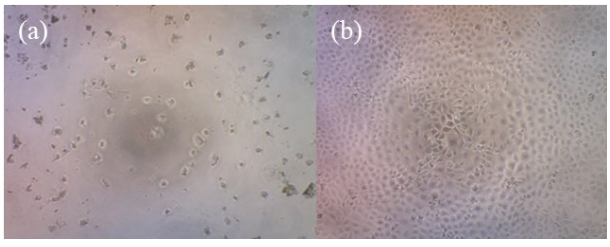

Figure 1. Morphologies of MCF 7 cells after being treated with *M. citrifolia* extract at (a) a concentration of 800 µg/mL and (b) without extract as a control

M. citrifolia leaf extract showed the highest cytotoxic effect with an IC_{50} value of 8.96 µg/mL. This differs from the previous results, with the highest activity from the root extract of 8.2 µg/mL [15]. The cytotoxic activity of *T. catappa* leaf extract has never been reported on MCF-7 cells; however, a cytotoxic effect on T47D cells has been reported [16]. The phytochemicals that play a role in inhibiting the growth of MCF-7 cells are thought to be

flavonoids and anthraquinones [15]. Research on the anticancer activity of *G. gnemon* leaf extract is still limited. Studies conducted on *G. gnemon* seed extract did not have an anticancer effect on MCF-7 cells [17], in contrast to the results in this study which showed a cytotoxic effect (137.16 µg/mL).

3.3. Insulinotropic Activities

The mechanism of decreasing blood sugar is by increasing insulin secretion from pancreatic beta cells, which can be proven by the secretion of an insulinoma (BRIN-BD11). The results of the three sample extracts are shown in Table 3.

Table 3. The insulinotropic activity of BRIN BD11 cells in response to KRB I (11.1 mMol glucose) and KRB III (16.75 mMol glucose) to the three leaf extracts

Leaf Extract	Concentration (mg/L)	Insulin Concentration (mg/mL)	
		KRB I	KRB III
M. citrifolia	15.65	22.79	36.8
	31.25	41.46	19.12
	62.5	41.61	15.65
G. gnemon	15.65	27.9	26
	31.25	42.29	34.86
	62.5	33.72	30.33
T. catappa	15.65	11.07	44.55
	31.25	21.54	47.1
	62.5	55.41	53.7
KRB		15.58	63.86
Glibenclamide		19.06	34.79

KRB: Kreb Ringer Buffer with 11.1 mMol glucose (KRB I) and 16.75 mMol glucose (KRB III) as negative controls; Glibenclamide (10 mM) served as the positive control.

Insulin release by BRIN BD-11 cells using *T. catappa* leaf extract showed the highest increase in insulin levels with an insulin level value of 55.41 mg/mL in KRB I and 53.7 mg/mL in KRB III with a leaf extract concentration of 62.5 mg/L (Table 3). *M. citrifolia* leaf extract showed a different response in triggering insulin secretion. The leaf extract concentration of 15.65 mg/L resulted in the highest insulin level of 36.8 mg/mL in KRB III. *G. gnemon* produced the highest insulin levels (34.86 mg/mL) at an extract concentration of 31.25 mg/L.

Increased insulin secretion was shown clearly by *T. catappa* leaf extract at a concentration of 15.65 mg/L after 16.75 mMol glucose administration. Plants with insulinotropic or secretagogue properties have been reported in several Leguminosae, Rutaceae, Rosaceae, and Annonaceae to improve pancreatic cell function [18]. Plant extracts containing alkaloids, terpenoids, flavonoids, and phenolics have shown antidiabetic potential through insulinotropic activity [19]. This study lacked analysis of the effect of the extract on the insulinotropic mechanism through inhibiting extracellular calcium [20]; therefore, the cellular mechanism could not be proven.

4. Conclusion

The results of this study indicate that the leaf extract of *T. catappa* has the highest antioxidant and insulinotropic activity and is proportional to its total phenolic content. The highest anticancer activity against MCF-7 cells was shown by *M. citrifolia* leaf extract. The extracts of *M. citrifolia*, *T. catappa*, and *G. gnemon* did not show cytotoxic activity in the Raji cell culture. *G. gnemon* leaf extract has the lowest activity in each activity test performed. *M. citrifolia* and *T. catappa* have the potential to be candidates for medicinal plants with high antioxidant, anticancer, and antidiabetic levels based on the test results in this study.

References

[1] Riset Kesehatan Dasar, *Pusat Data dan Informasi (Infodatin Diabetes Melitus)*, Kementerian Kesehatan RI, 2020

[2] Nyoto Santoso, Habitat Analysis and Dietary Potential of Long Tailed Macaques (*Macaca fascicularis*, Raffles) in Tinjil Island, *Media Konservasi*, 5, 1, (1996), 5-9

[3] Sadaf Mushtaq, Bilal Haider Abbasi, Bushra Uzair, Rashda Abbasi, Natural products as reservoirs of novel therapeutic agents, *EXCLI Journal*, 17, (2018), 420-451 http://dx.doi.org/10.17179/excli2018-1174

[4] Pankaj Bharali, Priyanka Dutta, Mohan Chandra Kalita, Arup Kumar Das, Hui Tag, Ananta Madhab Baruah, Evaluation of antioxidant and proximate compositions of the leaf extract of *Gnetum gnemon* L., *International Research Journal of Pharmacy*, 9, 10, (2018), 101-105 http://dx.doi.org/10.7897/2230-8407.0910234

