ANTI-INFLAMMATORY ACTIVITY OF *AGERATUM CONYZOIDES* LINN. , *LANTANA CAMARA* LINN. AND *PSIDIUM GUAJAVA* LINN. EXTRACTS

By

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ABSTRAK

Dalam percubaan untuk mendapatkan agen anti-inflamasi yang lebih poten tetapi mempunyai kurang kesan sampingan, fraksinasi berpandukan aktiviti dijalankan terhadap *Ageratum conyzoides* Linn., *Lantana camara* Linn., dan *Psidium guajava* Linn.. Ekstrak-ekstrak tersebut disaring dengan model haiwan tapak kaki belakang tikus aruhan '*carrageenan*'. Pada permulaannya, model ini dioptimasikan untuk memperolehi agen pengampaian yang paling baik untuk agen anti-inflamasi yang tidak larut air seperti indometasin / ekstrak dan menentukan jenis haiwan ujikaji yang paling peka terhadap perencatan pembentukan edema oleh indometasin. Didapati bahawa '*compound tragacanth'* (1 %) merupakan agent pengampaian yang terbaik dibandingkan dengan karboksi metil selulosa (CMC) (0.5 %), tween 80 (1 %), dan kombinasi tween 80 / etanol / air suling (5 : 5: 90) (1%). Haiwan yang menunjukkan perencatan terbesar terhadap pembentukan aruhan edema '*carrageenan'* oleh indometasin mengikut turutan berikut iaitu tikus betina > tikus jantan > mencit jantan > mencit betina. Bahan-bahan tumbuhan diekstrak secara bersiri dengan pelarut-pelarut berbeza seperti eter petroleum, kloroform, methanol. Ekstrak methanol daun *L. camara* didapati memberikan perencatan edema yang paling kuat berbanding dengan ekstrak lain. Oleh yang demikian, ekstrak methanol daun *L. camara* difraksikan kepada fraksi eter petroleum, kloroform, etil asetat, butanol, dan akuas (air suling) dengan cara partisi pelarut-pelarut. Didapati fraksi kloroform memberikan kesan perencatan yang paling ketara terhadap pembentukan edema di kaki belakang tikus betina. Fraksi kloroform ini kemudian difraksikan lagi

kepada empat subfraksi (CF1 - CF4) dengan menggunakan kromatografi turus. Keputusan yang diperolehi menunjukkan bahawa subfraksi CF4 adalah paling efektif dalam mengurangkan pembentukan edema yang diaruhkan oleh '*carrageenan'* dalam tikus betina. Kemudian, kesan subfraksi CF4 *L. camara* Linn. ini dikaji ke atas kontraksi sediaan terasing uterus tikus aruhan oksitosin dan asetilkolina, untuk mengkaji sama ada aktiviti anti-inflamasi ini disebabkan oleh perencatan terhadap sintesis prostaglandin. Keputusan menunjukkan bahawa subfraksi CF4 merencat aktiviti kontraksi yang diaruhkan oleh oksitosin dan asetilkolina secara tidak kompetitif yang mencadangkan bahawa aksi subfraksi CF4 adalah tidak spesifik. Kemungkinan penghalangan saluran kalsium yang dioperasikan voltan (VOCCs) dan saluran kalsium dalam sel oleh fraksi CF4 dikaji menggunakan sediaan terasing vas deferens tikus. Keputusan kajian ini mencadangkan bahawa subfraksi CF4 secara selektif menghalang saluran kalsium dalam sel berbanding dengan VOCC. Penyaringan fitokimia dengan kromatografi lapisan nipis telah dijalankan terhadap fraksi aktif *L. camara* Linn. untuk mengenalpasti kelas-kelas kompaun yang terdapat di dalam fraksi tersebut. Keputusan memaparkan bahawa subfraksi CF4 mengandungi glikosida kardiak, sebatian pahit, saponin, terpenoid, dan minyak pati. Kajian ini menyimpulkan bahawa esktrak *Lantana camara* berupaya memberi aktiviti anti-inflamasi melalui penghalangan saluran kalsium dalam sel.

ANTI-INFLAMMATORY ACTIVITY OF *AGERATUM CONYZOIDES* LINN. , *LANTANA CAMARA* LINN. AND *PSIDIUM GUAJAVA* LINN. EXTRACTS

ABSTRACT

In an attempt to seek for more potent and less side effects anti-inflammatory agent(s), activity guided fractionation was performed on *Ageratum conyzoides* Linn., *Lantana camara* Linn. and *Psidium guajava* Linn. extracts. The extracts were screened using animal model carrageenan induced hind paw oedema. The model was optimised first to find the best suspending agent for water insoluble anti-inflammatory agent indomethacin/extracts and to determine the type of experimental animals most sensitive to indomethacin inhibition of oedema. Compound tragacanth (1%) was found to be the best suspending agents compared to carboxymethyl cellulose (CMC) (0.5%), tween 80 (1%),and tween 80/ ethanol/ water (5: 5: 90) (1%). The animals that gave the largest inhibition of carrageenan-induced oedema to indomethacin was in the order of female rat > male rat > male mice > female mice. The plant materials were serially extracted with different solvent e.g. petroleum ether, chloroform and methanol. The methanolic extract of *L. camara* leaves was found to give the best inhibition of oedema compared to other extracts. Therefore, the methanolic *L. camara* leaves extract was fractionated into petroleum ether, chloroform, ethyl acetate, butanol and aqueous fractions using solvent-solvent partition method. It was found that the chloroform fraction caused the strongest inhibition on female rat hind paw oedema. The choloroform fraction was further fractionated into four subfractions (CF1 to CF4) using column chromatography. Results showed that CF4 was the most effective in reducing the oedema induced by carrageenan in female rats. The effect of CF4 of *L. camara* Linn. was then

examined on the oxytocin and acetylcholine induced contraction in isolated rat uterus preparation to investigate whether the anti-inflammatory activity is due to the inhibition of prostaglandins synthesis. Result showed that CF4 inhibited the contraction induced by both oxytocin and acetylcholine in non-competitive manner which suggests that the action of CF4 is non specific. The possible blockade of voltage-operated calcium channels (VOCCs) and intracellular calcium channels of CF4 fraction was investigated using isolated rat vas deferens preparation. Results obtained suggested that CF4 selectively blocks intracellular calcium channel rather than VOCCs. The phytochemical screening using thin layer chromatography was performed on the active fractions of *Lantana camara* to identify the classes of compounds present in this fraction. The result indicated that CF4 contains cardiac glycosides, bitter principles, saponins, terpenoids, and essential oil. This study concluded that the *Lantana camara* extracts are able to exert anti-inflammatory activity through blockage of the intracellular calcium channel.

