Original Article

Effects of various receptor antagonists on the peripheral antinociceptive activity of aqueous extracts of *Dicranopteris linearis*, *Melastoma malabathricum* and *Bauhinia purpurea* leaves in mice

Zainul Amiruddin Zakaria[1,*], Nurul Husna Sodri[2], Halmy Hassan[2], Khairiyah Anuar[2], Fatimah Corazon Abdullah[3]

[1]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia;* [2]*Faculty of Pharmacy, Science and Technology Complex, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia;* [3]*Institute of Bio-IT Selangor, Universiti Selangor, Jalan Zirkon A7/A, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia*

ABSTRACT

The present study aimed to determine the possible mechanisms of the peripheral antinociception of the aqueous extracts of *Dicranopteris linearis* (AEDL), *Melastoma malabathricum* (AEMM) and *Bauhinia purpurea* (AEBP) leaves in mice. Briefly, the antinociceptive profile of each extract (300, 500, and 1000 mg/kg; subcutaneous (s.c.)), was established using the abdominal constriction test. A single dose (500 mg/kg) of each extract (s.c.) was pre-challenged for 10 min with various pain receptors' antagonists or pain mediators' blockers and 30 min later subjected to the antinociceptive assay to determine the possible mechanism(s) involved. Based on the results obtained, all extracts exerted significant ($p < 0.05$) antinociceptive activity with dose-dependent activity observed only with the AEMM. Furthermore, the antinociception of AEDL was attenuated by naloxone, atropine, yohimbine and theophylline; AEMM was reversed by yohimbine, theophylline, thioperamide, pindolol, reserpine, and 4-chloro-DL-phenylalanine methyl ester hydrochloride; and of AEBP was inhibited by naloxone, haloperidol, yohimbine and reserpine. In conclusion, the antinociceptive activity of those extracts possibly involved the activation of several pain receptors (i.e. opioids, muscarinic, α_2-adrenergic and adenosine receptors, adenosine, H_3-histaminergic and $5HT_{1A}$, dopaminergic receptors).

Keywords *Dicranopteris linearis, Melastoma malabathricum, Bauhinia purpurea,* aqueous extract, peripheral antinociception, abdominal constriction test, receptor antagonists

INTRODUCTION

Plants have been used traditionally to treat various types of ailments including those associated with pain, and some of them have been scientifically studied (Panghal et al., 2010). With Malaysia being considered to be among the most biologically diverse countries in the world, it is important to highlight that there are approximately 12,000 species of flowering plants, including species of valuable and marketable timber and fruit (Jamal et al., 2010). Of these species, approximately 1,300 species of the higher plants found in Peninsular Malaysia have been reported to have medicinal properties for the treatment of various diseases and ailments (Jantan, 1998). Nevertheless, hitherto only about one hundred have been investigated fully for their medicinal potential (Jamal et al., 2010). Moreover, some of these plant species are underutilized and considered neglected as there is a lack of traditional claims of their medicinal uses among the Malaysian people, particularly the Malays. Based on this fact, we have decided to study the pharmacological potentials of several underutilized/considered neglected plants, namely *Dicranopteris linearis* (L.), *Bauhinia purpurea* L. and

Melastoma malabathricum L.

