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*Research Article*

# Cardiovascular Activity of Labdane Diterpenes from *Andrographis paniculata* in Isolated Rat Hearts

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The dichloromethane (DCM) extract of *Andrographis paniculata* Nees was tested for cardiovascular activity. The extract significantly reduced coronary perfusion pressure by up to 24.5 ± 3.0 mm Hg at a 3 mg dose and also reduced heart rate by up to 49.5 ± 11.4 beats/minute at this dose. Five labdane diterpenes, 14-deoxy-12-hydroxyandrographolide (**1**), 14-deoxy-11,12-didehydroandrographolide (**2**), 14-deoxyandrographolide (**3**), andrographolide (**4**), and neoandrographolide (**5**), were isolated from the aerial parts of this medicinal plant. Bioassay-guided studies using animal model showed that compounds, (**2**) and (**3**) were responsible for the coronary vasodilatation. This study also showed that andrographolide (**4**), the major labdane diterpene in this plant, has minimal effects on the heart.

## 1. Introduction

*Andrographis paniculata* Nees, family Acanthaceae, has been used since time immemorial in Ayurvedic medicine, mainly for liver problems and dysentery [1]. The plant is also known as "Indian Echinacea" and "King of bitter." Phytochemical screening on this herbal plant showed that it contains a lot of flavonoids and terpenoids while moderate in alkaloids and tannins compounds [2]. This plant has been featured in at least 26 Ayurvedic formulae, whereas in traditional chinese medicine, *A. paniculata* is an important "cold property". In Malaysia, *A. paniculata* is more commonly known as "hempedu bumi" and is widely used in traditional medicine, especially for the treatment of cardiovascular disorders.

Previous researches have shown that *A. paniculata* extract and its labdane diterpenes have a broad range of pharmacological effects such as the ability to inhibit replication of the HIV virus [3, 4], prevent common cold [5–7], antimalarial [8], prevent diarrhea [9], antibacterial [10], anti-inflammatory [11–13], antihyperglycemic effect [14, 15], suppress various cancer cells [16–18], and antifertility and pregnancy-terminating effects [19].

In earlier cardiovascular studies, *A. paniculata* extracts significantly reduced atherosclerotic artery stenosis and lowered restenosis rates after angioplasty in rabbits [20] decreases platelet aggregation *in vitro* [21] and were reported to be antihypertensive in rats [22]. Nevertheless, the scientific basis for the use of *A. paniculata* in treating "heart problems" is still unclear. To our knowledge, its direct effects on the isolated heart of an animal model are not known. Therefore, in this study, the dichloromethane (DCM) extract of *A. paniculata* was tested against coronary vessels, cardiac muscle contractility, and heart rate in Langendorff perfused rat hearts. Our preliminary study showed that the dichloromethane extract caused coronary vasodilation, reduced heart rate while not affecting the cardiac contractility of isolated perfused rat hearts. Further isolation work was performed on the dichloromethane extract in order to identify the active compound(s) responsible for the cardiovascular activity observed.

## 2. Materials and Method

*2.1. General.* All spectral data were obtained on various instruments; infrared (IR) spectrum was recorded on the Perkin Elmer FT-IR (fourier transform) spectrometer RX1, ultraviolet (UV) spectrum was taken on a Shimadzu UV-160A UV-visible recording spectrophotometer, nuclear magnetic resonance spectrum (NMR) was obtained from JEOL JNM-LA400 FT-NMR spectrometer (400 MHz), and mass spectrum (MS) was recorded on a Shimadzu GC-MS (gas chromatograph—mass spectrometry) spectrometer (HP 6890 Series Mass Selective Detector and HP 6890 Series GC System). The cardiovascular activity was measured using polygraph model 7D Grass, peristaltic pump model Compact Drive MasterFlex, and pressure transducer model GOULD Statham USA.

*2.2. Plant Material.* The aerial parts of *Andrographis paniculata* were supplied by the Herbarium Unit, Department of Botany, University of Malaya, with herbarium series number KL4930.