[5] Analucia G. Terças, Andrea de Souza Monteiro, Eduardo B. Moffa, Julliana R. A. dos Santos, Eduardo M. de Sousa, Anna R. B. Pinto, Paola C. da Silva Costa, Antonio C. R. Borges, Luce M. B. Torres, Allan K. D. Barros Filho, Elizabeth S. Fernandes, Cristina de Andrade Monteiro, Phytochemical characterization of *Terminalia catappa* linn. Extracts and their antifungal activities against *Candida* spp., *Frontiers in Microbiology*, 8, 595, (2017), https://doi.org/10.3389/fmicb.2017.00595

[6] D. S. Mohale, A. P. Dewani, A. V. Chandewar, C. D. Khadse, A. S. Tripathi, S. S. Agrawal, Brief review on medicinal potential of *Terminalia catappa*, *Journal of Herbal Medicine and Toxicology*, 3, 1, (2009), 7-11

[7] A. Muhammad, S. Y. Mudi, Phytochemical screening and antimicrobial activities of *Terminalia catappa*, leaf extracts, *Biokemistri*, 23, 1, (2011), 35-39

[8] Kalyarat Kruawan, Kaew Kangsadalampai, Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs, *Thai Journal of Pharmaceutical Sciences*, 30, (2006), 28-35

[9] Ricardo Salazar-Aranda, Luis Alejandro Pérez-Lopez, Joel Lopez-Arroyo, Blanca Alicia Alanís-Garza, Noemí Waksman de Torres, Antimicrobial and antioxidant activities of plants from northeast of Mexico, *Evidence-Based Complementary and Alternative Medicine*, 2011, 536139, (2011), https://doi.org/10.1093/ecam/nep127

[10] Paul W. Sylvester, Optimization of the Tetrazolium Dye (MTT) Colorimetric Assay for Cellular Growth and Viability, in: S.D. Satyanarayanajois (Ed.) *Drug Design and Discovery: Methods and Protocols*, Humana Press, Totowa, NJ, 2011, https://doi.org/10.1007/978-1-61779-012-6_9

[11] Sridevi Nagalingam, Changam Sheela Sasikumar, Kotturathu Mammen Cherian, Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit, *Asian Journal of Pharmaceutical and Clinical Research*, 5, 2, (2012), 179-181

[12] T. Oki, M. Masuda, S. Furuta, Y. Nishiba, N. Terahara, I. Suda, Involvement of anthocyanins and other phenolic compounds in radical - scavenging activity of purple - fleshed sweet potato cultivars, *Journal of Food Science*, 67, 5, (2002), 1752-1756 https://doi.org/10.1111/j.1365-2621.2002.tb08718.x

[13] Midadul Haq, Wirakarnain Sani, A. B. M. S. Hossain, Rosna Mat Taha, K. M. Monneruzzaman, Total phenolic contents, antioxidant and antimicrobial activities of *Bruguiera gymnorrhiza*, *Journal of Medicinal Plants Research*, 5, 17, (2011), 4112-4118

[14] Simone Caramel, Marco Marchionni, Sergio Stagnaro, *Morinda citrifolia* plays a central role in the primary prevention of mitochondrial-dependent degenerative disorders, *Asian Pacific Journal of Cancer Prevention*, 16, 4, (2015), 1675-1675 https://doi.org/10.7314/APJCP.2015.16.4.1675

[15] Afika Putri Dzakianda, Irma Herawati Suparto, Eti Rohaeti, Kandungan Fitokimia dan Aktivitas Antikanker Tanaman Mengkudu (*Morinda citrifolia*) Secara In Vitro terhadap Sel Lestari MCF-7, *Undergraduate thesis*, Chemistry Department, IPB University, Bogor, 2021

[16] Barinta Widaryanti, Nur Khikmah, Nunung Sulistyani, Efek Sitotoksik Ekstrak Daun Ketapang (*Terminalia catappa* L.) Pada Sel Kanker Payudara T47D, *Jurnal Biologi Papua*, 8, 2, (2016), 68-71 https://doi.org/10.31957/jbp.54

[17] Sonya Yunita, Valentina Yurina, Diana Lyrawati, Uji Sitotoksik Kombinasi Ekstrak Etanol Daun Kemangi (*Ocimum sanctum*) dan Biji Melinjo (*Gnetum gnemon*) terhadap Sel Kanker MCF-7, *Master Thesis*, Farmasi, Universitas Brawijaya, Malang, 2021

[18] Dariush Shanehbandi, Habib Zarredar, Milad Asadi, Venus Zafari, Shiva Esmaeili, Ensiyeh Seyedrezazadeh, Zahra Soleimani, Hamed Sabagh Jadid, Shirin Eyvazi, Sara Feyziniya, Anticancer impacts of *Terminalia catappa* extract on SW480 colorectal neoplasm cell line, *Journal of Gastrointestinal Cancer*, 52, (2021), 99-105 https://doi.org/10.1007/s12029-019-00349-z

[19] D. K. Patel, S. Kumar Prasad, R. Kumar, S. Hemalatha, An overview on antidiabetic medicinal plants having insulin mimetic property, *Asian Pacific Journal of Tropical Biomedicine*, 2, 4, (2012), 320-330 https://doi.org/10.1016/S2221-1691(12)60032-X

[20] Aris Wibudi, Bambang Kiranadi, Wasmen Manalu, Adi Winarto, Slamet Suyono, The traditional plant, *Andrographis paniculata* (Sambiloto), exhibits insulin-releasing actions in vitro, *Acta medica Indonesiana*, 40, 2, (2008), 63-68