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Traditionally used Thai medicinal plants: *In vitro* anti-inflammatory, anticancer and antioxidant activities

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ABSTRACT

Aims of the study: In order to assess traditional Thai claims about the therapeutic potential of medicinal plants and to select plants for future phytochemical research, nine plant species with anti-inflammatory uses were selected from Thai textbooks and assessed for their in vitro anti-inflammatory, antiproliferative and antioxidant activities.
Methods: Nuclear factor-kappaB (NF-κB) inhibitory effects in stably transfected HeLa cells were determined by luciferase assay, and effects on LPS-induced pro-inflammatory mediators prostaglandin E2 (PGE2), interleukin (IL)-6, IL-1β, and tumour necrosis factor (TNF)α in primary monocytes were assessed by ELISA. Cytotoxic activities were examined against HeLa cells, human leukaemia CCRF-CEM cells and the multidrug-resistant CEM/ADR5000 subline using the MTT and XTT tests. However, a redox status has been linked with both inflammation and cancer, antioxidant effects were also assessed using the DPPH, lipid-peroxidation, and Folin-Ciocalteau methods.
Results: Among all the nine species, *Gynura pseudochina var. hispida* and *Oroxylum indicum* showed the most promising NF-κB inhibitory effects with the lowest IC_{50} values (41.96 and 47.45 μg/ml, respectively). *Muehlenbeckia platyclada* did not inhibit the NF-κB activation but effectively inhibited the release of IL-6, IL-1β and TNF-α with IC_{50} values ranging between 0.28 and 8.67 μg/ml. *Pouzolzia indica* was the most cytotoxic against CCRF-CEM cells and the multidrug-resistant CEM/ADR5000 cells (9.75% and 10.48% viability, at 10 μg/ml, respectively). *Rhinacanthus nasutus* was the most potent cytotoxicity against HeLa cells (IC_{50} 3.63 μg/ml) and showed specific cytotoxicity against the multidrug-resistant CEM/ADR5000 cells (18.72% viability at 10 μg/ml, $p < 0.0001$ when compared to its cytotoxicity against CCRF-CEM cells). Moreover, *Oroxylum indicum* showed a high level of antioxidant activity by inhibiting lipid-peroxidation (IC_{50} 0.08 μg/ml).
Conclusions: This study provides in vitro evidence for the use of the Thai plants, most importantly *Gynura pseudochina var. hispida*, *Oroxylum indicum* and *Muehlenbeckia platyclada* as Thai anti-inflammatory remedies and these plants are now a priority for further phytochemical research.

1. Introduction

Traditional medicine is used widely throughout Thailand. It is a system which relies on a wide range of practices. Both, ready-made preparations and herbal drugs are used. Many Thai medicinal plants have provided the foundation for modern pharmaceuticals and drug leads (Farnsworth and Bunyapraphatsara, 1992). Recently, the Thai medicinal and food plant *Garcinia mangostana* L. has become popular which has been linked a wide range of in vitro activities (Obolskiy et al., 2009). However, in Europe and North America it is generally considered to be a food supplement. In this study we assess plants recorded in Thai textbooks with uses linked to potential anti-inflammatory effects in a panel of in vitro assays which are of direct

Abbreviations: AP, Aerial parts; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; EtAc, Ethyl acetate; EtOH, Ethanol; EX, Extract/extraction; FL, Flowers; IL, Interleukin; LPS, Lipopolysaccharide; LV, Leaves; MeOH, Methanol; MTT, 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; N/A, Data not available; ND, Not determined; NF-κB, Nuclear factor-KappaB; PE, Petroleum ether; PGE2, Prostaglandin E2; PMA, Phorbol myristate acetate; RB, Root bark; RT, Roots; SB, Stem bark; ST, Stem; T, Thailand; XTT, 2,3-Bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide.

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Table 1
Biological and pharmacological activities of the selected plant species reported in the previous publications (N/A = data not available).

Plant species (family)	Parts used/extracts	Active compounds	Traditional uses/ethnobotanical data	Biological & pharmacological activities	References
Basella alba L. (Basellaceae)	AP, LV, FL	N/A	AP were eaten to alleviate symptoms of appendicitis, smallpox fevers, and as laxatives (T) LV crushed, used against topical skin problems e.g. wounds, itching, or abscesses FL juice, used for the treatment of smallpox fevers, and skin inflammation	N/A	Theangburanatham (2005)
	Juice of LV	N/A	External use for eye infections (India)	N/A	Ignacimuthu et al. (2008)
	Aqueous EX from a mixture of *Basella alba* and *Hibiscus macranthus* LV	N/A	In Cameroon, traditional healers use a mixture of *Basella alba* and *Hibiscus macranthus* to prepare a crude EX which improves male virility and to cure male sexual asthenia and infertility	The testosterone production in testes slice was increased after incubated with aqueous EX of a mixture of *Basella alba* and *Hibiscus macranthus*. *Basella alba* aqueous EX significantly enhanced testosterone production in bull and rat Leydig cells in a concentration-dependent manner	Moundipa et al. (2005, 2006)
	LV, masticated in mouth	N/A	LV are masticated and kept in the mouth for sometime to get relief from aphthae (mouth ulcers)	N/A	Hebbar et al. (2004)
	Fresh LV	N/A	As an abortifacient (Cameroon), two spoons of the crushed LV-juice is drunk as needed. It was indicated that this drug often led to multiple tears (lacerations) of the vulva	N/A	Noumi and Tchakonang (2001)
	Aqueous EX of LV	N/A	N/A	Weak to moderate mutagenicity on *Salmonella typhimurium*	Yen et al. (2001)
Basella rubra L. (Basellaceae)	AP, LV, FL	N/A	Similar to the uses of *Basella alba* as above (T)	N/A	Theangburanatham (2005)
	LV crushed, mixed with cheese	N/A	For infected skin/urticaria or skin burns	N/A	Saikia et al. (2006)
	LV ground with sour buttermilk and salt, preparing as a poultice	N/A	Habitual intake of *Basella rubra* could cure 'arbuda' – one type of tumour in Ayurveda	N/A	Balachandran and Govindarajan (2005)
	Saline EX of seeds	α-Basrubrin and β-basrubrin	N/A	Antifungal activity	Wang and Ng (2004, 2001)
	Aqueous EX of LV	N/A	N/A	Antiulcer activity	Deshpande et al. (2003)
	LV crushed	N/A	LV crushed with black gram and paste is applied externally to boils (India)	N/A	Harsha et al. (2003)
Cayratia trifolia (L.) Domin. (Vitaceae)	LV	N/A	The LV have been used externally for nose ulcers, muscle pains, and abscesses (T)	N/A	Chuakul et al. (2000)
	LV, ST and RT	N/A	LV and RT are used against fever, and as an astringent. The ST is used as an expectorant, carminative and blood purifier. Heated LV applied to treat inflammatory conditions (T)	N/A	van Valkenburg and Bunyapraphatsara (2001)
	Plant part N/A	N/A	For asthma, catarrhal affection, headache (India)	N/A	Singh and Pandey, 1998 in Jain et al. (2005)
	LV, RT and stem	LV, RT and ST contain cyanic acid. LV contain cyanidin, delphinidin, kaempferol, myricetin and quercetin. AP contain triterpene epifriedelanol	In Peninsular Malaysia and East New Britain, LV poultice used against ulcers of the nose, LV or RT used as rubefacient, decoction of LV or RT used against fever In Java, juice of LV with young pineapple are used as antidandruff	N/A	van Valkenburg and Bunyapraphatsara (2001)

Table 1 (*Continued*)