*2.3. Extraction and Isolation.* Dried and ground aerial parts (650.0 g) of the plant were extracted with dichloromethane for 6 h using a Soxhlet apparatus at 45°C. The extract was concentrated under reduced pressure to yield 26.5 g of dichloromethane (DCM) crude extract. DCM (3 g) was chromatographed on a silica gel column with a $CH_2Cl_2$-MeOH gradient solvent system to give 80 fractions, which were combined into 4 subfractions (A–D) based on TLC (thin-layer chromatography) patterns and $^1$H NMR spectra. Subfraction B was subjected to column chromatography on silica gel and gave 14-deoxy-11,12-dihydroandrographolide (**2**) (264.6 mg, $CH_2Cl_2$/MeOH, 98 : 2) and 14-deoxyandrographolide (**3**) (352.8 mg, $CH_2Cl_2$/MeOH, 99 : 1). Subfractions A, C, and D underwent further isolation by column chromatography analyses over silica gel. Sub fraction C gave andrographolide (**4**) (882.3 mg, $CH_2Cl_2$/MeOH, 96 : 4), sub fraction D gave neoandrographolide (**5**) (44.1 mg, $CH_2Cl_2$/MeOH, 90 : 10), and sub fraction A gave 14-deoxy-12- hydroxyandrographolide (**1**) (176.4 mg, $CH_2Cl_2$/MeOH, 97 : 3). The structure of each compound was confirmed by comparison of its NMR, IR, UV, and MS data with literature values [12].

*2.4. Cardiovascular Activity on Isolated Rat Hearts.* The extracts and isolated compounds were screened for their cardiovascular effects in isolated perfused rat hearts by using the Langendorff-perfused model and method, with modification [23]. The Polygraph model (Grass Instrument Co. model 7D) was calibrated to measure heart rate, developed tension, and coronary perfusion pressure of the heart. The modified Krebs-Henseleit solution (37°C) was pumped to flow at a constant rate (10 mL/min) by using a peristaltic pump (Compact Drive Masterflex), and the gas (95% $O_2$ and 5% $CO_2$) was allowed to pass through this solution. The Krebs-Henseleit solution contained the following: NaCl 118 mM, D-glucose 11.6 mM, $NaHCO_3$ 25 mM, KCl 4.7 mM, $KH_2PO_4$ 1.2 mM, $MgSO_4$ 1.2 mM, and $CaCl_2$ 1.23 mM.

Sprague Dawley rats (250–300 g) were anaesthetized with sodium pentobarbitone (72 mg/1000 g) and killed by cervical dislocation. The heart was isolated and put in a Petri dish containing cold Krebs-Henseleit solution to stop the heartbeat. After the fat and connective tissues had been removed from the heart, the aorta was cannulated and perfused with the Krebs-Henseleit solution. The coronary vessel tone was indicated by coronary perfusion pressure, which was measured with a pressure transducer (GOULD Statham USA) and monitored with the Grass Model 7D Polygraph.

The apex of the left ventricle was then attached with a hook and connected to the isometric tension transducer with a piece of thread, which passed through a pulley system. The resting heart tension was adjusted to 2 g to the optimum contractile force. The contractility was monitored by an isometric tension transducer and recorded as developed tension. Heart rate was also detected through this transducer.

The perfused isolated rat heart (PIH) was stabilised for about 20 min before any drug samples were injected. All injections were made at the rubber tubing near the cannula into the Krebs-Henseleit buffer. The labdane diterpenes compounds of *A. paniculata* were prepared as 1 mg/mL solutions. The first compound at 100 $\mu$L was injected into the PIH soon after its heart rate, contractility, and coronary perfusion pressure stabilized. The effect of the first compound/dose was allowed to disappear, and then administered with the second compound until the last compound followed by 40% ethanol as a vehicle control and finally 1000 nmoles isoprenaline and sodium nitroprusside as positive controls, all in the same heart. The compounds administred to the next heart were arranged randomly in order to avoid bias.

