UNIVERSITI PUTRA MALAYSIA

ANTINOCICEPTIVE ACTIVITY OF BOESENBERGIA ROTUNDA (L.) MANSF. HEXANE EXTRACT AND ITS MECHANISMS OF ACTIONS IN MICE

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By

NOR 'ADILAH BINTI MAKHTAR

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Boesenbergia rotunda, fingerroot or locally known as "temu kunci" is commonly used in Southeast Asia as food ingredient and folk medicine for relieving pain related to the stomach, abdomen, joint, and muscle. Currently, there is lack of studies on the analgesic properties of the plant. Hence this study is an attempt to investigate the antinociceptive activity of the *Boesenbergia rotunda* hexane extract (BRHE) using various models of chemicals- and thermal-induced nociception in mice and thus promote and support the analgesic claim of *B. rotunda*. Results from the antinociceptive study showed that oral administration of BRHE produced significant ($p<0.05$) inhibition of mice writhing response with the highest dose of 300 mg/kg resulting in 82.19% inhibition. Results from the hot plate test also showed that BRHE produced significant ($p<0.05$) increase in the latency time compared to control groups. Additionally, in the formalin test, the nociceptive activity was inhibited significantly ($p<0.05$) at both phases by BHRE with the highest dose 300 mg/kg resulting in 67.7% inhibition of the first phase and 85.9% inhibition of the second phase. Oral administration of BRHE also significantly ($p<0.05$) reduced nociceptive activity caused by capsaicin and glutamate with the highest dose 300 mg/kg causing inhibition of 92.3% and 92% respectively. Pretreatment of the mice with naloxone (non-selective opioid antagonist), beta-funaltrexamine (mu opioid receptor antagonist), norbinaltorphimine (kappa opioid receptor antagonist), L-arginine (kappa opioid receptor antagonist), tetraethylammonium (non-selective voltage-dependent K^+ channel blocker), and charybdotoxin (large conductance Ca^{2+} activated K^+ channel blocker) at designated doses significantly ($p<0.05$) reversed BRHE-induced antinociception (300 mg/kg) in the acetic acid-induced writhing test. Together, these results suggested that BRHE may exert its antinociceptive activity through activation of mu opioid receptor and kappa opioid receptor. It also indicates that BRHE-induced antinociception was possibly related to its ability to inhibit the L-arginine/nitric oxide pathway, together with the activation of voltage-dependent K^+ channel and Ca^{2+} activated K^+ channel. In addition, no signs of toxicity or mortality were observed in the preliminary acute toxicity test. Furthermore, no significant alteration of mice motor

performance in rota-rod test was exhibited, ruling out the sedative effect of BRHE. The antinociceptive action demonstrated in the present study supports, at least in part, the ethnomedical uses of this plant.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains.