Chapter 1 Introduction

1.1 Introduction

Inflammation is a normal, protective, complex, homeostastic process. When tissue injury is caused by a single, non-lethal, finite event, such as mechanical trauma, a thermal or chemical burn, or a single exposure to a non-replicating antigen, the inflammatory and reparative processes begin smoothly from injury to healing. In this circumstance, the whole inflammatory process is truly beneficial and provides an example of normal, healthy state. In contrast, when the response becomes too great, it may be far worse than the disease state that its counteracted, and in extreme cases, it may be fatal. These defensive responses may be inappropriately directed against innocuous substance from outside the body (e.g. pollen) or against the tissues of body itself, and the response themselves may then produce damage and may indeed constitute part of the disease process either acutely as in anaphylaxis, or chronically as in asthma or rheumatoid arthritis, or in atherosclerosis. Therefore, anti-inflammatory agents are required for these conditions. It is believed that aging processes are all inflammatory responses (Turner, 1965). The importance of anti-inflammatory agents cannot be exaggerated because of their utility, often as life- saving drugs (Turner, 1965), in many diseases such as arthritis, lupus erythematosus, pemphigus, gout, psoriasis, osteoarthritis and rheumatic fever (Paulus, 1974).

Currently, the main anti-inflammatory agents available on the market are the glucocorticoids and the nonsteroidal anti-inflammatory drugs (NSAIDs). Glucocorticoids have powerful anti-inflammatory activity by inhibiting not only initial redness, heat, pain and swelling, but also involve in the later stages of wound healing and repair, and the proliferative reactions seen in chronic inflammation. The consequence of these powerful actions of the glucocorticoids is that they can be of immense value when used to treat certain conditions in which there is

hypersensitivity and unwanted inflammation, but they carry the hazard that they can suppress the necessary protective responses to infection and can decrease essential healing processes. The incidence of cataracts is higher after prolonged administration of the glucocorticoids in patients with rheumatoid arthritis. Other toxic effects, which have been reported, are glaucoma, raised intracranial pressure, hypercoagulability of the blood, fever and disorders of menstruation (Rang *et al.*, 2007). As a result, there are severe limitations to the use of glucocorticoids in the chronic inflammatory disease.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used of all therapeutic agents. They are frequently prescribed for “rheumatic” musculoskeletal complaints and the common inflammatory joint disease. However, virtually all currently available NSAIDs can have significant unwanted effects, especially in the elderly. When NSAIDs are used in joint diseases (which usually necessitates fairly large doses and long-continued use), there is a high incidence of side effects --- more particularly in the gastro-intestinal tract but also in the liver, kidney, spleen, blood and bone marrow (Rainsford and Velo, 1992). Common gastro-intestinal side effects are dyspepsia, diarrhoea (but sometimes constipation), nausea and vomiting. It has been estimated that one in five chronic users of NSAIDs will have gastric damage, which can be silent but carries a small but definite risk of serious haemorrhage and / or perforation. The second most common unwanted effect of NSAIDs is skin reactions, such as mild rashes, urticaria and photosensitivity reactions to more serious and potentially fatal diseases (Rang *et al.*, 2007). Although some recent anti-inflammatory drugs claim to have less of these effects, usually it also meant reduced anti-inflammatory activity (Asmawi *et al.*, 1993).

Clearly, there is a tremendous need for the introduction of more effective and less toxic anti-inflammatory agents into clinical use and particularly which have novel modes of action. Efforts to develop new, clinically effective pharmaceutical agents traditionally have relied most primarily on the use of existing agents, i.e. derivatisation of existing agents, synthesis of additional analogs of existing agents, use of combination therapy of existing agents with other drugs, improvement of delivery of existing agents to target site, and discovery of new prototype pharmaceutical agents. However, the chemical synthesis approach is time consuming and very costly since the rate of new invented drugs, which are proven to be beneficial for clinical use, is very low. It seems unlikely that efforts to modify the structures of existing agents will provide any new products that do not share similar toxicity. Ethnopharmacology provide scientists with an alternative approach for the discovery of anti-inflammatory agents, namely the study of medicinal plants with a history of traditional use as a potential source of substances with significant pharmacological and biological activities. This strategy may increase the likelihood of identifying new prototype drugs with quite different chemical structures and mechanisms of action and hence, lowers likelihood of similar toxicities.

In search for novel prototype anti-inflammatory agents, it seems reasonable to assume that new agents to be found have different structures with different or supplemental activities from the ones in current use or development. In any event, the identification of a novel structure, with or without a novel mechanism of action, provides a prototype agent from which a new class of anti-inflammatory drugs may be derived. By the same token, compounds which possess novel mechanism(s) of action, regardless of whether they become clinically useful, may serve as model compounds for synthetic or semisynthetic structure modifications and optimisation. In particular, the higher plants are a logical choice, chiefly because of their seemingly infinite variety of novel organic molecules. Anti-inflammatory agents are widely distributed among the higher plants, but very few have been evaluated.