*2.5. Statistical Analyses.* Data obtained were expressed as mean standard error (± S.E.M.). The data were analyzed for statistical significance using Student's *t*-test. *P* values less than 0.05 were considered to be significant ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

## 3. Results and Discussion

The DCM extract of *A. paniculata* [24] significantly reduced the coronary perfusion pressure by up to 24.5 ± 3.0 ($P < 0.05$) and 29.4 ± 8.5 mm Hg ($P < 0.05$) at doses of 3 mg and 1 mg, respectively, and the heart rate by up to 49.5 ± 11.4 beats/minute ($P < 0.05$) at the 3 mg dosage. Four isolated rat hearts were used to investigate the effect of the DCM. Further isolation work was performed on the dichloromethane extract in order to identify the compound(s) responsible for the cardiovascular activity. Fractionation and purification of the DCM extract led to the isolation and purification of five labdane diterpenes, identified as 14-deoxy-12-hydroxyandrographolide (**1**), 14-deoxy-11,12-dihydroandrographolide (**2**) (*trans* isomer), 14-deoxyandrographolide (**3**), andrographolide (**4**), and neoandrographolide (**5**) (Figure 1). The structure of each compound was identified by comparison of their NMR, IR, UV, and MS data with literature values [25]. At least seven rat hearts were used to investigate the effect of each diterpenoid

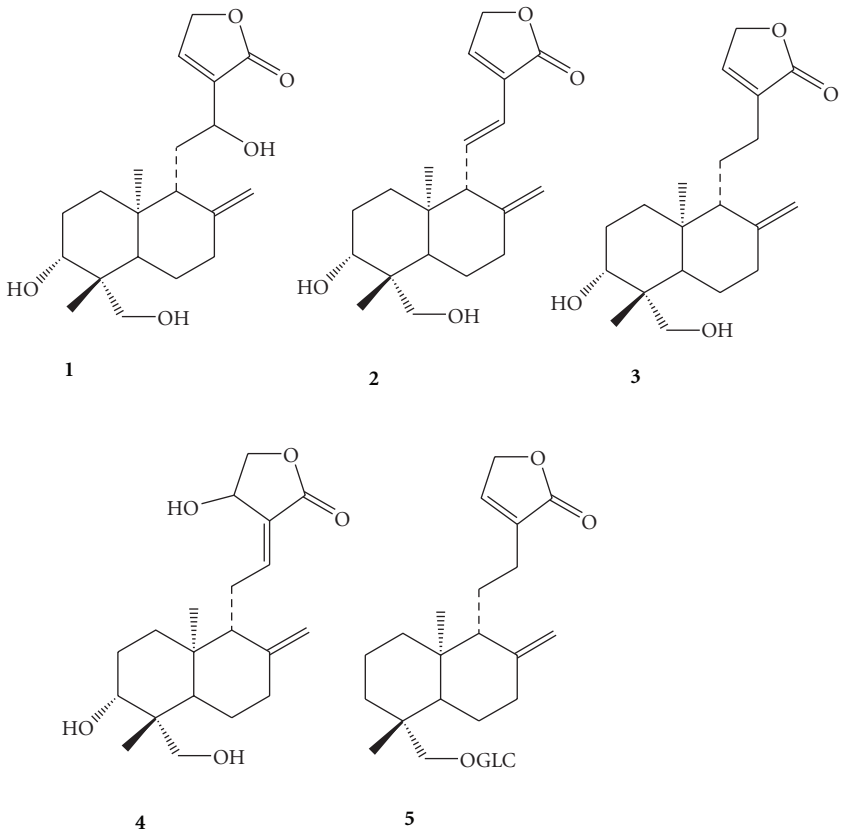


Figure 1: Chemical structure of compounds **1–5**.