Plant species (family)	Parts used/extracts	Active compounds	Traditional uses/ethnobotanical data	Biological & pharmacological activities	References
Gynura pseudochina (L.) DC. *var. hispida* Thv.(Asteraceae)	Fresh LV, rhizome, and RT, alcoholic or water EXs	N/A	Fresh LV and rhizome are used externally against inflammation and viral infections (herpes). The RT can be used internally for pain and fever (T)	N/A	Saralamp et al. (2000), Lemmens and Bunyapraphatsara (2003)
	LV, water EX	N/A	Prescribed for treating AIDS (T)	Moderate HIV-1 reverse transcriptase inhibitory activity	Woradulayapinij et al. (2005)
Gynura pseudochina (L.) DC. (Asteraceae)	Underground RT	N/A	RT have been used as an anti-inflammatory, relieving hot pain symptoms, fevers, and treating herpes infections (T)	N/A	Plant Genetic Conservation Project (2009)
	RT, LV, LF – poultice	N/A	RT are used against bruises; LV poultice is applied against pimples. LV and RT are used as a haemostatic and against breast tumours (Java) In Vietnam, RT are used as a tonic, LV are used as emollient and LV sap used to treat sore throat	N/A	Lemmens and Bunyapraphatsara (2003)
Muehlenbeckia platyclada (F. Muell.) Meisn. (Polygonaceae)	AP of mixed with whisky or alcohol	N/A	An alcoholic EX has been applied externally for skin swelling, sore, and insect bites (T)	N/A	Chuakul et al. (2000)
	Plant part N/A	N/A	Treatment of poisonous snakes' bites and fracture injuries, alleviating fever and detoxification (Taiwan and China)	N/A	Je-Chian et al., 1961 in Yen et al. (2009)
	MeOH-EX (plant part N/A)	Morin-3-*O*-α-rhamnopyranoside (1), kaempferol-3-*O*-β-glucopyranoside (2), (+)-catechin (3), and kaempferol-3-*O*-α-rhamnopyranoside (4)	N/A	Anti-inflammatory effects; compounds (1) (2) (3) inhibited the release of neutrophil elastase and compound (4) showed moderate inhibition of superoxide anion generation	Yen et al. (2009).
Oroxylum indicum (L.) Kurz. (Bignoniaceae)	SB, water or alcoholic EXs	N/A	The SB is used against abscesses, skin inflammation, purifying blood, and expectorant (T) SB mixed with alcohol can be used in children for treating fevers, tongue inflammation, bruises and swellings Fresh SB mixed with citric acid used against vomiting, and diabetes (used in combination with other herbal medicines)	N/A	Wuthithamvech (1997)
	Plant part N/A	N/A	Treatment of 'granthi' – one type of tumour in Ayurvedic medicine	N/A	Balachandran and Govindarajan (2005)
	Polyherbal formula of 17 plants (plant part not indicated)	N/A	Used in Indukantha Ghritha-polyherbal ayurvedic formula of 17 plants – for respiratory disorders, fevers, gastric disorders, cough, dyspnoea, etc	Inducer of immune responses by stimulating leucopoiesis, the non-specific and specific immune mechanisms	George et al. (2008)
	RB, alcoholic EX	N/A	N/A	Gastroprotective effects against EtOH and WIRS-induced gastric ulcer in rats	Zaveri and Jain (2007)
	MeOH-EX of FR	Baicalein	In China, it widely used as anti-inflammatory, antipyretic and antihypersensitivity	Antiproliferative effects on human cancer cells HL-60	Roy et al. (2007)
	RB, alcoholic EX	Baicalein, as it was found as majority in the active fractions	N/A	Antiulcer activity	Khandhar et al. (2006)
	RT	N/A	In Central Laos, the RT is mixed with the RT of *Bi kheuy ton*, and the RT of *Kok bi hon*, used against diabetes	N/A	Libman et al. (2006)
	RB, alcoholic EX, *n*-butanol fraction	N/A	N/A	Immunomodulatory activity enhanced specific immune responses both humural and cell-mediated	Zaveri et al. (2006)

	Plant part N/A	Chrysin (5, 7-dihydroxy flavone), and three series of chrysin analogues.	N/A	Antibacterial activities (moderate degree) against a panel of susceptible and resistant Gram-positive and Gram-negative organisms	Babu et al. (2006).
	N/A	Lapachol and beta-lapachone	Widely used as antimalarial, antibacterial and antiviral. (Ayurveda)	In combination with *Catharanthus alba*, *Commiphora mukul* and *Cynodon dactyton* was significant increase in the life span, WBC, RBC and TLC count in Dalton's lymphoma ascites tumour cell lines transplanted Swiss albino mice	Sam and Ganesh (2005)
	SB decoction or juice	N/A	In Sikkim and Darjeeling of Himalayan region, SB decoction (15–20 ml) or juice (5–10 ml) taken 2–3 times daily are used as antidiabetes	N/A	Chhetri et al. (2005)
	SB, EtOH-EX	N/A	N/A	Anticancer activity against B-16 (murine melanoma), HCT-8 (human colon carcinoma), CEM and HL-60 (leukaemia) tumour cell lines	Costa-Lotufo et al. (2005)
	SB, boiled in water	N/A	To treat arthritis (T)	Anti-inflammatory by inhibited the release of myeloperoxidase	Laupattarakasem et al. (2003)
	RB and SB, dichloromethane EX	Lapachol	N/A	Antifungal activity against the dermatophyte, *Microsporum gypsum*	Ali et al. (1998)
	RB and SB, dichloromethane EX	Lapachol, oroxylin A	N/A	Antibacterial activity	Houghton et al. (1997)
	Nitrosated fraction (plant part N/A)	N/A	N/A	Genotoxic and cell proliferative activities in the pyrolic mucosa of rat stomach in vivo	Tepsuwan et al. (1992)
	LV	N/A	N/A	Analgesic activity in writhing test and hot plate test	Upaganlawar et al. (2007)
	SB, alcoholic EX	N/A	N/A	Anti-inflammatory activity against carrageenan induced rats' paw oedema	Prasad et al. (1989)
	LV	N/A	N/A	Antioxidant and hepatoprotective activities (in vitro)	Tenpe et al. (2009)
	RB, *n*-butanol EX	N/A	N/A	Immunostimulant/immunomodulatory activity	Gohil et al. (2009)
	Bark, aqueous EX	N/A	Uses in Similipal Biosphere Reserve – Orissa for diarrhoea, rheumatism and stomachache	Antimicrobial activity against *Shigella flexneri*	Thatoi et al. (2008)
	Shoot	N/A	N/A	Antioxidant activity	Yang et al. (2006)
	SB, EtOH-EX	N/A	N/A	Antiproliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines	Lambertini et al. (2004)
	FL and FR, MeOH-EX	Baicalein	For stomach disorders, diarrhoea, dysentery and rheumatic swelling	Antimutagenicity activity against Trp-P-1 in an Ames test	Nakahara et al. (2001, 2002)
	Seed, boiled in water for 10 min	N/A	Against cough, antitumor, diarrhoea, tonic	Antioxidant activity in vitro by inhibition of Heinz bodies induction caused by oxidants, and also improved ABTS radical cation decolourization assay	Palasuwan et al. (2005)
Pouzolzia indica (L.) Gaudich. (Urticaceae)	AP and LV	N/A	The LV have been applied externally as anti-inflammatory, while the AP used internally as emmenagogue, diuretic and insecticide (T)	N/A	Saralamp et al. (2000)
	LV, RT, and whole plant	N/A	In Malaysia, a poultice of LV is used against stomachache and sores	N/A	van Valkenburg and Bunyapraphatsara (2001)

Table 1 (*Continued*)

Plant species (family)	Parts used/extracts	Active compounds	Traditional uses/ethnobotanical data	Biological & pharmacological activities	References
			In Indonesia, a poultice of LV is used against ulcers In Java, juice of LV or a decoction is used as a galactogogue In Vietnam, the WP is used against cough, sore throat, diuretic and galactogogue In the Philipines, LV are used against gangrene In India, the WP is used against gonorrhoea, syphilis and wounds In China, RT are used against sores, abscesses, and swellings		
Rhinacanthus nasutus (L.) Kuntze. (Acanthaceae)	Fresh LV mixed with alcohol	N/A	An alcoholic solution was reported to be an excellent treatment for various skin conditions such as ringworm, severe eczema and *Tinea* infections (T)	N/A	Saralamp et al. (2000), Suchawan (1989)
	LV and RT, soak in vinegar or alcohol, pounded with lemon or tamarind, or made into decoction	N/A	Treatment of skin disorders such as ringworm, eczema, scurf, skin infection e.g. herpes, antipyretic, anti-inflammatory and detoxicant. It is used against hypertension (Vietnam) and against cancers (T)	N/A	de Padua et al. (1999), Farnsworth and Bunyapraphatsara (1992)
	RT, MeOH-EX	Rhinacanthins C, N and Q	Used against cancers (T)	Induction of apoptosis in human cervical carcinoma (HeLaS3) cells by associating with the activation of caspase-3 pathway	Siripong et al. (2006)
	EtOH-EX of RT and aqueous EX of LV	Rhinacanthin C	In the treatment of hepatitis, diabetes, hypertension (South China and India) and skin diseases (Taiwan)	Antiproliferative activity	Gotoh et al. (2004)
	ST and LV, water and EtOH-EXs	N/A	The plant has been used in the treatment of mental disorders, inflammation, rheumatism, circulatory problems, asthma and bronchitis, epilepsy and immune system deficiencies	Modest increase in TNF-α expression but did not change iNOS	Punturee et al. (2004)
	ST and LV, water and EtOH-EXs	N/A		Immunomodulatory activity	Punturee et al. (2005)
	LV, EtOH-EX	N/A	In teas for treating colds, fever, refreshes the lungs. Relieves early stage of TB, headache from hypertension, reduces blood pressure, sore throat, constipation (T)	Moderate antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus* K147 methicillin-sensitive, *Escherichia coli* (wild), *Pseudomonas aeruginosa* 187 (wild)	Cheeptham and Towers (2002)
	LV, EtAc-EX	Rhinacanthins C, D and N	N/A	Potent antiallergic activity by inhibiting TNF-α and IL-4 gene expression in antigen-induced TNF-α and IL-4 releases on from RBL-2H3 cells	Tewtrakul et al. (2009a)
	LV, EtAc-EX	Rhinacanthins C, D and N	N/A	Anti-inflammatory activity against LPS-induced release of nitric oxide, PGE2 and TNF-α from RAW264.7 cells by inhibiting iNOS and COX-2 gene expressions	Tewtrakul et al. (2009b)
	LV-EX	N/A	N/A	Antifungal activity against some dermatophytes; *Trichophyton* spp., and *Microsporum canis*	Darah and Jain (2001)
	AP	Rhinacanthins E and F	N/A	Antiviral activity against influenza type A	Kernan et al. (1997)