AKTIVITI ANTINOSISEPTIF EKSTRAK HEKSANA *BOESENBERGIA ROTUNDA* (L.) MANSF. DAN MEKANISMA TINDAKANNYA DALAM MENCIT

Oleh

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Boesenbergia rotunda, atau pun temu kunci biasanya digunakan di kawasan Asia Tenggara sebagai bahan makanan dan ubatan tradisional bagi kesakitan yang berkaitan dengan perut , abdomen, sendi dan otot. Pada masa ini, kajian terhadap aksi analgesik tumbuhan ini adalah kurang. Keputusan daripada kajian antinosiseptif menunjukkan bahawa administrasi oral ekstrak heksana *Boesenbergia rotunda* (BRHE) menyebabkan penyekatan signifikan (p<0.05) respon penggeliatan mencit dengan dos tertinggi 300 mg/kg menghasilkan sekatan sebanyak 82.19%. Keputusan daripada ujian plat panas juga menunjukkan bahawa BRHE menghasilkan peningkatan signifikan (p<0.05) pada kependaman respon berbanding dengan kumpulan kawalan. Tambahan pula, dalam ujian formalin, aktiviti nosiseptif disekat secara signifikan (p<0.05) pada kedua-dua fasa oleh BRHE dengan dos tertinggi 300 mg/kg menghasilkan penyekatan sebanyak 67.7% pada fasa pertama dan 85.9% pada fasa kedua. Administrasi oral BRHE juga mengurangkan aktiviti nosiseptif yang diaruhkan oleh kapsaisin dan glutamat secara signifikan (p<0.05) dengan dos tertinggi 300 mg/kg menghasilkan penyekatan masing-masing 92.3% dan 92%. Mencit yang diprarawat dengan naloxone (antagonis opioid tidak selektif), beta-funaltrexamine (reseptor antagonis opioid mu) dan nobinaltorphimine (reseptor antagonis opioid kappa), L-arginin (prekursor nitric oksida), tetraethyl-ammonium (penyekat tidak selektif terusan potassium yang diaktifkan oleh voltan), dan charybdotoxin (penyekat terusan kalium konduktan besar yang diaktifkan oleh ion kalsium) pada dos yang digunakan menyongsangkan antinosisepsi yang dihasilkan oleh BRHE secara signifikan (p<0.05) pada ujian penjilatan tapak kaki yang diaruhkan oleh asid asetik. Sekali gus, keputusan–keputusan mencadangkan BRHE mungkin menjalankan aktiviti antinosiseptif melalui pengaktifan reseptor opioid mu dan kappa. Keputusan juga menunjukkan antinosisepsi yang diaruhkan oleh BRHE mungkin berkaitan dengan keupayaan BRHE untuk menyekat laluan L-arganin/ nitrik oksida , bersama-sama dengan pengaktifan terusan kalium yang diaktifkan oleh voltan dan ion kalsium, tanpa melibatkan cGMP. Disamping itu, tiada tanda-tanda toksik atau kematian dilihat pada ujian toksik akut. Tambahan pula, tiada perubahan persembahan motor

mencit meminggirkan kesan sedatif BRHE. Kesan antinosiseptif yang ditunjukkan pada kajian ini menyokong sekurang-kurangnya sebahagian daripada kegunaan etnomedikal tumbuhan ini.

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Declaration by graduate student

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LIST OF ABBREVIATIONS

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
ASA	acetylsalicylic acid
Ca^{2+}	calcium
cGMP	cyclic guanosine monophosphate
CGRP	calcitonin gene-related peptide
CNS	central nervous system
COX	cyclooxygenase
DH	dorsal horn
DRG	dorsal root ganglion
Fig.	Figure
H^+	hydrogen
BRHE	*Boesenbergia rotunda* hexane extract
i.p.	intraperitoneal
i.pl.	intraplantar
K^+	potassium
KA	kainate
L-NOARG	L-NG-nitro arginine
LTP	long-term potentiation
min	minutes
Na^+	sodium
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-Methyl-D-aspartic acid
NO	nitric oxide
NSAIDs	non-steroidal anti-inflammatory drugs
PAF	peripheral afferent fibre
p.o.	*per os*
PGE_2	prostaglandin E_2
S.E.M	standard error mean
TGF-α	transforming growth factor alpha
TRPV1	transient receptor potential vanilloid receptor

CHAPTER 1

INTRODUCTION

The International Association for The Study of Pain (IASP) characterized pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Loeser and Treede, 2008). Pain is very essential to human survival. It serves as protective function by signaling the presence of harmful, tissue-damaging conditions (Tortora and Derrickson, 2006). A withdrawal reflex response to an acute noxious stimulus is an apprehensible and crucial reaction that has an obvious protective function (Clarke and Harris, 2004). Moreover, the experience of pain may lead to the avoidance of potentially detrimental situations and possible injury. Immobility and withdrawal due to pain may assist to accommodate an environment in which healing and recovery of function can occur. Thus the severe deformities developed by individuals with a rare congenital insensitivity to pain portray the useful protective function provided by the sensation of pain (Hudspith *et al*, 2005).

However, chronic pain such as that following nerve injury, the pain associated with migraine, or where pain persists after healing of injury chronic pain serves no biological function and rather than being the symptom of a disease process, chronic pain is itself a disease process (Hudspith *et al*, 2005). Pathologic pain can be a major problem and greatly affect quality of life, and it is essential for patients to obtain relieve from the pain.

Nociception, the perception of noxious stimuli, is initiated by stimuli that activate the peripheral terminals of nociceptors, a highly specialized subset of primary sensory neurons that respond only to intense stimuli. Any agent or substance that is able to suppress the perception of pain is said to have antinociceptive properties.

Medicinal herbs with antinociceptive properties have been used as a form of therapy for the relief of pain throughout history (Almeida *et al*, 2001). Treatment of diseases by plants or extracts thereof is as old as the history of mankind. As early as 400 BC, Hippocrates recommended leaves of the willow tree for medical purposes (Schrör, 2008a). Essentially, natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy.