D. linearis belongs to a family of ferns from the family Gleicheniaceae and is locally known to the Malays as '*Resam*'' (Derus, 1998; Polunin, 1987). Our literature search indicated that there is a lack of research on *D. linearis* traditional uses all over the world: only three reports described its use to reduce body temperature and to control fever by the Malay people of Malaysia (Derus, 1998; Polunin, 1987), to treat external wound, ulcers and boils by the people of Papua New Guinea (Chin, 1992), to get rid of intestinal worms by the people of Indochina (Chin, 1992) and to treat asthma and woman's sterility by the tribes on Indian mountain (Vasuda, 1999). In the Malay traditional medicine, the leaf of *D. linearis* is squeezed in water and subsequently drank or applied as poultice on the body (Derus, 1998; Polunin, 1987). *M. malabathricum* is a shrub that belongs to the family *Melastomataceae* and known to the Malays as '*Senduduk*'. According to Malay folklore medicine, various parts of this plant possess medicinal properties (Burkill 1966, Jaganath and Ng, 2000). Depending on the way the plant is prepared, the leaves in particular may be used as a tonic or astringent, or to treat burn, diarrhea, dysentery, wounds, pox scars, and to relieve the discomfort of hemorrhoids (Ahmad et al., 1993; Burkill 1966; Institute of Medical Research, 2002; Jaganath and Ng 2000). Despite being given a 'herb' status among the Malay community due to the medicinal properties of its various parts, there is a lack of scientific study on the *M. malabathricum* pharmacological potential. *B. purpurea*, or '*Tapak kerbau*' as it is known to the Malays, belongs to the

family Fabaceae (Zakaria et al., 2007). The root, stem, bark, flower and leaf of this plant have been traditionally used by the Indian and Pakistani to treat infections, jaundice, leprosy, cough, pain, fever, ulcers, boils, diabetes, stomach cancer, rheumatism, convulsions, delirium, and septicaemia (Asolker et al., 2000; Chopra et al., 1956; Janardhanan et al., 2003; Kirthikar and Basu, 2001; Morais et al., 2005; Parrota, 2001) with no claim recorded in Malay traditional medicine.

We have earlier reported on the antinociceptive activity of the leaves of *D. linearis*, *M. malabathricum*, and *B. purpurea* (Zakaria et al., 2008, 2006, 2007). Interestingly, these extracts exhibited an antinociceptive activity that was comparable to standard analgesics like acetylsalicylic acid. This finding together with the fact that no attempt has been made to elucidate the possible mechanisms of antinociception of those extracts have triggered the present study. Thus, the aim of the present study was to determine the effect of various pain receptors' antagonists and pain mediators' blockers on the peripheral antinociceptive activities of the aqueous extracts of *D. linearis* (AEDL), *M. malabathricum* (AEMM) and *B. purpurea* (AEBP) leaves using the abdominal constriction test as the nociceptive model.

MATERIALS AND METHODS

Plant material
The leaves of *D. linearis*, *M. malabathricum* and *B. purpurea* were collected from their natural habitats around Seksyen 7, Shah Alam, Selangor, Malaysia between January and February, 2008 with the respective voucher specimens SK 855/05, SK 507/03 and SK 1095/05 previously preserved at the Herbarium of the Laboratory of Natural Product, Institute of Bioscience, UPM, Serdang Selangor (Zakaria et al., 2008, 2006, 2007).

Preparation of extracts
Preparations of the AEDL, AEMM and AEBP from 40 g of dried powdered samples were carried out according to previously described methods (Zakaria et al., 2008, 2006, 2007) and resulted in yields of 2.0 g (5.0%), 1.98 g (4.95%) and 2.1 g (5.2%) crude extract, respectively. Briefly, the respective leaves were washed and rinsed with water to remove all the dirt and unwanted particles and then oven-dried for 1 - 2 weeks at the temperature of $40^{\circ}C$. The dried leaves were then grinded into small particles, weighed and mixed with distilled water (dH$_2$O) in the ratio of 1:20 (w/v). This mixture was then left for 72 h with occasional stirring and the supernatant was collected and filtered using a Whatman No. 1 filter paper while the remaining plant residue was discarded. Each of the supernatants obtained was subjected to the freeze-drying process to determine the amount of crude dried extract present in each supernatant. Once the amount had been determined, each extract was prepared in doses of 300, 500, and 1000 mg/kg for the antinociceptive study.