Stem and LV, MeOH-EX	Naphthopyran derivatives, naphthoquinone derivatives	Treatments of ringworm and other skin diseases caused by fungi	Antifungal activity against *Pyricularia oryzae*, the pathogen of rice blast disease	Kodama et al. (1993); Awai et al. (1995)
AP, MeOH-EX	N/A	N/A	Hepatoprotective effect from paracetamol induced-liver damage in rats	Suja et al. (2004)
AP, EtOH-EX	N/A	N/A	Analgesic activity in the acetic acid induced-writhing test	Karunambigai et al. (2005)
AP, hot percolation using PE	Rhinacanthone (3,4-dihydro-3,3-dimethyl-2H-naphtho-[1,2-B] pyran-5,6-dione)	N/A	Antitumour activity against Dalton's ascetic lymphoma in Swiss albino mice	Thirumurugan et al. (2000)
RT, MeOH-EX	Rhinacanthin derivatives	N/A	Antiplatelet aggregation and cytotoxicity activities in the P-388, A-549, HT-29 and HL-60 test systems	Wu et al. (1998)
RT, MeOH-EX	Rhinacanthin N, rhinacanthin Q, naphthoquinone esters	N/A	Cytotoxicities against human carcinoma cell lines (epidermoid carcinoma, HeLa, and HepG2) and vero cell line (African green monkey kidney cell)	Kongkathip et al. (2004)
AP, EXed with 1:1 CH_2Cl_2-2-propanol	Rhinacanthin C, rhinacanthin D	RT and LV, pounded with vinegar or alcohol applied to herpes infections or other skin eruptions	Antiviral activity against cytomegalovirus (CMV)	Sendl et al. (1996)

Abbreviations: AP – aerial parts, FL – flowers, LV – leaves, RB – root bark, RT – roots, SB – stem bark, ST – stem, WP – whole plant, EtAc – ethyl acetate, EtOH – ethanol, EX – extract/extraction, MeOH – methanol, T - Thailand.

relevance in the context of treating acute or chronic conditions.

In spite of their long history of uses for inflammatory conditions, few studies have been reported on the selected species' potential modulatory effect on the NF-κB signalling pathway which is clearly established as one of most important targets of today's drug discovery for the treatment of a wide variety of inflammatory diseases, autoimmune diseases as well as cancers (Bork et al., 1997; Baud and Karin, 2009; Sun and Ley, 2008; Sarkar et al., 2008; Aggarwal and Gehlot, 2009). As already identified in many studies, NF-κB controls the expression of genes encoding for pro-inflammatory cytokines (e.g. IL-1, IL-2, IL-6, TNF-α, etc.), chemokines (e.g. IL-8, MIP-1α, eotaxin, etc.), adhesion molecules (e.g. ICAM, VCAM, E-selectin), inducible enzymes (COX-2 and iNOS), growth factors, and immune receptors. NF-κB is also recognised as a redox-sensitive transcriptional factor (Srivastava and Ramana, 2009). Oxidative stress-induced abnormal activation of NF-κB has also been demonstrated in many diseases (Kumar et al., 2004) providing an important link between NF-κB-modulatory and antioxidant effects.

Therefore, here we focus on the modulation of the NF-κB signalling pathway activated by PMA, and on the release of pro-inflammatory mediators; IL-6, IL-1β, TNF-α and PGE2 as in vitro models of anti-inflammatory. Cytotoxicity tests were carried out using three different cancer cell lines: CCRF-CEM leukaemia cells, multidrug-resistant CEM/ADR5000 leukaemia cells, and cervix cancer (HeLa) cells. Antioxidant effects were assessed using two different assays: DPPH, and lipid-peroxidation. In addition total phenolic content was determined by the Folin-Ciocalteau method.

2. Materials and methods

2.1. Plant collection

Fresh leaves of *Pouzolzia indica* and aerial parts of *Muehlenbeckia platyclada* were collected from the Sirirukhachart Botanical Garden, Mahidol University, Thailand. Stem bark of *Oroxylum indicum* and leaves of *Cayratia trifolia* were collected in suburban areas, while leaves of *Basella alba*, *Basella rubra*, *Gynura pseudochina*, *Gynura pseudochina var. hispida* and *Rhinacanthus nasutus* were collected from farmland in the north-eastern part of Thailand, mainly in Buriram Province. The plants were gathered during September–October 2006. The fresh and dried plants were identified by comparison with the plant specimens at the Forest Herbarium of the Thai Royal Forest Department, Bangkok, Thailand. Voucher specimens are deposited at the Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London (accession numbers NS06/00001–NS06/00009).

2.2. Extract preparation

All plant materials were collected, washed with water, and dried under shade at about 35–40 °C for several days, then ground to a fine powder using a laboratory scale mill. The 20 g of dried powder of each plant was extracted with petroleum ether (PE), ethyl acetate (EtAc) and methanol (MeOH) in a serial manner. Each solvent extraction was repeated 3 times and each of the extract solutions then combined and dried under pressure using a rotary evaporator. All dried extracts were then kept in tightly fitting stopper bottles in a freezer (−20 °C) until used for the pharmacological testing. Thereafter, the extracts were re-dissolved in 96% ethanol at a concentration of 20 mg/ml then cold sterilization by filtration through a mini-disk filter (0.45 μm), and stored in amber glass bottles for bioassays.

2.3. Measurement of anti-inflammatory activity

2.3.1. Determination of anti-inflammatory activity by IL-6/luciferase assay on HeLa cells

HeLa cells were stably transfected with a luciferase reporter gene controlled by the IL-6 promoter which is one of the target genes for activated NF-κB. The cells were seeded into 24-well plates and the extracts were tested in several concentrations (from 200 to 0.2 μg/ml). As positive controls we used the solvents to dissolve the samples (ethanol) and as negative controls we used unstimulated cells. Parthenolide (Sigma) was used as a standard reference. The enzymatic reaction was made with luciferase reagent (Promega), measured and recorded using a Lucy-1 luminometer/photometer (Anthos). More experimental details can be found in Bremner et al. (2004, 2009).

2.3.2. Determination of anti-inflammatory activity on human monocytes

Monocytes from healthy human donors were prepared following a standardised protocol (Ficoll gradient preparation, Amersham Biosciences) in completely endotoxin-free culture conditions. Cells were treated with the extracts (at the concentrations of 50, 10, and 1 μg/ml) followed by LPS treatment (10 ng/ml) for 24 h. Hydrocortisone (Sigma) was used as a standard reference. Measurements of the levels of the pro-inflammatory mediators: IL-6, IL-1β, TNF-α, and PGE2, were performed by ELISA and EIA kits (for details see Bremner et al., 2004).

2.4. Measurement of cytotoxic activity

2.4.1. MTT reduction assay on human cervix cancer (HeLa) cells

Cytotoxic activity was assessed using the MTT assay (Mosmann, 1983). HeLa cells were seeded in 96-well plates at the density of 10,000 cells/well. Starting concentrations of the extracts were added to the cells and serial dilutions were made. After incubation for 24 h, the MTT solution was added and the plates were incubated again for 2 h. The MTT solution was removed and the formazan product was solubilized using 10% DMSO plus 90% isopropanol. Absorbance was measured at 570 nm using a plate reader (Lucy-1, Anthos). The viability was determined based on a comparison with untreated cells. Doxorubicin hydrochloride (Sigma) was used as a positive (cytotoxic) control.

2.4.2. XTT reduction assay on human leukaemia cells

Cytotoxicity was assessed using the standard XTT assay kit (Roche, Indianapolis, IN), which measures the metabolic activity of viable cells (Konkimalla et al., 2008). Leukaemia cells were seeded in 96-well plates at a density of 1×10^5 cells/ml. After incubated the cells with the extracts, XTT reagent was added and the plates were incubated again for about 3 h then read out by an ELISA plate reader (Bio-Rad, München, Germany) at 490 nm with a reference wavelength of 655 nm. The viability of the treatment was determined as percentage of viability compared to untreated cells. Anticancer agents, doxorubicin and vincristine were used as reference standards as described by Efferth et al. (2008a,b).