Salicin, methyl salicylate and wintergreen oil are the natural products from higher plants, which have reached clinical utility. Moreover, salicin that is isolated from willow barks has served as a template for synthesis of aspirin (acetylsalicylic acid). A diverse group of chemical structures including withanolides, coumarins, noelignans, peptides, steroids, triterpenoids, glycosides, alkaloids, phenolic compounds, tannins, flavonoids, cannabinoids and polysaccharides have been shown to possess anti-inflammatory activities (Sener & Bingöl, 1988; Duwiejua & Zeitlin, 1993; Bingöl & Sener, 1995). Therefore, question arises as to whether anti-inflammatory agent also can be isolated from Malaysian traditional plants.

In folk medicine, various indigenous herbal drugs are used, in single and or in combined forms, for treating different types of inflammatory and arthritic conditions, with considerable success. The use of the plants to treat inflammation and many other diseases in Malaysia is similar to many other parts of the world. For instance, rhizome *Kaempferia galanga*, rhizome *Zingiber zerumbet* and *Elephantopus scaber* (whole plant) are used in decocted form to treat pain and swelling (Asmawi *et al.*, 1993). Although the use of these herbal drugs has a sound tradition, and their medicinal uses and general safety are well known to native peoples, their place has yet to be rationalized in therapeutics, in the current setting. Scientific studies are therefore required to assess their efficacy and some of the medicinal properties as popularly claimed. As part of study to seek new, more potent anti-inflammatory agent(s) with less side effects from plant origin, several Malaysian medicinal plants were screened for their ability to inhibit carrageenan induced hind paw oedema in rats. The plants studied were traditionally used to treat swelling, fever, pain, and rheumatism, i.e. indications for the use of nonsteroidal anti-inflammatory drugs.

1.2 Aims of the study

The present study is part of a project undertaken to investigate the anti-inflammatory activity of some traditional plants found in Malaysia. Three medicinal plants, different plants parts and different extracts, were evaluated. The plants and part studied were whole plant of *Ageratum conyzoides* Linn., leaves and the combination of other parts of the plant (flowers, stems, fruits) of *Lantana camara* Linn., and leaves of *Psidium guajava* Linn.. The specific objectives of this study are as follows:

1. to optimise carrageenan-induced oedema, anti-inflammatory models so that it requires less extract sample for screening.
2. to verify whether the traditional anti-inflammatory plants, *Ageratum conyzoides* Linn., *Lantana camara* Linn., *Psidium guajava* Linn. possess anti-inflammatory activity using carrageenan induced rats hind paws oedema as the anti-inflammatory screening model.
3. to perform an anti-inflammatory activity guided fractionation on the *Lantana camara* Linn. leaves.
4. to investigate the possible prostaglandin antagonist activity of active fractions of *Lantana camara* Linn., using oxytocin and acetylcholine induced contraction in isolated rat uterus preparation.
5. to investigate the effect of active fraction of *Lantana camara* Linn. on the possible involvement of voltage-operated calcium channels (VOCCs) and intracellular channels in the isolated rat vas deferens preparations.
6. to screen phytochemically the classes of compounds present in the active fractions of *Lantana camara* Linn. responsible for the anti-inflammatory activity.

Chapter 2 Literature Survey

2.1 Inflammation

Inflammation may be considered a protective mechanism that on occasion becomes deregulated and leads to a chronic inflammation. Inflammations are characterised into 5 cardinal symptoms i.e. heat, redness, pain, swelling and loss of function. The systems of an inflammation are results of complex patho-physiological processes that include increased blood flow and vascular permeability, activation of humoral and cellular defence mechanisms, sensibilization and activation of nociceptors. These processes are mediated by a variety of inflammatory mediators which are listed in Table 2.1. The inflammatory process is triggered by several interrelated cascade systems in the body. These are including the complement system, coagulation system, plasmakinin system and arachidonic acid cascade (Fig. 2.1).