In Asia, various spices, herbs, and condiments have been utilized not only to flavor food but also to maintain health and promote wellness. *Boesenbergia rotunda* (L.) Mansf. (local name: temu kunci), is a perennial rhizomatous herb of the family Zingiberaceae distributed from north-eastern India to south-east Asia, especially in Indonesia, Thailand

and Malaysia (Ng *et al*, 2013). The fresh rhizomes have a characteristic aroma and a slightly pungent taste. It is commonly used in Southeast Asia as a food ingredient, a folk medicine for the treatment of several diseases such as aphthous ulcer, dry mouth, stomach discomfort, leukorrhea, and dysentery (Ching *et al*, 2007).

The rhizomes of *Boesenbergia rotunda* have been reported to contain essential oil (Sukari *et al*, 2008), pinostrobin (Tan, 2005), cardamonin (Shindo *et al*, 2006), boesenbergin A (Sukari *et al*, 2007), 3,5,7-Trimethoxyflavone (Jaipetch *et al*, 1983), 1,8-cineole (Sukari *et al*, 2008) , and panduratin A (Tewtrakul *et al*, 2009). *B. rotunda* and its constituents have been shown to exhibit anti-inflammatory (Tuchinda *et al*, 2002), anti-amoebic (Sawangjaroen *et al*, 2005), anti-carcinogenesis (Kirana *et al*, 2007), suppression of melanogenesis (Cho *et al*, 2009) and inhibition of HIV-1 protease activity (Tewtrakul *et al*, 2009).

Currently, two major classes of drugs are used in the pharmacological therapy of pain, namely non-steroidal anti-inflammatory drugs, and opioids. However, undesirable adverse effects such as gastrointestinal damage, renal toxicity, sedation, tolerance and respiratory depression has led to a search for new pharmacologically potent analgesic compounds with minimum adverse effects compared to current pharmacological therapy of pain.

Pharmacologically, any effective anti-inflammatory treatment in turn will also inhibit the accompanying event of pain (Schrör, 2008b). Since *B. rotunda* have been pharmacologically proven to exhibit anti-inflammatory action, it is of interest to examine the antinociceptive properties of *B.rotunda.* Moreover, the basis for the traditional use of this plant in the management of pain related conditions is yet to be scientifically verified. The present study was aimed at investigating the possible antinociceptive activity of *B. rotunda* oil and to explore its possible mechanism of action using chemical and thermal models of nociception, thus verifying its traditional use as pain reliever.

1.1 General Objective

To investigate the antinociceptive effect of BRHE in various models of induced-nociception and its possible mechanism(s) of action.

1.2 Specific Objectives:

1. To investigate the antinociceptive effects of *Boesenbergia rotunda* (Roxb.) Schltr.'s hexane extract (BRHE) against central and peripheral nociception in mice.

2. To investigate the involvement of BRHE in endogenous opioid antinociceptive mechanism.
3. To investigate the participation of TRPV1 receptor and glutamatergis system in the BRHE antinociceptive mechanism.
4. To explore BRHE's antinociceptive interaction with NO-cyclic GMP/potassium channel pathway.

1.3 Hypothesis

Boesenbergia rotunda hexane extract exerts dose-dependent antinociceptive effect against central and peripheral nociception in mice through modulation of the endogenous opioid antinociceptive mechanism and NO/cGMP/K^+ pathways.

REFERENCES

Abacioglu, N., Demir, S., Cakici, I., Tunctan, B. and Kanzik, I. 2000. Role of guanylyl cyclase activation via thiamine in suppressing chemically-induced writhing in mouse. *Arzneimittelforschung,* 50(6): 554-558.

Ahern, G. P., Hsu, S. F., and Jackson, M. B. 1999. Direct actions of nitric oxide on rat neurohypophysial K+ channels. *Journal of Physiology,* 520(1): 165-176.

Akil, H., Watson, S.J., Young, E., Lewis, M.E., Khachaturian, H. and Walker, J. M. 1984. Endogenous opioids: biology and function. *Annual Review Neuroscience,* 7: 223-255.

Ali, M.S., Maum, A.S.M., Bachar, S.C., and Islam, M.S. 2004. Analgesic Activity of the Rhizomes of *Curcuma zedoaria. Dhaka University of Pharmaceutical,* 3(1).

Almeida, R. N., Navarro, D. S., and Barbosa-Filho, J. M. 2001. Plants with central analgesic activity. *Phytomedicine,* 8(4): 310-322.