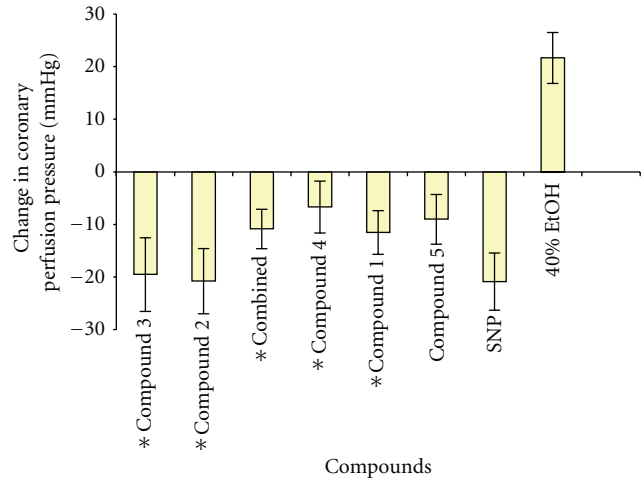


Figure 2: The effects of diterpenoids isolated from the dichloromethane extract of *A. paniculata*, sodium nitroprusside (SNP), and 40% EtOH, on coronary perfusion pressure of isolated rat hearts. The results are expressed as mean ± s.e.m., $^*P < 0.05$, student's paired *t*-test, compounds versus vehicle (40% ethanol), $n = 7$. Basal coronary perfusion pressure = 72.1 ± 5.6 mm Hg.

on coronary perfusion pressure (a measure of coronary tone), developed tension (a measure of cardiac contractility), and heart rate.

All compounds were prepared as 1 mg/mL solutions in 40% ethanol. The dose used for each compound was 0.1 mg. All of these diterpenoids reduced coronary perfusion pressure (CPP) significantly with compound **3** showing the largest reduction (−19.5 ± 7.0 mm Hg, $P < 0.05$) (Figure 2). This value was comparable with sodium nitroprusside (positive control), which reduced CPP by 22.0 ± 5.4 mm Hg ($P < 0.05$). The combination of all five diterpenoids in the same amounts reduced CPP by 10.8 ± 3.7 mm Hg ($P < 0.05$).

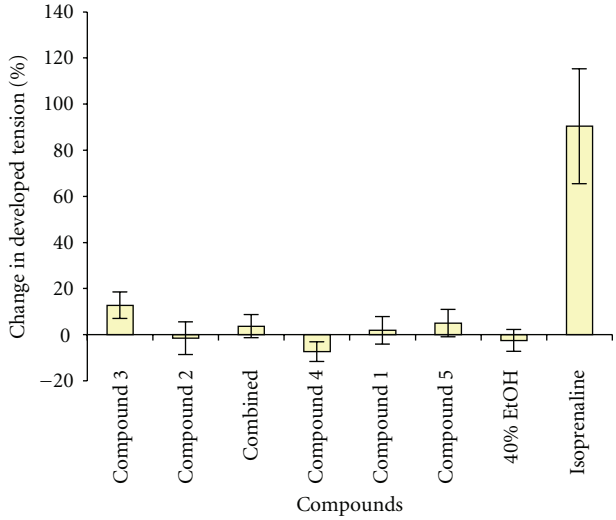


Figure 3: The effects of diterpenoids isolated from the dichloromethane extract of *A. paniculata*, 40% EtOH and isoprenaline on developed tension of isolated rat hearts. The results are expressed as mean ± s.e.m, $n = 7$. Basal developed tension = 3.9 ± 0.3 g.