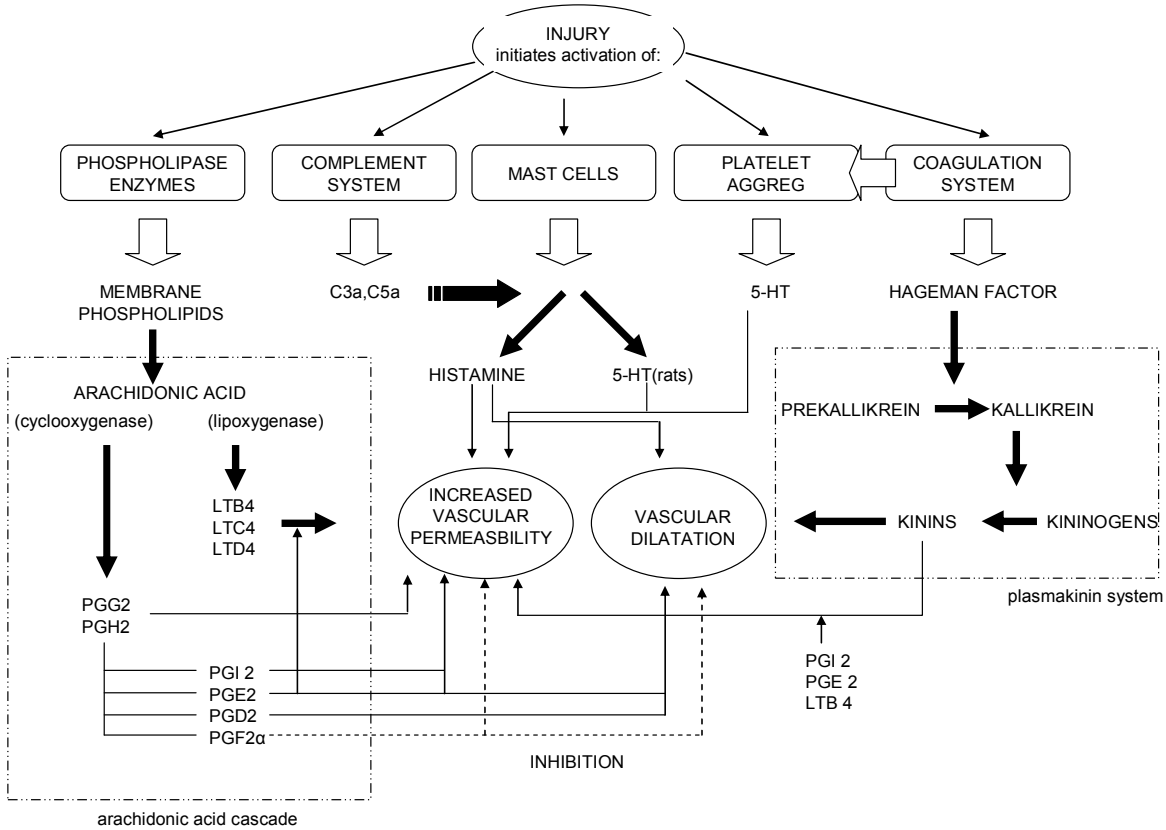

Fig. 2.1 Schematic representation of the interactions between and subsequent effects of inflammatory substances on vasculature (Dawson & Willoughby, 1985; Rang *et al.*, 2007).

Table 2.1 Mediators of inflammation (Ammon *et al.*, 1993; Dawson & Willoughby, 1985; Rang *et al.*, 2007).

Mediator	Origin	Inflammation systems
Serotonin (5-hydroxytryptamine)	Mast cell	Increased vascular permeability
Histamine	Mast cell, Basophilic granulocytes.	Vasodilatations, increased vascular permeability, pain
Bradykinin	Plasma	Vasodilatations, increased vascular permeability, pain
Prostagladins	Ubiquitous	Vasodilatations, pain sensitization against bradykinin and histamine
Thromboxane	Thrombocytes, other cells	Platelet activation
Leucotrienes	Leucocytes	Chemotactic for leucocytes, increased vascular permeability
Lysosomal enymes	Leucocytes, synovial cells	Necrotic, Chemotactic for leucocytes,
Platelet-activating factor	Polymorphonuclear leukocytes, Macrophages, Eosinophils, Mast cells	Vasodilatations, increased vascular permeability, Aggregation of platelets, Chemotactic for leucocytes, increased enzyme secretion, initiation of free radical release.
Complement components	Blood	Increased vascular permeability, chemoattration, immune adherence, leukocytosis
Cytokines (lymphokines, chemokines, interferon, interleukins, tumour necrosis factor)	Leukocytes, Macrophages	Adhesion molecule expression, cell growth, division, differentiation, or programmed cell death, immunoglobulin production, chemotaxis.

2.2 Cycloxygenase pathway and prostaglandin

Cycloxygenase (COX) pathways have long been targeted for the treatment of inflammatory, initially through the use of NSAIDs. Cyclooxygenase activity is known to be the consequence of the activition of different isoenzymes, including COX 1, COX 2, and a COX 1 variant, COX 3 (Gale et al, 2007). These enzymes use arachidonic acid as a substrate to form prostaglandins. COX isozymes are membrane-associated and internalize adjacent arachidonic acid, which is released when membrane damage occurs. COX 1 is expressed constitutively in most tissues, and is responsible for the production of PGs that control normal physiologic

functions including of PGs that control normal physiologic functions including flow, and platelet aggregation. COX 2 is not constitutively expressed, but is rapidly induced by both inflammatory and mitogenic stimuli resulting in increased PG synthesis in neoplastic and inflamed tissues (Wu, 2005).

Prostaglandins are synthesized by most cells in the human body. The biosynthesis of prostaglandin via the cyclooxygenase (COX) pathway is initiated by the release of arachidonic acid (AA) from membrane phospholipids by phospholipases, followed by the conversion of AA to prostaglandin G_2 (PGG_2) by the COX activity of the COX enzymes, PGG_2 is then immediately converted into PGH_2 by the hydroperoxidase activity of COX. The synthesis of PGH_2 by COX is coupled with modification by downstream cellspecific isomerase enzymes, resulting in the production of prostaglandin products in a tissue-specific manner. Prostaglandins act at or near the sites of synthesis by binding to specific receptors on the cell surface which results in a wide range of biological effects, including the relaxation or contraction of blood vessels, platelet aggregation as well as pain and fever generation (Gale et al, 2007).

2.3 Principles of anti-inflammatory drug action

Pharmacological control of inflammation can be achieved by two separate mechanisms: (i) by antagonizing or preventing the release of mediators involved in initiating or amplifying inflammation, (ii) by direct action on inflammatory cells affecting their response of function (Sedgwick & Willoughby, 1985). Anti-inflammatory compounds can act on many steps of the pathophysiological processes. It might block the biosynthesis of proinflammtory mediators by direct interaction with a key enzyme (e.g., inhibition of cyclooxgenase-II or lipooxygenases) or by decreasing enzyme expression (e.g., steroidal anti-inflammatory compounds) or by reducing substrate levels (e.g., decreased of arachidonic acid). Alternatively or in addition, it can act by inhibiting the release of

preformed, stored mediators (e.g., histamine release) or block mediator receptors interaction on target cells (e.g., leukotrience receptor antagonists). An anti-inflammatory compound may also act by immunostimulation (e.g., maturation of myeloid cells, stimulated phagocytosis) that in turn promotes an increased removal of the insulting signal molecules (organisms or alternatively, by immunosupression) that results in less aggressive inflammatory response to allergen challenge (Safayhi & Sailer, 1997). The prevention of inflammation is simplified in Fig. 2.2.