Alves, D. and Duarte, I. 2002. Involvement of ATP-sensitive K(+) channels in the peripheral antinociceptive effect induced by dipyrone. *European Journal of Pharmacology,* 444(1-2): 47-52.

Beirith, A., Santos, A.R., and Calixto, J.B. 2002. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. *Brain Research,* 924(2), 219-228.

Biophysical Research Communications, 390(3), 500-505.

Bleakman, D., Alt, A., and Nisenbaum, E. S. 2006. Glutamate receptors and pain. *Seminars in Cell and Developmental Biology,* 17(5): 592-604.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J. and Cohen, R.A. 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature*, 368(6474): 850-853.

Boonjaraspinyo, S., Boonmars, T., Aromdee, C. and Kaewsamut, B. 2010. Effect of fingerroot on reducing inflammatory cells in hamster infected with Opisthorchis viverrini and N-nitrosodimethylamine administration. *Parasitology Research,* 106(6): 1485-1489.

Brooks, J. and Tracey, I. 2005. From nociception to pain perception: imaging the spinal and supraspinal pathways. *Journal of Anatomy,* 207: 19-33.

Brownstein, M. J. (1993). A brief history of opiates, opioid peptides, and opioid receptors. *Proceedings of the National Academy of Sciences of the United States,* 90(12): 5391-5393.

Brzozowski, T., Konturek, S.J., Sliwowski, Z., Drozdowicz, D., Zaczek, M. and Kedra, D. 1997. Role of L-arginine, a substrate for nitric oxide-synthase, in gastroprotection and ulcer healing. *Journal of Gastroenterology,* 32(4): 442-452.

Burgess, P. R. and Perl, E. R. . (1967). Myelinated afferent fibres responding specifically to noxious stimulation of the skin. *Journal of Physiology*, 190, 541-562.

Butler, M. S. 2005. Natural products to drugs: natural product derived compounds in clinical trials. *Natural Product Reports,* 22(2): 162-195.

Caterina, M. J. and Julius, D. 2001. The vanilloid receptor: a molecular gateway to the pain pathway. *Annual Review in Neuroscience,* 24: 487-517.

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D. and Julius, D. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature,* 389(6653), 816-824.

Charoensin, S., Punvittayagul, C., Pompimon, W., Mevatee, W., and Wongpoomchai, R. 2010. Toxicological and clastogenic evaluation of pinocembrin and pinostrobin isolated from *Boesenbergia rotunda* in Wistar rats. *Thai Journal of Toxicology,* 25(1): 29-40.

Ching, A. Y. L., Tang, S. K., Sukari, M. A., Ee, G. C. L., Rahmani, R., and Khalid, K. 2007. Characterization of Flavonoid Derivatives from *Boesenbergia rotunda* (L.). *The Malaysian Journal of Analytical Sciences,* 11(1), 154-159.

Cho, M., Ryu, M., Jeong, Y., Chung, Y.H., Kim, D.-E., Cho, H.S., Kang, S., Han, J.S., Chang, M.Y., Lee, C.K., Jin, M., Kim, H.J., Oh, S. 2009. Cardamonin suppresses melanogenesis by inhibition of Wnt/[beta]-catenin signaling. *Biochemichal & Biophysical Research Communications,* 390(3):500-505.

Clarke, R.W. and Harris, J. 2004. The organization of motor responses to noxious stimuli. ***Brain Research Reviews***, 46(2): 163-172.

Cragg, G.M., Newman, D.J., Snader, K.M. 1997. Natural products in drug discovery and development. *Journal of Natural Products*, 60 (52-60).

Devaraj, S., Esfahani, A. S., Ismail, S., Ramanathan, S., and Yam, M. F. 2010. Evaluation of the antinociceptive activity and acute oral toxicity of standardized ethanolic extract of the rhizome of *Curcuma xanthorrhiza* Roxb. *Molecules,* 15(4): 2925-2934.

Dray, A. 1995. Inflammatory Mediators of Pain. *British Journal of Anaesthesia*, 75: 125-131.

Du, X., Wang, C. and Zhang, H. 2011. Activation of ATP-sensitive potassium channels Antagonize nociceptive behavior and hyperexcitability of DRG neurons from rats. *Molecular Pain, 7*: 35.

Dubin, A.E. and Patapoutian, A. 2010. Nociceptors: the Sensors of Pain Pathway. *The Journal of Clinical Investigation*, 120(11): 3760-3772.