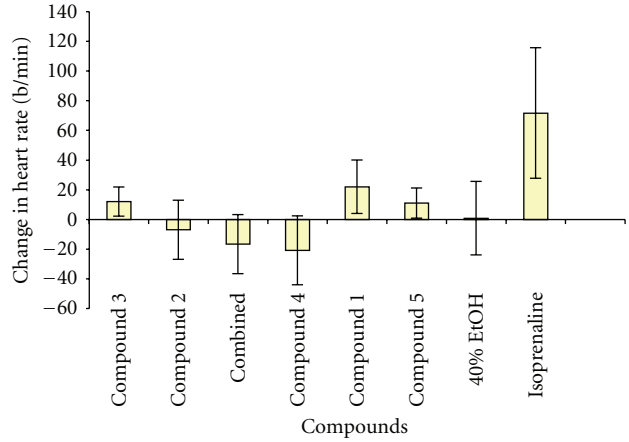


Figure 4: The effects of diterpenoids isolated from the dichloromethane extract of *A. paniculata*, 40% EtOH and isoprenaline on heart rate (beats/min) of isolated rat hearts. The results are expressed as mean ± s.e.m, $n = 7$. Basal heart rate = 251 ± 13 beats/min.

None of the five diterpenoids showed any significant effect on cardiac contractility and heart rate as compared with the positive control, isoprenaline (Figures 3 and 4).

Many studies have been reported on the cardiovascular activity of *A. paniculata* [20–28] and on other pharmacological aspects [3–19]. However, no cardiovascular activity study involving a direct effect on the isolated hearts of rats has been published previously. In this current study, the DCM crude extract showed potent cardiovascular activity on the isolated perfused rat heart, except that it did not affect cardiac contractility. Fractionation and purification of the DCM extract led to the isolation of five active compounds (**1–5**), with 14-deoxy-11,12-dihydroandrographolide (**2**) and 14-deoxyandrographolide (**3**) showing the largest reduction in coronary perfusion pressure (−18.96 ± 6.19 and −19.5 ± 7.00 Mm Hg, resp.) ($P < 0.05$). The results from our study support the previous finding by Zhang and Tan [26] that showed 14-deoxy-11,12-dihydroandrographolide (**2**) and 14-deoxyandrographolide (**3**) caused significant falls in mean arterial pressure (MAP) in the hearts of anaesthetized rats. However, none of the five diterpenes showed any significant effect on heart rate. This observation suggests that compound/s other than compounds **1–5** may be responsible for this activity.

The decreased coronary perfusion pressure is a measurement for coronary artery vasorelaxation of an isolated rat heart. Due to the constant perfusion of Krebs-Henseleit into the heart during the experiment, any change in coronary perfusion pressure therefore would reflect change in vascular resistance [28]. Activation of $\beta$-adrenoceptors caused vasorelaxation of coronary channel.

Another mechanism involved in the cardiovascular activity of 14-deoxy-11,12-dihydroandrographolide (**2**) and 14-deoxyandrographolide (**3**) has been reported [22–24, 26]. The ability of *A. paniculata* extract to dilate coronary vessels has not been reported before, but both 14-deoxy-11,12-dihydroandrographolide (**2**) and 14-deoxyandrographolide (**3**) have been reported to dilate aortic rings [26]. The compound 14-deoxy-11,12-dihydroandrographolide (**2**) exhibited hypotensive effect in anaesthetized rats [29]. Both compounds exert their vasorelaxant activity by the release of nitric oxide (NO) and activation of the guanylate cyclase pathway, as well as the blockade of $Ca^{2+}$ influx through both voltage- and receptor-operated $Ca^{2+}$ channels [30].

## 4. Conclusions

In conclusion, the ability of 14-deoxy-11,12-dihydroandrographolide (**2**) and 14-deoxyandrographolide (**3**) to dilate coronary vessels and aortic rings [23] supports the traditional use of *A. paniculata* in the treatment of cardiovascular disorder and also in alleviating hypertension. These results also suggest that both compounds (**2**) and (**3**) might be good drug candidates for the treatment of angina and hypertension. Therefore, further *in vivo* pharmacological, pharmacokinetic, and toxicological studies need to be undertaken. This result also shows that the major compound, andrographolide (**4**), is not the major contributor to the cardiovascular activity of this plant.

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## References


[1] R .P. Samy, M. M. Thwin, and P. Gopalakrishnakone, "Phytochemistry, pharmacology and clinical use of *Andrographis paniculata*," *Natural Product Communications*, vol. 2, pp. 607–618, 2007.