Table 2
Inhibitory effects of the extracts on PMA-induced activation of NF-κB in HeLa cells, and on LPS-induced IL-1β, IL-6, TNF-α and PGE2 release in human monocytes (values represent means, $n = 3$).

Species	Extracts	Yield (%)	IC_{50} (μg/ml)				
			NF-κB	PGE2	IL-6	IL-1β	TNF-α
Basella alba	PE	2.27	>200.00	↑	46.74	↑	>50.00
	EtAc	2.41	83.28	↑	36.40	↑	30.42
	MeOH	5.07	>200.00	↑	32.38	36.73	37.68
Basella rubra	PE	2.76	157.31	↑	44.49	>50.00	>50.00
	EtAc	0.62	162.83	↑	38.87	36.76	31.72
	MeOH	5.44	139.21	↑	>50.00	36.49	>50.00
Cayratia trifolia	PE	1.20	>200.00	↑	>50.00	>50.00	>50.00
	EtAc	0.58	>200.00	26.04	25.47	42.04	20.83
	MeOH	7.61	83.16	47.14	19.53	>50.00	28.45
Gynura pseudochina var. hispida	PE	0.90	>200.00	25.23	15.30	36.32	12.63
	EtAc	1.12	60.18	32.35	8.14	24.87	1.49
	MeOH	0.91	41.96	>50.00	12.01	2.46	21.24
Gynura pseudochina	PE	0.98	↑	↑	22.23	>50.00	>50.00
	EtAc	0.81	83.20	41.77	11.63	15.44	1.04
	MeOH	2.99	159.76	>50.00	28.62	16.11	33.28
Muehlenbeckia platyclada	PE	0.20	190.25	↑	24.95	↑	22.59
	EtAc	1.59	72.94	>50.00	0.28	3.27	0.86
	MeOH	1.50	>200.00	>50.00	3.38	0.73	8.67
Oroxylum indicum	PE	0.23	↑	↑	37.13	↑	>50.00
	EtAc	0.33	47.45	26.98	27.98	44.12	20.33
	MeOH	8.45	↑	>50.00	>50.00	>50.00	>50.00
Pouzolzia indica	PE	0.52	↑	↑	46.51	↑	42.52
	EtAc	0.44	↑	↑	>50.00	↑	15.68
	MeOH	4.01	134.69	↑	>50.00	↑	>50.00
Rhinacanthus nasutus	PE	0.62	138.16	↑	>50.00	>50.00	>50.00
	EtAc	0.36	104.04	↑	>50.00	>50.00	43.83
	MeOH	3.52	118.03	↑	>50.00	>50.00	>50.00
Parthenolide	–	–	1.97	ND	ND	ND	ND
Hydrocortisone	–		ND	0.77	0.32	1.44	0.89

↑ – Activating effects or increased biosynthesis at all the tested concentrations. ND = not determined.

2.5. Measurement of antioxidant activity

DPPH free radical scavenging activity was determined following procedures described by Bafna and Mishra (2005). Lipid-peroxidation test was performed following the methods of Houghton et al. (1995) and Burits and Bucar (2000) using liposomal suspension from type VII folch bovine brain extract and thiobarbituric acid reactive substance (TBARS). Trichloroacetic acid and 2,6-di-*t*-butyl-*p*-kresol were also used to precipitate interfering substances. Total phenolic contents of the plant extracts were determined using the Folin-Ciocalteau reagent following the method described by Lowry et al. (1951). Trolox® (Fluka): a standard vitamin E analogue and quercetin (Sigma) were used as positive controls for DPPH and lipid-peroxidation tests. Caffeic acid (Sigma) was used as a standard control for phenolic content test.

3. Results and discussions

3.1. Plant selection

Nine plant species (Table 1) were selected on the basis of their anti-inflammatory uses reported in Thai textbooks, particularly in Saralamp et al. (2000) who summarise commonly used Thai medicinal plants: *Basella alba* L. (Basellaceae), *Gynura pseudochina* (L.) DC. *var. hispida* Thv. (Asteraceae), *Oroxylum indicum* (L.) Kurz. (Bignoniaceae), *Pouzolzia indica* (L.) Gaudich. (Urticaceae), and *Rhinacanthus nasutus* (L.) Kuntze. (Acanthaceae). *Pouzolzia indica* and *Rhinacanthus nasutus* were also reported in Suchawan (1989) and *Oroxylum indicum* is included in Wuthithamvech (1997) as an easy growing food plant of the north and north-east of Thailand. *Cayratia trifolia* (L.) Domin. (Vitaceae) and *Muehlenbeckia platyclada* (F.) Muelll, Meisn. (Polygonaceae) have been reported in Chuakul et al. (2000): an ethnobotanical survey in 14 provinces of Thailand. *Basella rubra* L. (Basellaceae) was documented in Theangburanatham (2005) and *Gynura pseudochina* (L.) DC. (Asteraceae) was reported in Plant Genetic Conservation Project (2009).

Their traditional uses, and relevant/characteristic secondary metabolites, plant parts used and/or ways of extract preparations are reported in Table 1 and a total of 27 extracts from the nine species were assessed for their anti-inflammatory, cytotoxicity and antioxidant activities.

3.2. NF-κB inhibitory activities and effects on LPS-induced pro-inflammatory mediators PGE2, IL-6, IL-1β and TNF-α release.

Gynura pseudochina var. hispida (MeOH) and *Oroxylum indicum* (EtAc) showed the strongest NF-κB inhibitory effects and they also inhibited the release of IL-1β and PGE2 (Table 2). Interestingly, *Muehlenbeckia platyclada* (EtAc and MeOH) showed the highest level of inhibition on the release of several pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α, but did not present any inhibitory effects on the activation of NF-κB. In addition, a number of extracts activated NF-κB or increased the synthe-

Table 3
Cytotoxic effects of the extracts in the MTT assay (HeLa cells) and the XTT assay (leukaemia cells CCRF-CEM and a multidrug-resistant CEM/ADR5000 subline) (values represent means ± S.D., $n = 3$).

Species	Extracts	Hela cells		Leukemia cells (% viability at 10 μg/ml)	
		IC_{50} (μg/ml)	% Viability at 10 μg/ml	CCRF-CEM	CEM/ADR5000
Basella alba	PE	197.23 ± 1.23	91.22 ± 1.09	77.66 ± 0.01	75.56 ± 0.02**
	EtAc	130.89 ± 1.09	89.15 ± 0.78	49.52 ± 0.37	39.97 ± 0.13**
	MeOH	1024.24 ± 0.87	98.35 ± 0.67	82.13 ± 0.18	45.91 ± 0.18**
Basella rubra	PE	145.39 ± 0.81	96.40 ± 1.06	39.56 ± 0.13	41.51 ± 0.19
	EtAc	114.89 ± 1.37	89.58 ± 1.77	85.63 ± 0.32	73.82 ± 0.21**
	MeOH	711.56 ± 2.34	97.18 ± 2.09	87.48 ± 0.46	38.37 ± 0.24**
Cayratia trifolia	PE	128.37 ± 4.09	84.47 ± 1.23	39.41 ± 0.26	33.88 ± 0.12**
	EtAc	194.70 ± 0.19	83.33 ± 1.76	37.74 ± 0.02	60.49 ± 0.15
	MeOH	127.35 ± 1.34	93.89 ± 2.01	85.87 ± 0.53	55.37 ± 0.05**
Gynura pseudochina var. hispida	PE	93.38 ± 1.39	87.82 ± 0.54	54.67 ± 0.60	31.66 ± 0.04**
	EtAc	114.05 ± 1.84	100.00 ± 1.23	31.42 ± 0.42	23.50 ± 0.12**
	MeOH	181.85 ± 2.71	93.31 ± 0.89	16.72 ± 0.13	33.69 ± 0.22
Gynura pseudochina	PE	96.81 ± 0.80	88.63 ± 0.76	32.74 ± 0.27	24.47 ± 0.03**
	EtAc	119.56 ± 1.41	96.67 ± 0.34	30.29 ± 0.01	45.53 ± 0.12
	MeOH	397.15 ± 2.55	97.41 ± 0.66	68.56 ± 0.37	57.03 ± 0.04**
Muehlenbeckia platyclada	PE	123.59 ± 1.15	91.27 ± 0.32	49.52 ± 0.24	34.51 ± 0.17**
	EtAc	194.34 ± 1.65	93.63 ± 1.66	26.13 ± 0.09	32.45 ± 0.18
	MeOH	605.66 ± 5.33	92.88 ± 2.65	75.61 ± 0.08	65.31 ± 0.23**
Oroxylum indicum	PE	96.18 ± 1.32	85.61 ± 0.99	24.35 ± 0.26	60.37 ± 0.16
	EtAc	55.22 ± 0.58	87.81 ± 0.47	29.35 ± 0.02	38.99 ± 0.04
	MeOH	417.95 ± 1.77	100.00 ± 0.55	83.32 ± 0.39	89.94 ± 0.27
Pouzolzia indica	PE	214.27 ± 1.39	88.14 ± 0.79	9.75 ± 0.29	10.48 ± 0.12
	EtAc	199.72 ± 2.07	95.51 ± 0.46	35.12 ± 0.52	31.30 ± 0.19*
	MeOH	1108.54 ± 2.82	99.71 ± 0.59	56.35 ± 0.18	53.59 ± 0.22**
Rhinacanthus nasutus	PE	24.88 ± 0.69	45.05 ± 0.74	76.49 ± 0.57	77.03 ± 0.48
	EtAc	3.63 ± 1.99	36.77 ± 0.81	38.13 ± 0.25	31.81 ± 0.24**
	MeOH	171.21 ± 2.41	92.38 ± 0.49	60.10 ± 0.10	18.72 ± 0.10**
Doxorubicin		0.11 ± 0.33	0.00 ± 0.25	(IC_{50} = 11.8 nmol/L)[a]	(IC_{50} = 12.2 mmol/L)[a]
Vincristine		ND	ND	(IC_{50} = 1.7 nmol/L)[a]	(IC_{50} = 1,043 nmol/L)[a]