Experimental animals
Two hundred and sixty-four (264) male Balb-C mice (25 - 30 g; 5 - 7 weeks) used in the present study were obtained, acclimatized and taken care of as previously described (Zakaria et al., 2008, 2006, 2007). The animal ethics approval was obtained from the Animal Ethics Committee, UPM with reference no: UPM/FPSK/USH/0481. Eleven groups of animals were used to establish the antinociceptive profile of each test solution. The three extracts (300, 500, and 1000 mg/kg), DH$_2$O (distilled water; vehicle) and acetylsalicylic acid (ASA; positive control) were administered subcutaneously (s.c.) into the mice (n = 11) and 30 min later, the animals were subjected

to the 0.6% acetic acid-induced abdominal constriction test (Zakaria et al., 2008, 2006, 2007). To determine the possible mechanisms of antinociception, thirteen groups of animals were used and each extract, only at 500 mg/kg, was pre-challenged with various receptors' antagonists or mediators' blockers as described elsewhere (Abdel-Salam, 2007; Alhaider, 1991; Ghelardini et al., 2000; Zakaria et al., 2005; Zarrindast et al., 1994).

Drugs and chemicals
All drugs and chemicals were purchased from Sigma-Aldrich Co., USA. Each of the antagonists or blockers was administered (s.c) followed 15 min later by the administration of AEDL, AEMM or AEBP (s.c.). Thirty (30) min later, the mice were subjected to the antinociceptive assay.

Antinociceptive assay
The acetic acid-induced abdominal constriction test was carried out according to the method described by Mat Jais et al. (1997) with a slight modification. The mice (n = 11) were pretreated subcutaneously with the respective test solutions and, thirty minutes later, injected via the intraperitoneal (i.p.) route with a phlogistic agent (0.6% acetic acid). The animals were immediately placed individually into glass cages and 5 min was allowed to elapse. The abdominal constriction resulting from the injection of acetic acid consisted of a contraction of the abdominal muscle together with a stretching of at least one hind limb. The number of abdominal constrictions produced in these animals was counted cumulatively for 25 min. Antinociceptive activity, indicated by the reduction in the mean of the number of abdominal constrictions in the test groups compared to the control group, was calculated as the percentage inhibition of abdominal constrictions (percentage of inhibitory level) using the formula below: [Mean of (control − test group) / control group × 100%].

Involvement of opioid and non-opioid receptors and pain mediators' blockers
To determine the role of opioid and non-opioid receptors and pain mediator blockers in the modulation of the antinociceptive activities of AEDL, AEMC and AEBP, a separate procedure as described by Zakaria et al. (2005) was adopted with slight modifications. Thirteen groups of animals (n = 11) were pretreated (i.p.) with the respective antagonist or blocker for 15 min followed by the s.c. administration of the most effective dose (500 mg/kg) of each extract. Thirty minutes later, the animals were subjected to the acetic acid-induced abdominal writhing test.

Statistical analysis
The results are presented as Mean ± Standard Error of Mean (S.E.M.). The one-way Analysis of Variance (ANOVA) test with post-hoc Dunnett test was used to analyze and compare the data, with $p < 0.05$ as the limit of significance.

RESULTS

Except for AEDL (30.7, 64.8, and 40.9% analgesia) and AEBP (40.0, 57.3 and 43.6% analgesia), AEMM, at the doses of 300, 500 and 1000 mg/kg, exhibited significant ($p < 0.05$) antinociceptive activity in a dose-dependent manner (26.3, 38.8, and 76.1%) (Table 1).

Furthermore, pretreatment with several pain receptor antagonists or pain mediator inhibitors significantly ($p < 0.05$) reversed the respective extract antinociceptive activity (Table 2). Of all the antagonists and blockers used, the antinocicpetive

Table 1. The antinociceptive profiles of aqueous extract of D. linearis, M. malabathricum and B. purpurea leaves assessed using the abdominal constriction test in mice

Treatment	Dose (mg/kg)	No. of writhing ± SEM
DH2O	-	33.5 ± 1.6
ASA	150	16.2 ± 1.2[a]
DLAE	300	23.2 ± 2.1[a,b]
	500	11.8 ± 1.4[a,b]
	1000	19.8 ± 1.9[a,b]
MMAE	300	24.7 ± 1.6[a,b]
	500	20.5 ± 0.5[a,b]
	1000	8.0 ± 2.0[a,b]
BPAE	300	20.1 ± 1.1[a,b]
	500	14.3 ± 1.2[a,b]
	1000	18.9 ± 0.4[a,b]