Dubuisson, D., & Dennis, S.G. 1977. The formalin test: A quantitative study of the Analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain,* 4: 161-174.

Eddy, N. B., Lembach, D. 1953. Synthesis, anti-inflammatory and analgesic activity of pyrido[2,1-b]quinazoline derivatives. *Journal of Pharmacology & Experimental Therapeutics, 707*: 385.

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D. and Guo, Z. 1985. Medicinal plants in therapy. *Bulletin of World Health Organisation,* 63(6), 965-981.

Fein, A. 2012. *Nociceptors and the Perception of Pain*. Farmington: University of Connecticut Health Centre.

Fozard, J. R. (1995). The 5-hydroxytryptamine-nitric oxide connection: the key link in the initiation of migraine?*Archives Internationales de Pharmacodynamie et de Theraphie,* 329(1): 111-119.

Franklin, K.B.J. and Abbott, F.B. 1990. Techniques for Assessing the Effects of Drugs on Nociceptive Responses. *Psychopharmacology*, 13: 145-216.

Fundytus, M.E. 2001. Glutamate receptors and nociception: implications for the drug treatment of pain. *CNS Drugs,* 15(1): 29-58.

Ganesan, A. 2008. The impact of natural products upon modern drug discovery. *Current Opinion in Chemical Biology, 12*(3), 306-317.

Gross, M. and Greenberg, L.A. 1948. *The Salicylates. A Critical Bibliographic Review*. New Haven, CT: Hillhouse Press

Holthusen, H. and Arndt, J.O. 1994. Nitric oxide evokes pain in humans on intracutaneous injection. *Neuroscience Letter,* 165(1-2): 71-74.

Hong Jin, Y., Takemura, M., Furuyama, A. and Yonehara, N. 2012. Peripheral Glutamate Receptors are Required for Hyperalgesia Induced by Capsaicin. *Pain Research and Treatment*, 2012: 915706.

Hudspith, M. J., Siddall, P. J. and Munglani, R. 2005. Physiology of Pain. In *Foundations of Anaesthesia : Basic Sciences for Clinical Practise*. ed. H. Hemmings and P. Hopkins, pp. 267-285. Mosby Ltd.

Hunskaar, S. 1987. Similar effects of acetylsalicylic acid and morphine on immediate responses to acute noxious stimulation. *Pharmacology and Toxicology,* 60(3): 167-170.

Isa, N.M., Abdelwahab, S.I., Mohan, S., Abdul, A.B., Sukari, M.A., Taha, M.M., Syam, S., Narrima, P., Cheah, S.Ch., Ahmad, S., Mustafa, M.R. 2012. In vitro anti-inflammatory, cytotoxic and antioxidant activities of boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (L.) (fingerroot). *Brazilian Journal of Medical and Biological Research*, 45(6) 524-530.

Jaipetch, T., Reutrakul, V., Tuntiwachwuttikul, P., and Santisuk, T. 1983. Flavonoids in the black rhizomes of *Boesenbergia rotunda*. *Phytochemistry*, 22(2): 625-626.

Jantan I., Chin C.B., Chen L.L. and Sim, N.L. (2003). Antifungal activity of the essential oils of nine Zingiberaceae species. *Phararmacology Biology,* 41: 392-397.

Jhaveri, M.D., Elmes, S.J., Kendall, D.A., Chapman, V. 2005. Inhibition of peripheral vanilloid TRPV1 receptors reduces noxious heat-evoked responses of dorsal horn neurons in naïve, carrageenan-inflamed and neuropathic rats. *European Journal of Neuroscience*, 22(2): 361-70.

Julius, D. and Basbaum, A.I. 2001. Molecular mechanisms of nociception. *Nature,* 413(6852): 203-210.

Jung, H.W., Yoon, C-H., Park, K.M., Han, H.S., and Park, Y-K. 2009. Hexane fraction of Zingiberis Rhizoma Crudus extract inhibits the production of nitric oxide and proinflammatory cytokines in LPS-stimulated BV2 microglial cells via the NF-kappaB pathway. *Food and Chemical Toxicology*, 47(6): 1190-1197.

Kirana, C., Jones, G., Record, I., & McIntosh, G. 2007. Anticancer properties of panduratin A isolated from *Boesenbergia rotunda* (Zingiberaceae). *Journal of Natural Medicines,* 61(2), 131-137.