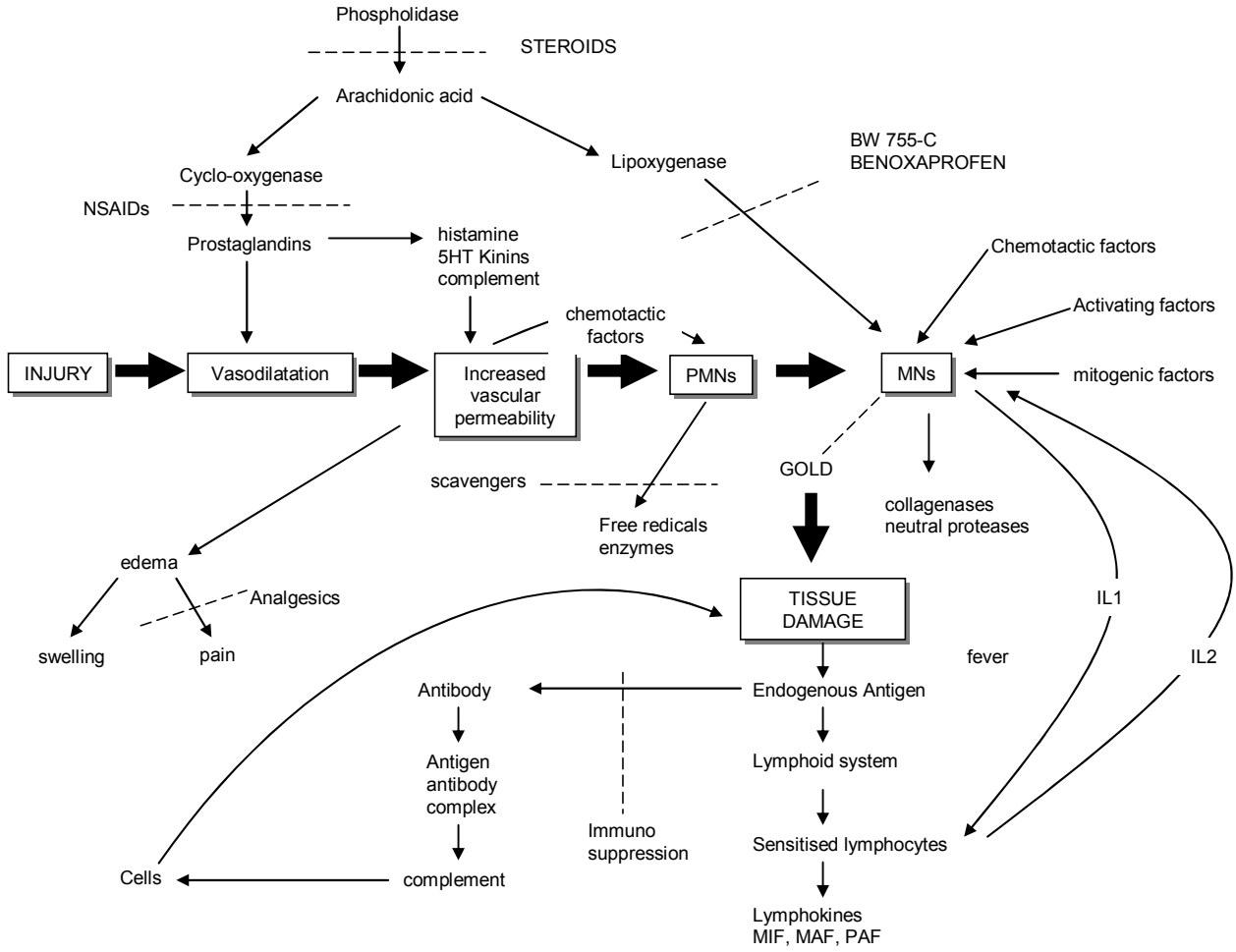

Fig. 2.2 Simplified diagram of the inflammatory response and its prevention (Rang *et al.*, 2007; Sedgwick & Willoughby, 1985).

2.4 Plant-derived medicinals

The importance of plant-derived medicinals in modern medicine is often underestimated. Such useful compounds as digitoxin, rutin, papain, morphine, codeine, papaverine, atropine, scopolamine, quinine, quinidine, reserpine, ergotamine, ergonovine, cocaine, vincaleukoblastine, leurocristine, *d*-tubocurarine, protoveratrines A and B, ephedrine, sparteine, physostigmine, pilocarpine, colchicines, and caffeine present a broad and representative range of pharmacologic activities. Amongst our most invaluable orthodox medicines derived from compounds in higher plants are analgesic agents (e.g. morphine and codeine), antimalarial treatments (e.g. quinine), antitumour drugs (e.g. vincristine and taxol) and asthma therapies (e.g. cromoglycate). An analysis of the top-selling pharmaceuticals for 1999 shows that 13 of the top-selling 30 medicines, with combined sales of over $26 billion, have active ingredients whose chemical structure is based on a compound found originally in nature (Table 2.2) (O' Neill & Lewis, 2002).

Table 2.2 Top-selling medicines for 1999 in $billion. Those in italics have a structure based on a compound discovered in nature (O' Neill & Lewis, 2002).

Medicines	$billion	Medicines	$billion
Omeprazole	5.85	Ciprofloxacin	1.64
Simvastatin	*4.37*	Risperidone	1.47
Atorvastatin	*3.68*	*Taxol*	*1.44*
Erythropoetin	3.68	Celecoxib	1.43
Pravastatin	*3.26*	*Cyclosporin*	*1.42*
Amlodipine	2.98	Losartan	1.36
Fluoxetine	2.93	*Clarithromycin*	*1.32*
Loratidine	2.82	*Human Insulin*	*1.30*
Enalapril	*2.24*	CSF	1.21
Sertraline	2.09	*Azithromycin*	*1.21*
Paroxetine	2.01	*Ceftriaxone*	*1.21*
Olanzapine	1.83	Lisinoprol	1.20
Conjugated oestrogens	*1.83*	Sumatriptan	1.09
Lansoprazole	1.80	*Interferon*	*1.09*
Amox-Clav	*1.69*	Nifedipine	1.09

2.5 *Ageratum conyzoides* Linn. (Asteraceae)

Species : *Ageratum conyzoides* Linn.

Family : Asteraceae or Compositae

Local Name : Rumput tahi ayam (Mustafa & Muhamad, 1994)

Plate 1.1 *Ageratum conyzoides* Linn.