[a]Data differed significantly ($p < 0.05$) when compared against the DH2O-treated group.
[b]Data differed significantly ($p < 0.05$) when compared against the ASA-treated group.

activity of AEDL was significantly ($p < 0.05$) reversed by naloxone, atropine, yohimbine and theophylline; while the antinociceptive activity of AEMM was significantly ($p < 0.05$) blocked by yohimbine, reserpine, theophylline, thioperamide, pindolol and 4-chloro-DL-phenylalanine methyl ester hydrochloride. Except for theophylline, thioperamide, propanolol, pindolol, alpha-methyl-L-tyrosine and 4-chloro-DL-phenylalanine methyl ester hydrochloride, which was not tested against AEBP in the present study, the antinociceptive activity of AEBP was significantly ($p < 0.05$) inhibited by naloxone, haloperidol, yohimbine and reserpine.

DISCUSSION

Natural products, particularly the medicinal plants, have been considered as an important source of new bioactive compounds with potential therapeutic effects. This is based on the fact that medicinal plants have been used to treat various ailments including pain-related diseases throughout history (Almeida et al., 2001). It is worth mentioning that the most important

analgesic drugs (e.g. acetylsalicylic acid and morphine) were originally developed from their respective plant sources (Shanmugasundaram and Venkataraman, 2005). Therefore, research related to plants with traditional claims of having pain-relieving properties should be viewed as a vital, reasonable and profitable strategy in the quest for new analgesic drugs with less, or possibly, no side effects (Karumi et al., 2003).

It is important to understand the actions of analgesic drugs within the body, a process called pharmacodynamics. Briefly, the drugs upon entering the human body tend to stimulate certain receptors, as well as ion channels, act on enzymes or transporter proteins, which cause the human body to react in a specific way. Depending on the types of drugs, agonists stimulate and activate the receptors while antagonists prevent the agonists from stimulating the receptors. Once activated, the receptors either trigger a particular response directly on the body, or trigger the release of hormones and/or other endogenous drugs in the body to stimulate a particular response (Katzung et al., 2012).

Nociceptive processes are mediated via various pathways, which include the activation of various types of receptors and the modulation of various neurotransmitters' release. According to Edmond Charlton (2005), the nociceptor is composed of numerous sensory and chemical receptors, which resulted in the polymodal nature of nociceptive sensory neurons. Various chemical mediators (e.g. bradykinin, serotonin and histamine) can activate nociceptors (Millan, 1999) and the receptors for all these chemicals are invariably multiple (Dray, 1997; Dray and Perkins, 1993; Dray et al., 1994; Hill, 1999; Julius and Basbaum, 2001). These chemicals together with several neuropeptides and prostaglandins participate in peripheral events leading to hyperalgesia and edema in inflammation (Besson and Chaouch, 1987; Dray and Perkins, 1993; Millan, 1999).

Various animal assays can be used to determine the antinociceptive activities of compounds/extracts. However, the abdominal constriction test was normally used as the preliminary screening tool to check whether those compounds/extracts possess the antinociceptive activity. It is considered as a very sensitive test due to its ability to detect the antinociceptive activities of compounds/extracts at the dose levels that may be inactive in the other tests (Bentley et al.,

Table 2. Effects of various receptor antagonists against the antinociceptive activities of 500 mg/kg AEDL, AEMM and AEBP