Koster R.. A.M., De Beer, E.J. 1959. Acetic Acid for Analgesic Screening. *Federation Proceedings,* 18: 412.

Levine, J.D., Fields, H.L. and Basbaum, A.I. 1993. Peptides and the Primary Afferent Nociceptor. *The Journal of Neuroscience*, 13(6): 2273-2286.

Lipp, J. 1991. Possible mechanisms of morphine analgesia. *Clinical Neuropharmacology,*14(2):131–147.

Loeser, J.D. and Treede, R-D. 2008. The Kyoto protocol of IASP Basic Pain Terminology. *Pain* 137(3): 473-477.

Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of Toxicology,* 54(4): 275-287.

Mansour, A., Watson, S. J., and Akil, H. 1995. Opioid receptors: past, present and future. *Trends in Neurosciences, 18*(2), 69-70.

Meller, S.T. and Gebhart, G.F. 1993. Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain,* 52(2), 127-136.

Millan, M.J. 1999. The Induction of Pain: an Integrative Review. *Progress in Neurobiology*. 57: 1-164.

Miller, R.J., Jung, H., Bhangoo, S.K., and White, F.A. 2009. Cytokine and Chemokine Regulation of Sensory Neuron Function. In *Sensory Nerves,* ed. Canning, B.J. and Spina, D. pp. 418-441, Heidelberg: Springer.

Ming-Tatt, L., Khalivulla, S.I., Akhtar, M.N., Mohamad, A.S., Perimal, E.K., Khalid, M. H., Akira, A., Lajis, N., Israf, D.A., Sulaiman, M.R. 2012. Antinociceptive activity of a synthetic curcuminoid analogue, 2,6-bis-(4-hydroxy-3-methoxybenzylidene) cyclohexanone, on nociception-induced models in mice. *Basic Clinical Pharmacolology and Toxicology,* 110(3): 275-282.

Mirchandani, A. and Akhtar, S. 2010. Pain Pathways and Pain Processing. In *The Essence of Analgesia and Analgesics*, ed. Sinatra, R.S., Jahr, J.S., and Watkins-Pitchford, J.M. pp. 7-17. Cambridge University Press.

Morad, A.F. *Boesenbergia rotunda (L.) Mansf.* 2013. www.flickr.com. Web. 13th August 2013.

Murray, C.W., Porreca, F. and Cowan, A. 1988. Methodological refinements to the mouse paw formalin test. An animal model of tonic pain. *Journal of Pharmacology Methods,* 20(2): 175-186.

Ng, B-K, Bustamam, A., Sukari, M.A., Abdelwahab, S.I., Mohan, S., Buckle, M.J., Kamalidehghan, B., Nadzri, N.M., Anasamy, T., A Hadi, A.H., and Rahman, H.S. 2013. Induction of selective cytotoxicity and apoptosis in human T4-lymphoblastoid cell line (CEMss) by boesenbergin a isolated from *Boesenbergia rotunda* rhizomes involves mitochondrial pathway, activation of caspase 3 and G2/M phase cell cycle arrest. *BMC Complementary and Alternative Medicine*, 13: 41.

Nociceptors. Retrieved from 17 January 2014 from http://hsan-iv.weebly.com/physiology.html

Ong, H.M., Mohamad, A.S., Makhtar, N., Khalid, M.H., Khalid, S., Perimal, E.K., Mastuki, S.N., Zakaria, Z.A., Lajis, N., Israf, D.A., Sulaiman, M.R. 2011. Antinociceptive activity of methanolic extract of *Acmella uliginosa* (Sw.) Cass. *Journal of Ethnopharmacology,* 133(1): 227-233.

Perimal, E.K., Akhtar, M.N., Mohamad, A.S., Khalid, M.H., Ming, O.H., Khalid, S., *et al.* 2011. Zerumbone-induced antinociception: involvement of the L-arginine-nitric oxide-cGMP -PKC-K+ ATP channel pathways. *Basic and Clinical Pharmacology and Toxicology,* 108(3): 155-162.

Pinho, J.P., Silva, A.S., Pinheiro, B.G., Sombra, I., Bayma Jde, C., Lahlou, S., and Magalhães, P.J. 2012. Antinociceptive and antispasmodic effects of the essential oil of *Ocimum micranthum*: potential anti-inflammatory properties. *Planta Medica,* 78(7): 681-685.