Ageratum conyzoides Linn., is an annual herb with a long history of traditional medicinal uses in many countries in the world (Table 2.3), especially in the tropical and subtropical regions. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from this species (Table 2.4). Extracts and metabolites from this plant have been found to possess pharmacological activities (Table 2.5). (Okunade, 2002)

Table 2.3 Traditional medicinal uses of *Ageratum conyzoides* Linn. (Okunade, 2002).

Region / Country	Uses
Githen	use as purgative, febrifuge, opthalmia, colic; treatment of ulcers and wound dressing
Senegal	antienteralgic and antipyretic
some African countries	treatment of mental and infectious diseases as well as headaches and dyspnoea
Cameroon	remedy for craw-craw
Nigeria	skin diseases and wound healing, treat diarrhoea and to relieve pain associated with navel in children
Central Africa	used to treat wounds caused by burns
Kenya (East Africa)	antiasthmatic, antispasmodic and haemostatic
India	treatment of leprosy and as an oil lotion for purulent opthalmia
Brazil	anti-inflammatory, analgesic and anti-diarrhoeic
Vietnam	treatment of gynaecological diseases

Table 2.4 The classes of chemical constituents isolated from *Ageratum conyzoides* Linn. (Okunade, 2002).

Class	Compound
essential oil	• (+)-sesamin • aurantiamide acetate • fumaric acid • caffeic acid • phytol • (Z)12-6-methyl-heptadecenoic acid
essential oil – monoterpenes	• sabinene • β-pinene • β -phellandrene • 1,8-cineole • limonene • terpinen-4-ol • α-terpineol • Ocimene • α-Pinene • eugenol • methyleugenol
essential oil – sesquiterpenes	• β-caryophyllene • δ-Cadinene • sesquiphellandrene • caryophyllene epoxide
essential oil – chromene	• 7-methoxy-2,2-dimethylchromene (precocene I) • 6,7-dimethoxy derivative, ageratochromene (precocene II) • ageratochromene dimer • encecalin • 6-vinyl-7-methoxy-2,2-dimethylchromene • dihydroencecalin • dihydrodemethoxyencecalin • demethoxyencecalin • demethylencecalin • 2-(1'-oxo-2'-methylpropyl)-2-methyl-6,7-dimethoxychromene
Chromene	• 2,2-dimethylchromene-7-O- β-glucopyranoside • 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene • 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene • 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene • 6-angeloyloxy-7-methoxy-2,2-dimethylchromene • Encecanescins
Benzofuran	• 2-(2'-methylethyl)-5,6-dimethoxybenzofuran • 14-hydroxy-2H β,3-dihydroeuparine

Table 2.4 The classes of chemical constituents isolated from *Ageratum conyzoides* Linn. (Okunade, 2002). (Continued)

Class	Compound
Chromone	• 3-(2'-methylpropyl)-2-methyl-6,8-dimethoxychrom-4-one • 2-(2'-methylprop-2'-enyl)-2-methyl-6,7-dimethoxychroman-4-one
Flavonoids	• 5'-methoxynobiletin • linderoflavone B • 5,6,7,3',4',5'-hexamethoxyflavone • eupalestin • scutellarein-5,6,7,4'-tetrahydroxyflavone • quercetin • quercetin-3-rhamnopiranoside • kaempferol • kaempferol -3- rhamnopiranoside • kaempferol 3,7-diglucoyside • isoflavone
Triterpene	• friedelin
Sterol	• β-sitosterol • stigmasterol • brassicasterol • dihydrobrassicasterol • spinasterol • dihydrospinasterol
pyrrolizidine alkaloids	• lycopsamine • echinatine

Table 2.5 Biological activity of *Ageratum conyzoides* Linn. (Okunade, 2002).

Extracts / Metabolites	Activity
crude extract of the whole plant	• as a wound dressing • possess neuromuscular blocking activity in isolated rats phrenic nerve-diaphragm • caused greater fall in diastolic pressure compared to systolic pressure in anaesthetized rats • possess calcium channels blocking activity
leaves extract	• treatment of chronic pain in osteoarthritic patients • analgesic activity
methanolic extract of the whole plant	• antimicrobial activity
aqueous extract of the leaves	• prevent coagulation of the whole blood while causing precipitation of some blood materials
water soluble fraction (WSF) of the plant extract	• possess a peripheral analgesic activity and an anti-inflammatory action • possess direct relaxing effect on smooth muscles and inhibit contraction induced by several agonists
essential oil	• anti-inflammatory - cotton pellet granuloma • analgesic - tail-flick and writhing test activity • antipyretic activity -brewer's yeast injection • antibacterial - 20 bacteria • antifungal activities - 4 fungi (*Candida albicans* SP-14, *Cryptococcus neoformas* SP-16, *Sclerotium rolfsii* SP-5 and *Trichophyton mentagrophytes* SP-12.)
demethoxyageratochromene	• against two of the fungi, *Penicillium chrysogenum* and *P. javanicum*

2.6 *Lantana camara* Linn. (Verbenaceae)

Species : *Lantana camara* Linn.

Family : Verbenaceae

Local Name : Bunga tahi ayam busuk, tahi ayam munai, bunga tahi anjing, bunga tahi asu, bunga tahi ayam, bunga pagar, bunga asam senyur (Mustafa & Muhamad, 1994)

Plate 1.2 *Lantana camara* Linn.

Lantana camara Linn. is regarded both as a notorious weed and a popular ornamental garden plant and has found various uses in folk medicine in many parts of the world (Table 2.6) (Ross, 1999). The *L. camara* Linn. plant contains different classes of constituents listed in Table 2.7. Some taxa of the widely variable *L. camara* Linn. complex are toxic to small ruminants and this effect has been associated with the types and relative amounts of some triterpene ester metabolites. However, *L. camara* linn. also produces a number of metabolites in good yields and some have been shown to possess useful biological activities (Table 2.8). (Ghisalberti, 2000)

Table 2.6 Traditional medicinal uses of *Lantana camara* Linn. (Ross, 1999).