Treatment	Dose (mg/kg)	Types of extracts		
		AEDL	AEMM	AEBP
DH2O	-	33.5 ± 1.6		
Aqueous extract	500	11.8 ± 1.4	20.5 ± 0.5	14.3 ± 1.2
Naloxone	3	25.0 ± 2.5[*]	20.3 ± 1.2	24.1 ± 0.7[*]
	10	32.2 ± 2.5[*]	24.2 ± 1.1	22.7 ± 0.3[*]
Atropine	5	23.0 ± 1.1[*]	18.7 ± 1.2	14.0 ± 1.9
Methysergide	5	17.0 ± 1.5	17.3 ± 1.5	15.2 ± 0.6
Haloperidol	1	12.8 ± 1.1	19.9 ± 1.9	29.4 ± 1.3[*]
Yohimbine	5	29.3 ± 1.0[*]	38.2 ± 2.4[*]	27.1 ± 2.4[*]
Reserpine	2	17.2 ± 2.6	31.2 ± 2.7[*]	27.9 ± 1.1[*]
Theophylline	25	29.2 ± 0.9[*]	33.0 ± 1.4[*]	ND
Thioperamide	10	16.5 ± 2.0	29.2 ± 1.5[*]	ND
Propanolol	4	12.7 ± 2.7	19.8 ± 1.5	ND
Pindolol	10	13.5 ± 1.7	27.8 ± 2.1[*]	ND
Alpha-methyl-L-tyrosine	200	10.7 ± 1.3	20.3 ± 1.1	ND
4-chloro-DL-phenylalanine methyl ester hydrochloride	200	13.0 ± 0.8	28.5 ± 1.0[*]	ND

ND – not determined
[*]Data differed significantly ($p < 0.05$) when compared against the extract-treated group of the respective column.

1981). The abdominal constriction test is thought to partly involve the activation of local peritoneal receptors located at the surface of the cells lining the peritoneal cavity, which to a certain extent represent the peripheral receptor system (Bentley et al., 1983). The abdominal constrictions seen are due to the acetic acid-induced irritation of the peritoneal cavity (Deraedt et al., 1980).

In order to elucidate the mechanisms of antinociceptive activities of compounds/extracts, particularly the types of receptors that modulate, various receptor antagonists were pre-challenged with the compounds/extracts (Omar et al., 2006; Zakaria et al., 2005). A receptor antagonist is a drug that does not incite a biological response itself once it binds to a receptor, but blocks agonist-mediated responses (Katzung et al, 2012). In other words, antagonists have an affinity but no efficacy for the particular receptors, and binding will disrupt the interaction of agonist with receptors leading to the inhibition of the function of an agonist (Hopkins and Groom, 2002). Most drug antagonists attain their effectiveness by competing with endogenous ligands or substrates at structurally defined binding sites on receptors.

The present study demonstrated the peripheral antinociceptive activities of AEDL, AEMM and AEBP. The AEDL and AEBP antinociceptions did not depend on the dose in comparison to the AEMM antinociception that depended on the dose used. Pre-challenging the extract antinociception with some of the antagonists or blockers did affect the respective extract peripheral antinociceptive activity. From the data obtained, it is plausible to suggest that the AEDL antinociceptive activity involved the activation of opioids, muscarinic, α_2-adrenergic and adenosine receptors, while the AEMM antinociceptive activity involved the activation of α_2-adrenergic, adenosine and H_3-histaminergic and $5HT_{1A}$ receptors, and an increase in catecholamine uptake and noradrenaline synthesis. On the other hand, the antinociceptive activity of AEBP is postulated to involve the activation of opioids, dopaminergic and α_2-adrenergic receptors, and an increase in catecholamine uptake.