Ramabadran, K. and Bansinath, M. 1990. The role of endogenous opioid peptides in the regulation of pain. *Critical Review in Neurobiology,* 6(1): 13-32.

Regulation of Sensory Neuron Function. In *Sensory Nerves*, ed. Canning, B.J., and Spina, D, pp. 418-441. Heidelberg: Springer.

Ricciotti, E. and FitzGerald, G.A. 2011. Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 31*(5), 986-1000.

Ridtitid, W., Sae-Wong, C., Reanmongkol, W. and Wongnawa, M. 2008. Antinociceptive activity of the methanolic extract of *Kaempferia galanga* Linn. in experimental animals. *Journal of Ethnopharmacology,* 118(2): 225-230.

Rosenbaum, T. and Simon, S.A. 2007. TRPV1 Receptors and Signal Transduction. In *TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades*, ed. Liedtke, W.B. and Heller, S. Boca Raton (FL): CRC Press.

Ruangsang, P. 2010. *Evaluation of Anti-inflammatory, Antinociceptive, and Antipyretic Activities of the Extract from* Curcuma mangga *Rhizome*. PhD Thesis, Prince of Songkhla University.

Sakurada, T., Matsumura, T., Moriyama, T., Sakurada, C., Ueno, S. and Sakurada, S. 2003. Differential effects of intraplantar capsazepine and ruthenium red on capsaicin-induced desensitization in mice. *Pharmacology Biochemistry and Behavior,* 75(1): 115-121.

Saraithong, P., Saenphet, S. and Saenphet, K. 2010. Safety evaluation of ethanol extracts from *Boesenbergia rotunda* (L.) Mansf. In male rats. *Trends Research in Science and Technology*, 2(1): 19-22.

Savegnago, L., Pinto, L. G., Jesse, C. R., Alves, D., Rocha, J. B., Nogueira, C. W., Zeni, G.. 2007. Antinociceptive properties of diphenyl diselenide: evidences for the mechanism of action. *European Journal of Pharmacology,* 555(2-3): 129-138.

Sawangjaroen, N., Subhadhirasakul, S., Phongpaichit, S., Siripanth, C., Jamjaroen, K., and Sawangjaroen, K. 2005. The in vitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand. *Parasitology Research,* 95(1), 17-21.

Schmidt, R.F. and Willis, W.D. 2007. *Encyclopaedia of Pain*. New York: Springer.

Schmidtko, A., Tegeder, I. and Geisslinger, G. 2009. No NO, no pain? The role of nitric oxide and cGMP in spinal pain processing. *Trends in Neuroscience,* 32(6): 339-346.

Schrör, K. 2008a. General Aspects. In *Acetylsalicylic Acid,* pp. 5. Weinheim: Wiley-Blackwell.

Schrör, K. 2008b. Pain, Inflammation and Fever. In *Acetylsalicylic Acid,* pp. 105-111. Weinheim: Wiley-Blackwell.

Schumacher, M.A., Basbaum, A.I., and Way, W.L. 2003. Opioid Analgesics and Antagonists. In *Basic and Clinical Pharmacology,* ed. Katzung, B.G., pp 497-516. New York: Lange.

Sherrington, C. S. 1906. *The Integrative Action of the Nervous System*. Charles Scribner's Sons.

Shindo, K., Kato, M., Kinoshita, A., Kobayashi, A. and Koike, Y. 2006. Analysis of antioxidant activities contained in the *Boesenbergia rotunda* Schult. Rhizome. *Bioscience, Biotechnology and Biochemistry*, 70(9): 2281-2284.

Sukari, M.A. Ching, A.Y.L., Lian, G.E.C., Rahmani, M., and Khalid, K. 2007. Cytotoxic constituents from *Boesenbergia rotunda* (Roxb.) Schltr. *Natural Product Sciences,* 13(2): 110-113.

Sukari, M.A., Sharif, N.W.M., Ching, A.Y.L., Lian, G.E.C., Rahmani, M., and Khalid, K. 2008. Chemical constituents variations of essential oils from rhizomes of four Zingiberaceae species. *The Malaysian Journal of Analytical Sciences,* 12(3): 638-644.

Sulaiman, M.R., Tengku Mohamad, T.A., Shaik Mossadeq, W.M., Moin, S., Yusof, M., Mokhtar, A.F., Zakaria, Z.A., Israf, D.A., Lajis, N. 2010. Antinociceptive activity of the essential oil of *Zingiber zerumbet*. *Planta Medica,* 76(2): 107-112.