Region / Country	Uses
Australia	• To treat neurodermatitis, eczema, rashes, psoriasis, tinea, chicken pox, boils, bites, whooping cough, catarrh, pulmonary problems, epidermic parotiditis, and fever • To stop bleeding in traumatic injuries.
Brazil	• To treat mange, malaria, fevers, colds, headache.
Canary Islands	• Taken by pregnant women as an abortifacient.
Colombia	• To facilitate childbirth and as an emmenagogue.
East Africa	• To treat sore throat, cough, toothache, headache, and colds. • As a diaphoretic, stimulant, for jaundice, chest diseases, and for rheumatism.
Guatemala	• Taken orally to treat rheumatism, constipation and eczema • Taken orally as a tonic and stimulant • Used externally for wounds, ulcers, bruises, and sores
India	• Used externally for chronic ulcers. • Applied to the eye for eye injuries.
Indonesia	• Taken orally to treat gonorrhoea, leucorrhoea, intestinal spasm, rheumatism, and cough. • Used as emetic, diaphoretic, tonic, diaphoretic, carminative, and antiseptic. • Applied to skin to treat boils and wounds.
Kenya	• Dried stem is used as toothbrush
Mexico	• Given orally to treat rheumatism, snakebite, vomit, dysentery, gastrointestinal pain, toothache, uterine haemorrhage, excess menstrual discharge, rashes, and coughs. • The whole plant is rubbed with cold water to treat chills. • Taken orally as a stomach tonic and as an appetizer.
Nigeria	• Taken orally as an antiasthmatic, tonic, and anticonvulsant.
Panama	• To treat colds, stomach afflictions, digestive disorders, and skin diseases.

Table 2.6 Traditional medicinal uses of *Lantana camara* Linn. (Ross, 1999). (Continued)

Region / Country	Uses
Rwanda	• Taken orally for malaria.
Southeast Asia	• To treat swelling on skin cuts, rheumatism, bilious fever.
Surinam	• Used as an herbal bath.
Tanzania	• Taken orally for stomachache and against vomiting. • Used externally for itching and rashes.
Thailand	• Taken orally for asthma. • Used as a poultice for anti-inflammatory infections.
Tonga	• Applied to cuts to prevent infection.
USA	• Taken orally as a carminative.
Venda	• Used as a eye drop for eye injury.
Venezuela	• Taken orally as an emmenagogue.
Vietnam	• Taken orally as an emmenagogue.
West Africa	• Taken orally to treat coughs, colds, jaundice, and chest pain. • Used as a diaphoretic, and also stimulant. • Used in bath for rheumatism
West Indies	• Taken orally for dysmenorrhoea, fever, cough, flu, and indigestion.

Table 2.7 The classes of chemical constituents isolated from *Lantana camara* Linn. (Ghisalberti, 2000).

Class	Compound	Class	Compound
essential oils	• safrole • humulene • davanone	sterol	• β–sitosterol • campesterol • stigmasterol • β-sitosterol glucoside
essential oils – sesquiterpenes	• β-curcumene • (E)-nuciferal • (Z)-nuciferol • (-)-ar-curcumen-15-al • γ-curcumene • ar-curcumene • (-)-epi-β-bisabolol • (-)-γ-curcumen-15-al • caryophyllene	phenyl ethanoid glycosides	• verbascoside • (Z)- verbascoside • isoverbascoside • martynoside • derhamnosyl-verbascoside • isonuomioside A • calceolarioside E
Triterpenes	• lantadene A • lantadene B • lantadene C • lantadene D • reduced lantadene A • lantanolic • lantic acid • icterogenin • oleanolic acid • Ursolic acid	flavonoids	• 3-methoxyquercetin • 3,7-dimethoxyquercetin • 3,7,4'-trimethoxyquercetin • hispidulin • pectolinarigenin 7-O- β-D-glucoside • flavone glycoside camaraside
iridoid glycosides	• theveside • theviridoside • geniposide • 8-epiloganin • shanzhside methyl ester • lamiridoside	oligo-saccharides	• ajugose • stachyose • verbascotetraose • verbascose • lantanose A • lantanose B
furanonaphtho-quinones	• quinone diodantunezone		

Table 2.8 Biological activity of metabolites isolated from *Lantana camara* Linn. (Ghisalberti, 2000).

Class	Compound	Biological activity
pentacyclic triterpenes	ursolate acetate	• antimicrobial - active (30 µg/disk) against Staphylococcus aureus and S. typhi • antimutagenic activity - reduced the number of micronucleated polychromatic erythrocytes induced by mitomycin C
	lantadene A	• inhibited Epstein–Barr virus activation in Raja cells induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) • possess inhibitory effects on the two-stage carcinogenesis of mouse skin papillomas
	lantadene B	• inhibited Epstein–Barr virus activation in Raja cells induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) • as an inhibitors of tumour promoters *in vivo* • possess inhibitory effects on the two-stage carcinogenesis of mouse skin papillomas • delayed the formation of papillomas on mouse skin, reduced the rate of papilloma-bearing mice (by 15% at 20 weeks) and reduced the average number of papillomas/mouse (50% at 20 weeks)
	lantadene C	• inhibited Epstein–Barr virus activation in Raja cells induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) • as an inhibitors of tumour promoters *in vivo*
	reduced lantadene A	• inhibited Epstein–Barr virus activation in Raja cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA)
	oleanolic acid	• as an inhibitors of human leucocyte elastase • possess inhibitory effects on inflammation and on various stages of tumour development • possess COX-2 inhibitory effect with an IC_{50} 295 µM and a COX-2/COX-1 selectivity ratio of 0.8
	ursolic acid	▪ as an inhibitors of human leucocyte elastase • possess inhibitory effects on inflammation and on various stages of tumour development • possess COX-2 inhibitory effect with an IC_{50} value of 130 µM and a COX-2/COX-1 selectivity ratio of 0.6