Note: Asterisks indicate significant greater cytotoxicity against multidrug-resistant CEM/ADR5000 cells compared to CCRF-CEM cells, ND = not determined.
* $p = 0.0003$.
** $p < 0.0001$.
[a] Efferth et al. (2008a).

Table 4
Antioxidant capacities and total phenolic contents of the plant extracts (values represent means, $n = 3$).

Species	Extracts	IC_{50} in DPPH assay (μg/ml)	IC_{50} in Lipid-peroxidation assay (μg/ml)	Total phenolic content[a]
Basella alba	PE	>100	>100	1.44 ± 0.97
	EtAc	5.32	56.65	7.25 ± 0.76
	MeOH	93.72	78.70	3.81 ± 1.51
Basella rubra	PE	82.64	>100	2.93 ± 0.32
	EtAc	34.58	69.59	6.17 ± 0.58
	MeOH	>100	>100	3.50 ± 0.07
Cayratia trifolia	PE	82.06	>100	3.51 ± 0.62
	EtAc	47.89	78.27	4.81 ± 0.55
	MeOH	0.48	1.36	28.14 ± 0.71
Gynura pseudochina var. hispida	PE	>100	42.76	3.43 ± 0.09
	EtAc	44.56	81.90	13.66 ± 0.32
	MeOH	39.27	73.63	5.27 ± 1.08
Gynura pseudochina	PE	>100	79.94	3.77 ± 0.63
	EtAc	>100	62.88	3.76 ± 0.76
	MeOH	>100	93.56	10.82 ± 0.25
Muehlenbeckia platyclada	PE	37.74	33.61	3.56 ± 0.71
	EtAc	2.45	49.87	7.89 ± 0.45
	MeOH	14.42	37.36	7.85 ± 0.12
Oroxylum indicum	PE	>100	74.28	2.01 ± 0.91
	EtAc	0.73	0.08	13.17 ± 1.01
	MeOH	13.39	1.05	5.59 ± 0.78
Pouzolzia indica	PE	83.37	>100	3.09 ± 1.90
	EtAc	67.23	>100	4.24 ± 0.23
	MeOH	0.60	5.44	10.25 ± 0.82
Rhinacanthus nasutus	PE	>100	74.01	3.72 ± 1.34
	EtAc	19.34	18.26	7.73 ± 1.09
	MeOH	0.78	43.56	8.45 ± 0.76
Quercetin		0.17	0.13	ND
Trolox®		0.31	0.28	ND

ND = not determined.
[a] Equivalent to caffeic acid 1 μmol and express in μg of extract (microgram of extract) (values represent means ± S.D., $n = 3$).

sis of the pro-inflammatory mediators which will require further investigation.

3.3. Cytotoxicity of the extracts on HeLa cells, human leukaemic CCRF-CEM cells and a multidrug-resistant CEM/ADR5000 subline

Pouzolzia indica (PE) strongly inhibited cell mitochondrial activity of both CCRF-CEM and CEM/ADR5000 cells at the concentration of 10 μg/ml followed by *Rhinacanthus nasutus* (MeOH) and *Gynura pseudochina var. hispida* (EtAc) which more specifically inhibited the multidrug-resistant CEM/ADR5000 subline. *Rhinacanthus nasutus* (EtAc and PE) also showed the highest cytotoxicity against HeLa cells, followed by *Oroxylum indicum* (EtAc) (Table 3). Some of the extracts showed cytotoxic effects on both leukaemia cells and cervix cancer cells, but some extracts only acted on one of the two cell lines. For example, *Rhinacanthus nasutus* (EtAc) expressed high cytotoxicity against HeLa cells, CCRF-CEM cells and CEM/ADR5000 cells, while *Pouzolzia indica* (PE) only showed high level of cytotoxicity on both leukaemia cells but not on HeLa cells.

3.4. Antioxidant activity and total phenolic content

The highest DPPH free radical scavenging activities were found in *Cayratia trifolia* (MeOH), followed by *Pouzolzia indica* (MeOH) and *Oroxylum indicum* (EtAc) (Table 4). On the other hand, *Oroxylum indicum* (EtAc and MeOH) showed the most potent inhibition of lipid-peroxidation, followed by *Cayratia trifolia* (MeOH) (Table 4). The Folin-Ciocalteau expressed as caffeic acid equivalents showed remarkably high amounts of phenolics in *Cayratia trifolia* (MeOH), *Gynura pseudochina var. hispida* (EtAc), and *Oroxylum indicum* (EtAc).

3.5. Comparative analysis of the species' activities

Our investigation identified *Gynura pseudochina var. hispida* as the most potent inhibitor of NF-κB activation and of the release of interleukins 1β and 6, as well as TNF-α. As far as we know, no pharmacological study has reported such anti-inflammatory activity of this species. The only available report related to the moderate HIV-1 reverse transcriptase inhibitory effect of the water extract of the leaves (Table 1). Therefore, our results provide in vitro support for the uses of the leaves for treating inflammations. No extracts of *Gynura pseudochina var. hispida* showed cytotoxicity against either HeLa cells or leukaemia cells at the concentrations tested, nor did they show relevant antioxidant effects, although the EtAc extract showed high level of total phenolic contents.

Oroxylum indicum was found to be the second most potent NF-κB inhibitor. The EtAc extract not only showed potent NF-κB inhibitory effect, but also inhibited PGE2 as well as the in vitro lipid-peroxidation. *Oroxylum indicum* contains baicalein which is known to suppress the growth of primary myeloma cells through the downregulation of NF-κB (Otsuyama et al., 2005) and to inhibit IL-6 and IL-8 production at the transcriptional level in human retinal pigment epithelial cells (Nakamura, 2003). *Oroxylum indicum* also contains lapachol, oroxylin A, and chrysin derivatives which are known to have a large number of therapeutic potentials (Balassino et al., 2005; Houghton et al., 1994; Binutu et al., 1996; Lima et al., 2004; de Andrade-Neto et al., 2004; Sacau et al., 2003; Chen et al., 2000; Dao et al., 2004; Woo et al., 2005).

Muehlenbeckia platyclada yielded the most active extracts inhibiting pro-inflammatory cytokines IL-1β, IL-6 and TNF-α release but had no effect in the NF-κB assay. These extracts possessed low antioxidant activity in both the DPPH radical scavenging and the lipid-peroxidation assays. In Taiwan and China, *Muehlenbeckia platyclada* has been used for alleviating fever and detoxification whilst in Thailand the alcoholic extract of the aerial parts has been applied on skin swellings, sores, and insect bites (Table 1). From our studies the overall anti-inflammatory effect is not mediated by NF-κB activation through the IL-6 promoter or by unspecific antioxidant mechanisms.

Pouzolzia indica did not show any in vitro anti-inflammatory or antioxidant activities. However, the PE extract exhibited the most potent cytotoxic activity against both CCRF-CEM cells and the multidrug-resistant CEM/ADR5000 cells. Despite reports indicating that the leaves of *Pouzolzia indica* have been used externally as anti-inflammatory in Thailand and other Asian countries (Table 1), our evidence suggests that such activity might process through other mechanisms, apart from the NF-κB signalling pathway and the inhibition of pro-inflammatory mediators which need to be further investigated.

The EtAc extract of *Gynura pseudochina* showed low level of NF-κB and PGE2 inhibition, but strong inhibition of TNF-α release. Previous studies reported the use of the roots as an anti-inflammatory (Table 1), and that, due to the limited amount of root material available for collection, we had to use leaves. Therefore, these results are not relevant to the validation of the popular and the low pharmacological activities may not be surprising. However, the PE extract showed intermediate, but specific, cytotoxic effects against multidrug-resistant CEM/ADR5000 cells, which are similar to that of cytotoxic effects of the EtAc extract of another subspecies *Gynura pseudochina var. hispida*. In Java, the leaves of this species are used against breast tumours (Table 1).