Sulaiman, M.R., Zakaria, Z.A., Adilius, M., Mohamad, A.S., Ismail, M., & Israf, D.A. 2009. Antinociceptive and anti-inflammatory effects of the ethanol extract of *Alpinia conchigera* Griff. leaves in various animal models. *Methods and Findings in Experimental and Clinical Pharmacology,* 31(4), 241-247.

Suzuki, T. and Misawa, M. 1997. Opioid receptor types and dependence. *Nihon Yakurigaku Zasshi,* 109(4): 165-174.

Szallasi, A. and Blumberg, P.M. 1999. Vanilloid (Capsaicin) Receptors and Mechanisms. *Pharmacological Reviews*, 51(2): 159-212.

Szliszka, E., Czuba, Z.P., Mazur, B., Paradysz, A. and Krol, W. 2010. Chalcones and dihydrochalcones augment TRAIL-mediated apoptosis in prostate cancer cells. *Molecules,* 15(8): 5336-5353.

Takeda, M., Tsuboi, Y., Kitagawa, J., Nakagawa, K., Iwata, K. and Matsumoto, S. 2011. Potassium channels as a potential therapeutic target for trigeminal neuropathic and inflammatory pain. *Molecular Pain, 7*: 5.

Tan, S.K. 2005. *Flavonoids from Boesenbergia rotunda (L.) Mansf.: chemistry, bioactivity and accumulation,*. PhD Thesis, Universiti of Malaya.

Tewtrakul, S., Subhadhirasakul, S., Karalai, C., Ponglimanont, C., and Cheenpracha, S. 2009. Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia rotunda*. *Food Chemistry*, 115(2): 534-538.

Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H. and Hole, K. 1992. The formalin test: an evaluation of the method. *Pain,* 51(1): 5-17.

Torres-Santos, E.C., Moreira, D.L., Kaplan, M.A., Meirelles, M.N. and Rossi-Bergmann, B. 1999. Selective effect of 2',6'-dihydroxy-4'-methoxychalcone isolated from *Piper aduncum* on Leishmania amazonensis. *Antimicrobcrobial Agents Chemotheraphy,* 43(5): 1234-1241.

Tortora, G.J. and Derrickson, B. 2006. Sensory, Motor, and Integrative Systems. In *Principle of Anatomy and Physiology.* pp. 552-553. United States of America: John Wiley and Sons, Inc.

Treede, R.D. 2009. The Adequate Stimulus. In *Science of Pain*, ed. Basbaum, A.I. and Busnell, M.C., pp. 1-4. Singapore: Elsevier Inc.

Tuchinda, P., Reutrakul, V., Claeson, P., Pongprayoon, U., Sematong, T., Santisuk, T., Taylor, W.C. 2002. Anti-inflammatory cyclohexenyl chalcone derivatives in *Boesenbergia rotunda*. *Phytochemistry,* 59(2), 169-173.

Turner, P.V., Brabb, T., Pekow, C., and Vasbinder, M.A. 2011. Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of American Association Laboratory Animal Science*, 50(5): 600–613.

Turner, R. A. 1965. *Screening methods in pharmacology*. New York: Academic Press.

Wagner, W., Khanna, P., and Furst, D.E. 2003. Nonsteroidal Anti-inflammatory Drugs, Disease Modifying Antirheumatic Drugs, Nonopioid Analgesics, & Drugs Used in Gout. In *Basic and Clinical Pharmacology,* ed. Katzung, B.G., pp. 576-603. New York: Lange.

Winarti, L. and Wantiyah. 2011. Examinationof analgetic effect of extract *Boesenbergia pandurata* (Roxb.) Schlechter to Swiss Furrow Male Mice. *Majalah Obat Tradisional*, 16(1): 26–33.

Xu, H., Blair, N.T. and Clapham, D. E. 2005. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. *Journal of Neuroscience,* 25(39): 8924-8937. Yaksh, T.L. 1997. Pharmacology and mechanisms of opioid analgesic activity. *Acta Anaesthesiologica Scandinavia,* 41(1): 94-111.

Yu, H.L., Zhang, F., Li, Y.J., Gong, G.H. and Quan, Z.S. 2012. Anti-inflammatory and antinociceptive effects of 6-(4-chlorophenoxy)-tetrazolo[5,1-a]phthalazine in mice. *Pharmacological Reports,* 64(5): 1155-1165.