[2] D. Sutha, R. Jegathambigai, P. Kumar, and S. Sudhakaran, "A study on the hepatoprotective effect of *Andrographis paniculata* (Burm.F) Nees on mice," *Journal of Phytology*, vol. 2, no. 11, pp. 25–30, 2010.

[3] W. Lü, "Prospect for study on treatment of AIDS with traditional Chinese medicine," *Journal of Traditional Chinese Medicine*, vol. 15, no. 1, pp. 3–9, 1995.

[4] C. Calabrese, S. H. Berman, J. G. Babish et al., "A phase I trial of andrographolide in HIV positive patients and normal volunteers," *Phytotherapy Research*, vol. 14, no. 5, pp. 333–338, 2000.

[5] D. D. Cáceres, J. L. Hancke, R. A. Burgos, and G. K. Wikman, "Prevention of common colds with *Andrographis paniculata* dried extract: a pilot double blind trial," *Phytomedicine*, vol. 4, no. 2, pp. 101–104, 1997.

[6] D. D. Cáceres, J. L. Hancke, R. A. Burgos, F. Sandberg, and G. K. Wikman, "Use of visual analogue scale measurements (VAS) to asses the effectiveness of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study," *Phytomedicine*, vol. 6, no. 4, pp. 217–223, 1999.

[7] E. S. Gabrielian, A. K. Shukarian, G. I. Goukasova et al., "A double blind, placebo-controlled study of *Andrographis paniculata* fixed combination Kan Jang in the treatment of acute upper respiratory tract infections including sinusitis," *Phytomedicine*, vol. 9, no. 7, pp. 589–597, 2002.

[8] P. Misra, N. L. Pal, P. Y. Guru, J. C. Katiyar, V. Srivastava, and J. S. Tandon, "Antimalarial activity of *Andrographis paniculata* (Kalmegh) against *Plasmodium berghei* NK 65 in *Mastomys natalensis*," *International Journal of Pharmacognosy*, vol. 30, no. 4, pp. 263–274, 1992.

[9] S. Gupta, J. N. S. Yadava, and J. S. Tandon, "Antisecretory (antidiarrhoeal) activity of Indian medicinal plants against Escherichia coli enterotoxin-induced secretion in rabbit and guinea pig ileal loop models," *International Journal of Pharmacognosy*, vol. 31, no. 3, pp. 198–204, 1993.

[10] A. Sule, Q. U. Ahmed, O. A. Samah, and M. N. Omar, "Screening for anti bacterial activity of *Andrographis paniculata* used in Malaysia folkloric medicine: a possible alternative for the treatment of skin infection," *Ethnobotanical leaflets*, vol. 14, pp. 445–456, 2010.

[11] W. L. Deng, "Preliminary studies on the pharmacology of the *Andrographis* product dihydroandrographolide sodium succinate," *Newsletters of Chinese Herbal Medicine*, vol. 8, pp. 26–28, 1978.

[12] W. F. Chiou, C. F. Chen, and J. J. Lin, "Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide," *British Journal of Pharmacology*, vol. 129, no. 8, pp. 1553–1560, 2000.

[13] K. Sheeja, P. K. Shihab, and G. Kuttan, "Antioxidant and anti-inflammatory activities of the plant *Andrographis paniculata* Nees," *Immunopharmacology and Immunotoxicology*, vol. 28, no. 1, pp. 129–140, 2006.

[14] X. F. Zhang and B. K. H. Tan, "Anti-diabetic property of ethanolic extract of *Andrographis paniculata* in streptozotocin-diabetic rats," *Acta Pharmacologica Sinica*, vol. 21, no. 12, pp. 1157–1164, 2000.

[15] R. Subramanian, M. Z. Asmawi, and A. Sadikun, "*In vitro* $\alpha$-glucosidase and $\alpha$-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide," *Acta Biochimica Polonica*, vol. 55, no. 2, pp. 391–398, 2008.