Basella alba and *Basella rubra* extracts did not show any anti-inflammatory effects except for the EtAc extract of *Basella alba* which is endowed with a moderate effect as an NF-κB inhibitor. This extract also showed moderate radical scavenging activity in the DPPH assay. However, the crushed leaves and the flowers juice of both species have been used against skin inflammations and the most active extracts of the plants are likely to be the aqueous extracts (Table 1). For instance, the aqueous extract of *Basella rubra* has demonstrated antiulcer activity and leaves masticated kept in mouth helped relief aphthae (Table 1). As a result, in order to gain a higher level of pharmacological activities of *Basella alba* and *Basella rubra*, an aqueous extraction could be of interest for further investigation.

The MeOH extract of *Cayratia trifolia* exhibited the most potent DPPH free radical scavenging activity and strongly inhibited lipid-peroxidation as well as containing the highest amount of phenolics. This plant has been reported to contain cyanic acid, cyanidin, delphinidin, kaempferol, myricetin, quercetin and a triterpene epifriedelanol, and the leaves have been used for inflammatory conditions (Table 1). The EtAc extract of *Cayratia trifolia* inhibited PGE2 production but did not inhibit NF-κB activation. It is known that reactive oxygen species have a regulatory role in the expression of COX-2 and the subsequent synthesis of PGE2 (Wang et al., 2004; Martinez et al., 2000). Therefore our findings may support the traditional uses of this plant although its mechanism of action need to be confirmed especially on unspecific antioxidant effects responsible for the induction of pro-inflammatory mediators.

The EtAc extract of *Rhinacanthus nasutus* demonstrated the most potent cytotoxicity against HeLa cells and the MeOH extract showed highly specific cytotoxicity against the multidrug-resistant CEM/ADR5000 cells compared to CCRF-CEM cells ($p < 0.0001$). Previous studies reported that *Rhinacanthus nasutus* contains naphthoquinone–rhinacanthin derivatives which possess many pharmacological potentials such as cytotoxic/anticancer, antiviral, as well as anti-inflammatory activities through inhibition of iNOS and COX-2 gene expressions against LPS-induced release of nitric oxide (NO), PGE2 and TNF-α in RAW264.7 cells (Table 1). However, *Rhinacanthus nasutus* showed poor inhibitory effects upon NF-κB in our stably transfected HeLa cells, as well as poor inhibitory effects on the release of IL-1β, IL-6, TNF-α or PGE2 in primary human monocytes.

4. Conclusions

Ethnopharmacological knowledge is beneficial in guiding which plants may have potentials to yield anti-inflammatory and/or anticancer products. Here, we found that *Gynura pseudochina var. hispida* (Asteraceae), *Oroxylum indicum* (Bignoniaceae), and *Muehlenbeckia platyclada* (Polygonaceae) could serve as leads for the development of future anti-inflammatory drugs while *Rhinacanthus nasutus* (Acanthaceae) and *Pouzolzia indica* (Urticaceae) might yield novel natural compounds as anticancer products. Interestingly, multidrug-resistant, P-glycoprotein expressing CEM/ADR5000 cells reveal high levels of resistance to doxorubicin, vinblastine, paclitaxel and many other established anticancer drugs (Efferth et al., 2008b), but no or only weak cross-resistance was found to the present panel of Thai medicinal plants. This suggests that the plant extracts might yield valuable adjuncts for use in standard chemotherapy in case of drug-resistance and refractory tumours. However, further detailed phytochemical, pharmacological and in vivo studies should be the next step in the identification of active compounds of the lead plants, particularly *Gynura pseudochina var. hispida*, *Muehlenbeckia platyclada* and *Pouzolzia indica*, which are currently ongoing.

References

Aggarwal, B.B., Gehlot, P., 2009. Inflammation and cancer: how friendly is the relationship for cancer patients? Current Opinion in Pharmacology 9, 351–369.

Ali, R.M., Houghton, P.J., Hoo, T.S., 1998. Antifungal activity of some Bignoniaceae found in Malaysia. Phytotherapy Research 12, 331–334.

Awai, N., Kuwahara, S., Kodama, O., Santisopasri, V., 1995. Synthesis of an antifungal naphthoquinone isolated from *Rhinacanthus nasutus* (Acanthaceae). Bioscience, Biotechnology, and Biochemistry 59, 1999–2000.

Babu, K.S., Babu, T.H., Srinivas, P.V., Kishore, H., Murthy, U.S.N., Rao, J.M., 2006. Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents. Bioorganic & Medicinal Chemistry Letters 16, 221–224.

Bafna, A.R., Mishra, S.H., 2005. In vitro antioxidant activity of methanol extract of rhizomes of *Curculigo orchioides* Gaertn. Ars Pharmaceutica 46, 125–138.

Balachandran, P., Govindarajan, R., 2005. Cancer – an ayurvedic perspective. Pharmacological Research 51, 19–30.

Balassino, I.T., de Paulo, S.A., Silva, N.H., Cabral, M.C., da Costa Carvalho, M.G., 2005. Demonstration of the lapachol as a potential drug for reducing cancer metastasis. Oncology Reports 13, 329–333.

Baud, V., Karin, M., 2009. Is NF-κB a good target for cancer therapy? Hopes and pitfalls. Nature Reviews Drug Discovery 8, 33–40.

Binutu, O.A., Adesogan, K.E., Okogun, J.I., 1996. Antibacterial and antifungal compounds from *Kigelia pinnata*. Planta Medica 62, 352–353.

Bork, P.M., Schmitz, M.L., Kuhnt, M., Escher, C., Heinrich, M., 1997. Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-κB. FEBS Letters 402, 85–90.

Bremner, P., Rivera, D., Calzado, M.A., Obón, C., Inocencio, C., Beckwith, C., Fiebich, B.L., Munoz, E., Heinrich, M., 2009. Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-KappaB and other pro-inflammatory mediators. Journal of Ethnopharmacology 124, 295–305.

Bremner, P., Tang, S., Fiebich, B.L., Munoz, E., Marquez, N., Rivera, D., Heinrich, M., 2004. Phenylpropanoid NF-κB inhibitors from *Bupleurum fruticosum*. Planta Medica 70, 914–918.

Burits, M., Bucar, F., 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research 14, 323–328.

Cheeptham, N., Towers, G.H.N., 2002. Light-mediated activities of some Thai medicinal plant teas. Fitoterapia 73, 651–662.

Chen, Y.C., Yang, L.L., Lee, T.J.F., 2000. Oroxylin A inhibition of lipopolysaccharide-induced iNOS and COX-2 gene expression via suppression of nuclear factor-κB activation. Biochemical Pharmacology 59, 1445–1457.