Based on the results obtained, several potential implications could be drawn with regards to the therapeutic applications of these extracts in humans. Firstly, the ability of each extract to exhibit an antinociceptive activity suggests their potential use as natural product-based pain-relieving agents. Their being free of toxic effect, which has been proven via an acute toxicity study elsewhere, makes them suitable candidates for future drug development and possibly as a replacement for some standard drugs. The ability of AEDL and AEBP to activate the opioid receptors makes them possible candidates for the replacement of morphine, which has been known to cause side effects like tolerance and dependence upon chronic usages, or acetylsalicylic acid (Katzung et al., 2012). The three extracts ability to activate the α_2-adrenergic might indicate their potential uses to lower blood pressure as seen with the antihypertensive drugs (Katzung et al., 2012) and to manage the withdrawal associated with heroin and methadone (Gowing et al., 2009). The ability of AEDL and AEMM to activate the adenosine receptors seems to imply that these extracts are also involved in an energy transfer during biochemical processes, signal transductions and cellular signaling, acting as an inhibitory neurotransmitter and anti-inflammatory (Trevethick et al., 2008), and promoting sleep and suppressing arousal (Hasko et al., 2008). The ability of AEDL to modulate the muscarinic receptors makes it a future candidate for the development of drugs to treat diseases like Alzheimer's (Fischer et al., 2002) and schizophrenia (Shekhar et al., 2008). The ability of AEMM to modulate the H_3-histaminergic receptors also implies that the extract was a suitable candidate for the development of drugs for the treatment of addiction to drugs of abuse and schizophrenia. The ability of AEMM to modulate the serotonin subtype $5HT_{1A}$ receptors implies the potential of the extract to be used as an antidepressant or anxiolytic agent (Dawson and Watson, 2009). The ability of AEBP to modulate the dopaminergic receptors suggested the ability of this extract to be used to treat Parkinson's disease and as an antidote for the overdosing of antipsychotic drugs (Avanzi et al., 2004; Youssef et al., 2010). The abilities of AEMM and AEBP to affect the uptake of catecholamine indicate their potential in the treatment of depressive disorders and seasonal affective disorders (Black, 2004).

CONCLUSION

In conclusion, the peripheral antinociceptive activities of crude AEDL, AEMM and AEBP involved the activation of several pain pathways, which require further in-depth studies. Furthermore, the non-opioid antinociceptive activities of AEMM should be further explored in our attempt to find better analgesics with less or, possibly, no side effects.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors have no conflicting financial interests.

REFERENCES

Abdel-Salam OM. Modulation of visceral nociception, inflammation and gastric mucosal injury by cinnarizine. Drug Target Insights. 2007;2:29-38.

Ahmad F, Hamid RA, Raji H. Medicinal Plants Used by Bajau Community in Sabah. In Trends in Traditional Medicine Research: Proceedings of the International Conference on the Use of Traditional Medicine and Other Natural Products in Health Care. Chan KL, Hussin A, Sadikun A, Yuen KH, Asmawi MZ and Ismail Z, ed. (Penang, Malaysia: The School of Pharmaceutical Sciences. Universiti Sains Malaysia), p. 494, 1993.

Alhaider AA. Antinociceptive effect of ketanserin in mice: involvement of supraspinal 5-HT2 receptors in nociceptive transmission. Brain Res. 1991;543:335-340.

Almeida RN, Navarro DS, Barbosa-Filho JM. Plants with central analgesic activity. Phytomedicine. 2001;8:310-322.

Asolker LV, Kakkar KK, Chakre OJ. Supplement to glossary of Indian Medicinal Plants, Part I (A-K). (New Delhi, India: National Institute of Science Communication), pp. 116-117, 2000.

Avanzi M, Uber E, Bonfa F. Pathological gambling in two patients on dopamine replacement therapy for Parkinson's disease. Neurol Sci. 2004;25:98-101.

Bentley GA, Newton SH, Starr J. Evidence for an action of morphine and the enkephalins on sensory nerve endings in the mouse peritoneum. Br J Pharmacol. 1981;73:325-332.

Bentley GA, Newton SH, Starr J. Studies on the antinociceptive action of α-agonist drugs and their interactions with opioid mechanisms. Br J Pharmacol. 1983;79:125-134.

Besson JM, Chaouch A. Peripheral and spinal mechanisms of nociception. Physiol Rev. 1987;67:67-186.

Black DW. An open-label trial of bupropion in the treatment of pathologic gambling. J Clin Psychopharmacol. 2004;24: 108-110.

Burkill IHM. A dictionary of economic products of Malay Peninsular. Ministry of Agriculture and Cooperatives Malaysia. 1966;66:1463-1465.

Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. (New Delhi, India: Publication and Information Directorate Hill Side Road), p. 35, 1956.