Table 2.8 Biological activity of metabolites isolated from *Lantana camara* Linn. (Ghisalberti, 2000). (Continued)

Class	Compound	Biological activity
iridoid glycosides	geniposide	• inhibited hepatoxicity and the DNA repair synthesis induced by aflatoxin B1 in rat primary hepatocytes • showed hypolipidemic activity in hyperlipidemic rats • showed cAMP phosphodiesterase inhibitory activity
Furanonaphtho quinones	diodantunezone	• possess cytotoxicity against KB epidermoid nasopharynx, K562 human leukaemia and P388 lymphocytic leukaemia cell lines and found to be active (IC_{50} 6.76, 9.2 and 7.94 µmol/l, respectively)
phenylethanoid glycosides	verbascoside (syn: acteoside, kusaginin)	• inhibited PKC in a concentration-dependent manner and showed an IC_{50} of 25 µM • inhibited rabbit lens aldolase reductase and lipid peroxidation • exhibited immunomodulating activity and immunosuppressive properties • potentiated the anti-tremor effect of DOPA • against murine P-388 lymphocytic leukaemia *in vivo* (ED50 2.6 µg/ml compared to isoverbascoside ED50 10 µg/ml) • antiproliferative effect *in vitro* against L-1210 cells (IC50 13 µM) • analgesic activity • protection against oxidative haemolysis • antibacterial activity (*E. coli*) and was active against the Aujeszky virus • as a cardiotonic and vasodilatory agent increased levels of cAMP and of prostacyclin • exhibited activity against respiratory syncytial virus (EC50 0.80 µg/ml; cytotoxicity, IC50 76.9 µg/ml; selective index, SI 85.4)
	martynoside	• increased (16%) chronotropism and coronary perfusion rate (44%) in the Langendorff isolated rat heart1
	isoverbascoside	• exhibited activity against respiratory syncytial virus (EC50 0.0.62 µg/ml; IC50 51.4 µg/ml; SI 84)

2.7 *Psidium guajava* Linn. (Myrtaceae)

Species : *Psidium guajava* Linn.

Family : Myrtaceae

Local Name : Jambu burung, jambu padang, jambu berasu, jambu bereksa, jambu buyawas, jambu melukut, jambu Portugal, jambu batu, jambu pelawas, jambu biji, jambu biyawas (Mustafa & Muhamad, 1994).

Plate 1.3 *Psidium guajava* Linn.

Psidium guajava Linn. also known as the guava plant, is used in ethnomedicine for the treatment of various human ailments (Table 2.9) (Ross, 1999). Phytochemical studies undertaken by different groups of workers on different parts of the plant have resulted in the isolation and identification of various terpenoids, flavonoids and tannins (Table 2.10) (Begum *et al.*, 2002; Ross, 1999). Extracts of *P. guajava* Linn. have been shown to possess several pharmacological actions (Table 2.11) (Begum *et al.*, 2002; Olajide *et al.*, 1999; Ross, 1999).

Table 2.9 Traditional medicinal uses of *Psidium guajava* Linn. (Ross, 1999).

Region / Country	Parts of the plant	Uses
Brazil	Fruit	• Taken orally to treat diarrhoea, stomachache, and diabetes.
Canary Islands	Fruit	• Used as an antihemorrhoidal.
China	Roots	• Taken orally to suppress libido.
Cook Islands	Leaves	• Relieve postpartum pain and rid the body of residual stale blood.
Fiji	Fruit	• Taken orally for constipation.
	Leaves	• Taken orally for diarrhoea, indigestion, dysentery, and upset stomach.
Guam	Leaves	• To treat vaginitis and to promote conception.
Guatemala	Bark and leaves	• Taken orally to treat fevers, respiratory ailments, and skin infections.
	Fruit	• Powdered and eaten for stomach cramps.
Haiti	Leaves and fruit	• Taken orally for diarrhoea.
India	Flowers and buds	• Taken orally as an anthelmintic. • Used for diarrhoea, high fever, headache, and jaundice. • Used as antiemetic, remedy for stomachache.
	Leaves	• Taken orally for diarrhoea and as an antiemetic.
	Fruits	• Used for jaundice.
	Bark	• Taken orally as a remedy for stomachache.
Indonesia	Leaves	• Taken orally as an emmenagogue.
Japan	Root	• Taken orally as a suppressant of libido.
Madagascar	Leaves	• Taken orally for diarrhoea.
Malaysia	Bark and leaves	• Taken orally to expel the placenta, as an emmernagogue, and for after-birth disorders.
Mexico	Bark	• Taken orally for dysentery.
	Fruits	• Taken orally as a digestive.
	Leaves	• Used externally as a treatment for mange. • Taken orally for diarrhoea.
Nigeria	Root	• Taken orally for diarrhoea.
Panama	Bark and Fruits	• Taken orally for diarrhoea.
	Flowers and fruits	• Taken orally as an emmenagogue.

Table 2.9 Traditional medicinal uses of *Psidium guajava* Linn. (Ross, 1999). (Continued)

Region / Country	Parts of the Plant	Uses
Papua-New Guinea	Leaves	• Taken orally for diarrhoea.
Peru	Bark, leaves and root	• Taken orally as an astringent, antihemorrhagic, antidiarrheal, and for stomach pain.
Philippines	Bark	• Used in steam baths postpartum.
Rarotonga	Leaves	• Taken orally for dysentery.
Rwanda	Leaves	• Taken orally for dysentery.
Senegal	Leaves and fruits	• Taken orally for diarrhoea, and dysentery.
Sierra Leone	Leaves	• Taken orally for diarrhoea during pregnancy.
Taiwan	Fruits	• Taken orally to treat diabetes mellitus.
	Branches	• Taken orally for liver diseases.
Tanzania	Leaves	• Taken orally for malaria, and skin diseases.
Thailand	Leaves	• Taken orally for diabetes.
Venda	Roots	• Taken orally for venereal diseases.