Chhetri, D.R., Parajuli, P., Subba, G.C., 2005. Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. Journal of Ethnopharmacology 99, 199–202.
Chuakul, W., Saralamp, P., Prathanturarug, S., 2000. Medicinal Plants in Thailand. Volume II: Siam-Phaisatchayapruek. Amarin Printing & Publishing, Mahidol University, Bangkok, Thailand.
Costa-Lotufo, L.V., Khan, M.H., Ather, A., Wilke, D.V., Jimenez, P.C., Pessoa, C., Moraes, M.E., 2005. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. Journal of Ethnopharmacology 99, 21–30.
Dao, T.T., Chi, Y.S., Kim, J., Kim, H.P., Kim, S., Park, H., 2004. Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives. Bioorganic & Medicinal Chemistry Letters 14, 1165–1167.
Darah, I., Jain, K., 2001. Efficacy of the *Rhinacanthus nasutus* Nees leaf extract on dermatophytes with special reference to *Trichophyton mentagrophytes var. mentagrophytes* and *Microsporum canis*. Natural Product Sciences 7, 114–119.
de Andrade-Neto, V.F., Goulart, M.O., da Silva Filho, J.F., da Silva, M.J., Pinto, M.C.F.R., Pinto, A.V., Zalis, M.G., Carvalho, L.H., Krettli, A.U., 2004. Antimalarial activity of phenazines from lapachol, beta-lapachone and its derivatives against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo. Bioorganic & Medicinal Chemistry Letters 14, 1145–1149.
de Padua, L.S., Bunyapraphatsara, N., Lemmens, R.H., 1999. Plant Resources of South-East Asia (12) 1: Medicinal and Poisonous Plants, vol. 1. Backhuys Publishers, Leiden, The Netherlands.
Deshpande, S., Shah, G.B., Deshpande, I., Parmar, N.S., 2003. Antiulcer activity of aqueous extract of *Basella rubra* in albino rats. Journal of Natural Remedies 3, 212–214.
Efferth, T., Kahl, S., Paulus, K., Adams, M., Rauh, R., Boechzelt, H., Hao, X., Kaina, B., Bauer, R., 2008a. Phytochemistry and pharmacogenomics of natural products derived from traditional Chinese medicine and Chinese Materia Medica with activity against tumor cells. Molecular Cancer Therapeutics 7, 152–161.
Efferth, T., Konkimalla, V.B., Wang, Y.F., Sauerbrey, A., Meinhardt, S., Zintl, F., 2008b. Prediction of broad spectrum resistance of tumors towards anticancer drugs. Clinical Cancer Research 14, 2405–2412.
Farnsworth, N.R., Bunyapraphatsara, N., 1992. Thai Medicinal Plant: Recommended for Primary Health Care System. Prachachon Company, Bangkok, Thailand.
George, S.K., Rajesh, R., Kumar, S.S., Sulekha, B., Balaram, P., 2008. A polyherbal ayurvedic drug – Indukantha Ghritha as an adjuvant of cancer chemotherapy via immunomodulation. Immunobiology 213, 641–649.
Gohil, P., Zaveri, M., Jain, S., 2009. Immumodulatory activity of *n*-butanol extract of *Oroxylum indicum*. Pharmaceutical Biology (Formerly International Journal of Pharmacognosy) 46, 914–919.
Gotoh, A., Sakaeda, T., Kimura, T., Shirakawa, T., Wada, Y., Wada, A., Kimachi, T., Takemoto, Y., Iida, A., Iwakawa, S., Hirai, M., Tomita, H., Okamura, N., Nakamura, T., Okumura, K., 2004. Antiproliferative activity of *Rhinacanthus nasutus* (L.) Kurz extracts and the active moiety Rhinacanthin C. Biological & Pharmaceutical Bulletin 27, 1070–1074.
Harsha, V.H., Hebbar, S.S., Shripathi, V., Hegde, G.R., 2003. Ethnomedicobotany of Uttara Kannada district in Karnataka, India – plants in treatment of skin diseases. Journal of Ethnopharmacology 84, 37–40.
Hebbar, S.S., Harsha, V.H., Shripathi, V., Hegde, G.R., 2004. Ethnomedicine of Dharwad district in Karnataka, India plants used in oral health care. Journal of Ethnopharmacology 94, 261–266.
Houghton, P.J., Ali, R.M., Azizol, M., 1997. Antimicrobial activity of extracts of some Bignoniaceae from Malaysia. Pharmaceutical & Pharmacological Letters 7, 96–98.
Houghton, P.J., Photiou, A., Uddin, S., Shah, P., Browning, M., Jackson, S.J., Retsas, S., 1994. Activity of extracts of *Kigelia pinnata* against melanoma and renal carcinoma cell lines. Planta Medica 60, 430–433.
Houghton, P.J., Zarka, R., de las Heras, B., Hoult, J.R., 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Medica 61, 33–36.
Ignacimuthu, S., Ayyanar, M., Sankarasivaraman, K., 2008. Ethnobotanical study of medicinal plants used by Paliyar tribals in Theni district of Tamil Nadu, India. Fitoterapia 79, 562–568.
Jain, A., Katewa, S.S., Galav, P.K., Sharma, P., 2005. Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan, India. Journal of Ethnopharmacology 102, 143–157.
Karunambigai, K., Sugumaran, M., Vetrichelvan, T., 2005. Analgesic activity of *Rhinacanthus nasutus*. Indian Journal of Natural Products 21, 36–38.
Kernan, M.R., Sendl, A., Chen, J.L., Jolad, S.D., Blanc, P., Murphy, J.T., Stoddart, C.A., Nanakorn, W., Balick, M.J., Rozhon, E.J., 1997. Two new lignans with activity against influenza virus from the medicinal plant *Rhinacanthus nasutus*. Journal of Natural Products 60, 635–637.
Khandhar, M., Shah, M., Santani, D., Jain, S., 2006. Antiulcer activity of the root bark of *Oroxylum indicum* against experimental gastric ulcers. Pharmaceutical Biology 44, 363–370.
Kodama, O., Ichikawa, H., Akatsuka, T., Santisopasri, V., Kato, A., Hayashi, Y., 1993. Isolation and identification of an antifungal naphthopyran derivative from *Rhinacanthus nasutus*. Journal of Natural Products 56, 292–294.
Kongkathip, N., Luangkamin, S., Kongkathip, B., Sangma, C., Grigg, R., Kongsaeree, P., Prabpai, S., Pradidphol, N., Piyaviriyagul, S., Siripong, P., 2004. Synthesis of novel rhinacanthins and related anticancer naphthoquinone esters. Journal of Medicinal Chemistry 47, 4427–4438.
Konkimalla, V.B., Blunder, M., Korn, B., Soomro, S.A., Jansen, H., Chang, W., Posner, G.H., Bauer, R., Efferth, T., 2008. Effect of artemisinins and other endoperoxides on nitric oxide-related signalling pathway in RAW 264.7 mouse macrophage cells. Nitric Oxide: Biology and Chemistry 19, 184–191.
Kumar, A., Takada, Y., Boriek, A.M., Aggarwal, B.B., 2004. Nuclear factor-κB: its role in health and disease. Journal of Molecular Medicine 82, 434–448.
Lambertini, E., Piva, R., Khan, M.T., Lampronti, I., Bianchi, N., Borgatti, M., Gambari, R., 2004. Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene. International Journal of Oncology 24, 419–423.
Laupattarakasem, P., Houghton, P.J., Houl, J.R.S., Itharat, A., 2003. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. Journal of Ethnopharmacology 85, 207–215.
Lemmens, R.H., Bunyapraphatsara, N., 2003. Plant Resources of South-East Asia (12) 3: Medicinal and Poisonous Plants, vol. 3. Backhuys Publishers, Leiden, The Netherlands.
Libman, A., Bouamanivong, S., Southavong, B., Sydara, K., Soejarto, D.D., 2006. Medicinal plants: an important asset to health care in a region of Central Laos. Journal of Ethnopharmacology 106, 303–311.
Lima, N.M.F., Correia, C.S., Leon, L., Machado, G.M.C., Madeira, M.F., Santana, A.E.G., Goulart, M.O.F., 2004. Antileishmanial activity of lapachol analogues. Memorias do Instituto Oswaldo Cruz 99, 757–761.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193, 265–275.
Martinez, J., Sanchez, T., Moreno, J.J., 2000. Regulation of prostaglandin E2 production by the superoxide radical and nitric oxide in mouse peritoneal macrophages. Free Radical Research 32, 303–311.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65, 55–63.
Moundipa, P.F., Beboy, N.S.E., Zelefack, F., Ngouela, S., Tsamo, E., Schill, W.B., Monsees, T.K., 2005. Effects of *Basella alba* and *Hibiscus macranthus* extracts on testosterone production of adult rat and bull leydig cells. Asian Journal of Andrology 7, 411–417.
Moundipa, P.F., Ngouela, S., Kamtchouing, P., Tsamo, E., Tchouanguep, F.M., Carreau, S., 2006. Effects of extracts from *Hibiscus macranthus* and *Basella alba* mixture on testosterone production in vitro in adult rat testes slices. Asian Journal of Andrology 8, 111–114.
Nakahara, K., Onishi-Kameyama, M., Ono, H., Yoshida, M., Trakoontivakorn, G., 2001. Antimutagenicity activity against Trp-P-1 of the edible Thai Plant *Oroxylum indicum* Vent. Bioscience, Biotechnology, and Biochemistry 65, 2358–2360.
Nakahara, K., Trakoontivakorn, G., Alzoreky, N.S., Ono, H., Onishi-Kameyama, M., Yoshida, M., 2002. Antimutagenicity of some edible Thai plants, and a bioactive carbazole alkaloid Mahanine, isolated from *Micromelum minutum*. Journal of Agricultural and Food Chemistry 50, 4796–4802.
Nakamura, N., 2003. Effects of baicalin, baicalein, and wogonin on interleukin-6 and interleukin-8 expression, and nuclear factor-kappaB binding activities induced by interleukin-1beta in human ratinal pigment epithelial cell line. Experimental Eye Research 77, 195–202.
Noumi, E., Tchakonang, N.Y.C., 2001. Plants used as abortifacients in the Sangmelima region of Southern Cameroon. Journal of Ethnopharmacology 76, 263–268.
Obolskiy, D., Pischel, I., Siriwatanametanon, N., Heinrich, M., 2009. *Garcinia mangostana* L.: a phytochemical and pharmacological review. Phytotherapy Research: PTR 23, 1047–1065.
Otsuyama, K.I., Ma, Z., Liu, S., Abroun, S., Asaoku, H., Kawano, M.M., 2005. PPAR beta-mediated suppression of the growth and survival in human myeloma cells conteracting NF-κB activity. Blood (American Society of Hematology Annual Meeting Abstracts) 106, 5053–15053.
Palasuwan, A., Soogarun, S., Lertlum, T., Pradniwat, P., Wiwanitkit, V., 2005. Inhibition of Heinz body induction in an in vitro model and total antioxidant activity of medicinal Thai plants. Asian Pacific Journal of Cancer Prevention: APJCP 6, 458–463.
Plant Genetic Conservation Project, 2009. Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. Available from: http://www.rspg.or.th/index_sub.html (accessed 01.04.10).
Prasad, A., Rezaei, N.N., Wahi, A.K., Gambhir, S.S., Agarwal, V.K., 1989. Preliminary study on anti-inflammatory activity of some medicinal plants. Journal of Natural Products 5, 14–15.
Punturee, K., Wild, C.P., Kasinrerk, W., Vinitketkumnuen, U., 2005. Immunomodulatory activities of *Centella asiatica* and *Rhinacanthus nasutus* extracts. Asian Pacific Journal of Cancer Prevention 6, 396–400.
Punturee, K., Wild, C.P., Vinitketkumneun, U., 2004. Thai medicinal plants modulate nitric oxide and tumor necrosis factor-α in J774.2 mouse macrophages. Journal of Ethnopharmacology 95, 183–189.
Roy, M.K., Nakahara, K., Na Thalang, V., Trakoontivakorn, G., Takenaka, M., Isobe, S., Tsushida, T., 2007. Baicalein, a flavonoid extracted from a methanolic extract of *Oroxylum indicum* inhibits proliferation of a cancer cell line in vitro via induction of apoptosis. Die Pharmazie 62, 149–153.
Sacau, E.P., Estevez-Braun, A., Ravelo, A.G., Ferro, E.A., Tokuda, H., Mukainaka, T., Nishino, H., 2003. Inhibitory effects of lapachol derivatives on epstein-barr virus activation. Bioorganic & Medicinal Chemistry 11, 483–488.
Saikia, A.P., Ryakala, V.K., Sharma, P., Goswami, P., Bora, U., 2006. Ethnobotany of medicinal plants used by Assamese people for various skin ailments and cosmetics. Journal of Ethnopharmacology 106, 149–157.
Sam, S., Ganesh, N., 2005. Short term in vivo study of *Oroxylum indicum* with the combination of *Catharanthus alba Commiphora mukul* and *Cynodon dactylon* in DLA transplanted Swiss albino mice to understand its anticancer property. Biosciences Biotechnology Research Asia 3, 131–136.