Dawson LA, Watson JM. Vilazodone: A 5-HT1A receptor agonist/serotonin transporter inhibitor for the teatment of affective disorders. CNS Neurosci Ther. 2009;15:107-117.

Deraedt R, Jougney S, Delevalcee F, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol. 1980;51:17-24.

Derus ARM Pengenalan dan Penggunaan Herba Ubatan. (Kuala Lumpur, Malaysia: Multiple Triple Vision), p. 75, 1998.

Dray A. Peripheral mediators of pain. In The Pharmacology of Pain. Handbook of Experimental Pharmacology. Vol. 130. Dickenson AH and Besson JM ed. (Erlangen, Germany: Springer-Verlag), pp. 21-42, 1997.

Dray A, Perkins MN. Bradykinin and inflammatory pain. Trends Neurosci. 1993;16:99-104.

Dray A, Urban L, Dickenson AH. Pharmacology of chronic pain. Trends Pharmacol Sci. 1994;15:190-197.

Edmond Charlton J. Core Curriculum for Professional Education in Pain. (Seattle, USA: IASP Press), 2005.

Fisher A, Brandeis R, Bar-Ner RH, Kliger-Spatz M, Natan N, Sonego H, Marcovitch I, Pittel Z. AF150(S) and AF267B: M1 muscarinic agonists as innovative therapies for Alzheimer's disease. J Mol Neurosci. 2002;19:145-153.

Ghelardini C, Galeotti N, Bartolini A. Antinociception induced by amitriptyline and imipramine is mediated by alpha2A-adrenoceptors. Jpn J Pharmacol. 2000;82:130-137.

Gowing L, Farrell M, Ali R, White JM. Alpha2-adrenergic agonists for the management of opioid withdrawal. Cochrane Database Syst Rev. 2009;15:CD002024.

Haskó G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov. 2008;7:759-770.

Hill RG. Peripheral analgesic pharmacology. In Pain 1999 - An Updated Review. Max M ed. (Seattle, USA: IASP Press), pp. 391-395, 1999.

Hopkins AL, Groom CR. The druggable genome. Nature Rev Drug Discov. 2002;1:727-730.

Herbal Medicine Research Centre, Institute of Medical Research. Compendium of medicinal plants used in Malaysia. Vol. 2 (Kuala Lumpur, Malaysia: Herbal Medicine Research Centre. Institute for Medical Research), pp. 131-132, 2002.

Jaganath IB, Ng LT. Herbs: The green pharmacy of Malaysia. (Kuala Lumpur, Malaysia: Vinpress), pp. 58-59, 2000.

Jamal P, Barkat AA, Amid A. Distribution of Phenolics in Various Malaysian Medicinal Plants. J Appl Sci. 2010;10: 2658-2662.

Janardhanan K, Vadivel V, Pugalenthi M. Biodiversity in Indian underexploited/tribal pulses. In Improvement Strategies for Leguminosae Biotechnology. Jaiwal KP and Singh RP ed. (Great Britain: Kluwer Academic Press), pp. 353-405, 2003.

Jantan I. The real value of medicinal plants in traditional health care. In Proceedings of the Seminar – Medicinal Plants: Cure for the 21th Century. Nair MNB and Ganapathi N ed. (Serdang, Malaysia: Universiti Putra Malaysia), pp. 20-24, 1998.

Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413:203-210.

Karumi Y, Onyeyili P, Ogugbuaja VO. Anti-inflammatory and antinociceptive (analgesic) properties of Momordical balsamina Linn. (Balsam apple) leaves in rats. Pak J Biol Sci. 2003;6: 1515-1518.

Katzung B, Masters S, Trevor A. Basic and Clinical Pharmacology (LANGE Basic Science) 12th ed. (New York, USA: McGraw-Hill Medical Publishers), 2012.

Kirthikar KR, Basu DB. Indian Medicinal Plants. Vol. 4. 3rd ed. (Dehradun, India: Oriental Enterprises), pp. 1255-1257, 2001.