[16] R. Ajaya Kumar, K. Sridevi, N. Vijaya Kumar, S. Nanduri, and S. Rajagopal, "Anticancer and immunostimulatory compounds from *Andrographis paniculata*," *Journal of Ethnopharmacology*, vol. 92, no. 2-3, pp. 291–295, 2004.

[17] M. Geethangili, Y. K. Rao, S. H. Fang, and Y. M. Tzeng, "Cytotoxic constituents from *Andrographis paniculata* induce cell cycle arrest in Jurkat cells," *Phytotherapy Research*, vol. 22, no. 10, pp. 1336–1341, 2008.

[18] K. Sheeja and G. Kuttan, "Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide," *Immunopharmacology and Immunotoxicology*, vol. 29, no. 1, pp. 81–93, 2007.

[19] M. S. Zoha, A. H. Hussain, and S. A. Choudhury, "Antifertility effect of *Andrographis paniculata* in mice," *Bangladesh Medical Research Council Bulletin*, vol. 15, no. 1, pp. 34–37, 1989.

[20] G. Zhi-ling, Z. Hua-yue, and Z. Xin-hua, "The effect of *Andrographis paniculata* Nees (APN) in alleviating the myocardial ischemic reperfusion injury," *Journal of Tongji Medical University*, vol. 14, no. 1, pp. 49–51, 1994.

[21] N. Yoopan, P. Thisoda, N. Rangkadilok et al., "Cardiovascular effects of 14-Deoxy-11,12-didehydroandrographolide and *Andrographis paniculata* extracts," *Planta Medica*, vol. 73, no. 6, pp. 503–511, 2007.

[22] C. Y. Zhang and B. Tan, "Hypotensive activity of aqueous extract of *Andrographis paniculata* in rats," *Clinical and Experimental Pharmacology and Physiology*, vol. 23, no. 8, pp. 675–678, 1996.

[23] O. Langendorff, "Untersuchungen am überlebenden Säugethierherzen," *Pflüger, Archiv für die Gesammte Physiologie des Menschen und der Thiere*, vol. 61, no. 6, pp. 291–332, 1895.

[24] M. D. Nadzri, *The effect of Andrographis paniculata and Garcinia sp. extracts on the isolated rat hearts*, Dissertation, Kuala Lumpur, Malaysia, University Kebangsaan Malaysia, 1999.

[25] T. Matsuda, M. Kuroyanagi, S. Sugiyama, K. Umehara, A. Ueno, and K. Nishi, "Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees," *Chemical and Pharmaceutical Bulletin*, vol. 42, no. 6, pp. 1216–1225, 1994.

[26] C. Y. Zhang and B. K. H. Tan, "Vasorelaxation of rat thoracic aorta caused by 14-deoxyandrographolide," *Clinical and Experimental Pharmacology and Physiology*, vol. 25, no. 6, pp. 424–429, 1998.

[27] C. Y. Zhang and B. K. H. Tan, "Effects of 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide on nitric oxide production in cultured human endothelial cells," *Phytotherapy Research*, vol. 13, no. 2, pp. 157–159, 1999.

[28] A. G. Stewart and P. J. Piper, "Vasodilator actions of acetylcholine, A23187 and bradykinin in the guinea-pig isolated perfused heart are independent of prostacyclin," *British Journal of Pharmacology*, vol. 95, no. 2, pp. 379–384, 1988.

[29] C. Zhang, M. Kuroyangi, and B. K. H. Tan, "Cardiovascular activity of 14-deoxy-11,12-didehydroandrographolide in the anaesthetised rat and isolated right atria," *Pharmacological Research*, vol. 38, no. 6, pp. 413–417, 1998.

[30] D. B. Hoover, "Muscarinic blocking drugs," in *Modern Pharmacology*, vol. 1, pp. 178–187, Little, Brown and Co., Boston, Mass, USA, 1990.