Saralamp, P., Chuakul, W., Prathanturarug, S., 2000. Medicinal Plants in Thailand. Volume I: Sirirukhachart Botanical Garden. Amarin Printing & Publishing, Mahidol University, Bangkok, Thailand.
Sarkar, F.H., Li, Y., Wang, Z., Kong, D., 2008. NF-κB signalling pathway and its therapeutic implications in human diseases. International Reviews of Immunology 27, 293–319.
Sendl, A., Chen, J.L., Jolad, S.D., Stoddart, C., Rozhon, E., Kernan, M., Nanakorn, W., Balick, M., 1996. Two new naphthoquinones with antiviral activity from *Rhinacanthus nasutus*. Journal of Natural Products 59, 808–811.
Siripong, P., Yahuafai, J., Shimizu, K., Ichikawa, K., Yonezawa, S., Asai, T., Kanokmedakul, K., Ruchirawat, S., Oku, N., 2006. Induction of apoptosis in tumor cells by three naphthoquinone esters isolated from Thai medicinal plant: *Rhinacanthus nasutus* Kurz. Biological & Pharmaceutical Bulletin 29, 2070–2076.
Srivastava, S.K., Ramana, K.V., 2009. Focus on Molecules: Nuclear factor-kappaB. Experimental Eye Research 88, 2–3.
Suchawan, P., 1989. Thai herbal medicine volume 6 (Thai language). Aksarapipat Publisher, Bangkok, Thailand.
Suja, S.R., Latha, P.G., Pushpangadan, P., Rajasekharan, S., 2004. Assessment of hepatoprotective and free radical scavenging effects of *Rhinacanthus nasutus* (Linn.) Kurz in Wister rats. Journal of Natural Remedies 4, 66–72.
Sun, S.C., Ley, S.C., 2008. New insights into NF-κB regulation and function. Trends in Immunology 29 (Suppl. 10), 469–478.
Tenpe, C.R., Upaganlawar, A., Brule, S., Yeole, P.G., 2009. In vitro antioxidant and preliminary hepatoprotective activity of *Oroxylum indicum* Vent leaf extracts. Pharmacologyonline 1, 35–43.
Tepsuwan, A., Furihata, C., Rojanapo, W., Matsushima, T., 1992. Genotoxicity and cell proliferative activity of a nitrosated *Oroxylum indicum* Vent fraction in the pyloric mucosa of rat stomach. Mutation Research Letters 281, 55–61.
Tewtrakul, S., Tansakul, P., Panichayupakaranant, P., 2009a. Anti-allergic principles of *Rhinacanthus nasutus* leaves. Phytomedicine 16, 929–934.
Tewtrakul, S., Tansakul, P., Panichayupakaranant, P., 2009b. Effects of rhinacanthins from *Rhinacanthus nasutus* on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha releases using RAW264.7 macrophage cells. Phytomedicine 16, 581–585.
Thatoi, H.N., Panda, S.K., Rath, S.K., Dutta, S.K., 2008. Antimicrobial activity and ethnomedicinal uses of some medicinal plants from Simillipal Biosphere Reserve Orissa. Asian Journal of Plant Sciences 7, 260–267.
Theangburanatham, W., 2005. Dictionary of Thai Herbal Medicine, 6th ed. (Thai language). Bangkok: Odienstore, Available from: http://thaiherb.most.go.th/plantdetail.php?id=118 (accessed 13.01.10).
Thirumurugan, R.S., Kavimani, S., Srivastava, R.S., 2000. Antitumour activity of rhinacanthone against Dalton's Ascetic lymphoma. Biological & Pharmaceutical Bulletin 23, 1438–1440.
Upaganlawar, A.B., Tenpe, C.R., Yeole, P.G., 2007. Analgesic activity of leaves of *Oroxylum indicum*. Indian Journal of Natural Products 23, 30–32.
van Valkenburg, J.L., Bunyapraphatsara, N., 2001. Plant Resources of South-East Asia No. 12 (2): Medicinal and Poisonous Plants, vol. 2. Blackhuys Publishers, Leiden, The Netherlands.
Wang, H., Ng, T.B., 2001. Novel antifungal peptides from Ceylon spinach seeds. Biochemical and Biophysical Research Communications 288, 765–770.
Wang, H., Ng, T.B., 2004. Antifungal peptides, a heat shock protein-like peptide, and a serine-threonine kinase-like protein from Ceylon spinach seeds. Peptides 25, 1209–1214.
Wang, T., Qin, L., Liu, B., Liu, Y., Wilson, B., Eling, T.E., Langenbach, R., Taniura, S., Hong, J.S., 2004. Role of reactive oxygen species in LPS-induced production of prostaglandin E2 in microglia. Journal of Neurochemistry 88, 939–947.
Woo, K.J., Jeong, Y.J., Inoue, H., Park, J.W., Kwon, T.K., 2005. Chrysin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression through the inhibition of nuclear factor for IL-6 (NF-IL6) DNA-binding activity. Federation of European Biochemical Societies Letters 579, 705–711.
Woradulayapinij, W., Soonthornchareonnon, N., Wiwat, C., 2005. In vitro HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and *Canna indica* L. rhizomes. Journal of Ethnopharmacology 101, 84–89.
Wu, T.S., Hsu, H.C., Wu, P.L., Teng, C.M., Wu, Y.C., 1998. Rhinacanthin-Q, a naphthoquinone from *Rhinacanthus nasutus* and its biological activity. Phytochemistry 49, 2001–2003.
Wuthithamvech, W., 1997. Encyclopedia of Thai Herbal Medicine and Fundamental of Thai Pharmaceutics. OS Printing House, Bangkok, Thailand.
Yang, R.Y., Tsou, S.C., Lee, T.C., Wu, W.J., Hanson, P.M., Kuo, G., Engle, L.M., Lai, P.Y., 2006. Distribution of 127 edible plant species for antioxidant activities by two assays. Journal of the Science of Food & Agriculture 86, 2395–2403.
Yen, C.T., Hsieh, P.W., Hwang, T.L., Lan, Y.H., Chang, F.R., Wu, Y.C., 2009. Flavonol glycosides from *Muehlenbeckia platyclada* and their anti-inflammatory activity. Chemical & Pharmaceutical Bulletin 57, 280–282.
Yen, G.C., Chen, H.Y., Peng, H.H., 2001. Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food & Chemical Toxicology 39, 1045–1053.
Zaveri, M., Gohil, P., Jain, S., 2006. Immunostimulant activity of *n*-butanol fraction of root bark of *Oroxylum indicum*, vent. Journal of Immunotoxicology 3, 83–99.
Zaveri, M, Jain, S., 2007. Gastroprotective effects of root bark of *Oroxylum indicum*, Vent. Journal of Natural Remedies 7, 269–277.