Koh HL, Chua TK, Tan CH. A guide to medicinal plants. (Singapore, Singapore: Singapore Science Centre), p. 24, 1992.

Mat Jais AM, Dambisya YM, Lee TL. Antinociceptive activity of Channa striatus (Haruan) extracts in mice. J Ethnopharmacol. 1997;57:125-130.

Millan MJ. The induction of pain: an integrative review. Prog Neurobiol. 1999;57:1-164.

Morais SM, Dantas JDP, Silva ARA, Magalhães EF. Ethno-medicinal plants of Tapeba Indians from the State of Ceará - Brazil. Rev Bras Farmacog. 2005;15:169-177.

Omar ME, Abdel Salam OM, El-Batran S, Baiuomy AR. Noradrenergic and dopaminergic modulation of melatonin visceral antinociception in mice. J Pharmacol Toxicol. 2006;1: 259-269.

Panghal M, Arya V, Yadav S, Kumar S, Yadav JP. Indigenous knowledge of medicinal plants used by Saperas community of

Khetawas, Jhajjar District, Haryana, India. J Ethnobiol Ethnomed. 2010;6:4.

Parrota JA. Healing plants of Peninsular India. (Wallingford, United Kingdom: CABI Publishing), 2001.

Polunin I. Plants and flowers of Singapore. (Singapore , Singapore: Time edition), p. 42, 1987.

Shanmugasundaram P, Venkataraman S. Antinociceptive activity of Hygrophila auriculata (Schum.) Heine. Afr J Trad CAM. 2005;2:62- 69.

Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dubé S, Mallinkrodt C, Bymaster FP, McKinzie DL, Felder CC. Selective muscarinic receptor agonist Xanomeline as a novel treatment approach for schizophrenia. Am J Psychiatry. 2008; 165:1033-1039.

Trevethick MA, Mantell SJ, Stuart EF, Barnard A, Wright KN, Yeadon M. Treating lung inflammation with agonists of the adenosine A2A receptor: problems and potential solutions. Br J Pharmacol. 2008;155:463-474.

Vasuda SM. Economic importance of pteridophytes. Ind Fern J. 1999;16:130-152.

Youssef MA, van Wely M, Hassan MA, Al-Inany HG, Mochtar M, Khattab S, van der Veen F. Can dopamine agonists reduce the incidence and severity of OHSS in IVF/ICSI treatment cycles? A systematic review and meta-analysis. Hum Reprod Update. 2010;16:459-466.

Zakaria ZA, Abdul Ghani ZDF, Raden Mohd. Nor RNS, Hanan Kumar G, Sulaiman MR, Mat Jais AM, Somchit MN, Arifah AK, Ripin J. Antinociceptive, anti-inflammatory and antipyretic properties of Dicranopteris linearis leaves aqueous extract in experimental animals. J Nat Med. 2008;62:179-187.

Zakaria ZA, Loo YW, Abdul Rahman NI, Abdul Ayub AH, Sulaiman MR, Hanan Kumar G. Antinociceptive, anti-inflammatory and antipyretic properties of Bauhinia purpurea leaves aqueous extract in experimental animals. Med Princ Pract. 2007;16:443-449.

Zakaria ZA, Raden Mohd. Nor RNS, Hanan Kumar G, Abdul Ghani ZDF, Sulaiman MR, Rathna Devi G, Mat Jais AM, Somchit MN, Fatimah CA. Antinociceptive, anti-inflammatory and anti-pyretic properties of Melastoma malabathricum leaves aqueous extract in experimental animals. Can J Physiol Pharmacol. 2006;84:1291-1299.

Zakaria ZA, Sulaiman MR, Mat Jais AM, Somchit MN. Effect of various antagonists on the Channa striatus fillet extract antinociception in mice. Can J Physiol Pharmacol. 2005;83: 635-642.

Zarrindast MR, Vahedy A, Heidari MR, Ghazi Khansari M. On the mechanism(s) of morphine-induced hypothermia. J Psychopharmacol. 1994;8:222-226.