ANTI-INFLAMMATORY, ANALGESIC AND PRELIMINARY CHEMICAL INVESTIGATIONS OF *CRINUM ASIATICUM* LEAVES

by

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In the name of ALLAH

The most beneficent and merciful

THIS THESIS IS DEDICATED

TO

MY MOST BELOVED PARENTS

MR. AND MRS. MOHAMMED ARAFAT

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LIST OF ABREVIATIONS

The following abbreviations have been used

(5-HT)	5-hydroxytryptamine
°C	Degree centigrade
15-LOX	15-Lipoxygenase
5-LOX	5-Lipoxygenase
A.D	Anno Damini
ad libitum	As needed
B_1 and B_2	Bradykinin receptors 1 and 2
BC	Before Christ
BK	Bradykinin
C_5a	Complement activating factor 5
Ca^{2+}	Calcium
cAMP	Cyclic adenosin monophosphate
cGMP	Cyclic guanosine 3'5'-monophosphate
Cl	Chloride
cm	Centimeter
Conc	Concentrated
COX	Cyclooxygenase
COX-1 and COX-2	Cyclooxygenase 1 and 2
D	Fraction of chloroform fraction

DA	Dopamine agonist
DP	Receptors activated by PGD
EP	Receptors activated by PGE
et.al.	Else where or add others
etc	Et cetera
f	Chloroform fraction
Fig.	Figure
FMLP	N-Formyl-L-Methionyl-L-Leucyl-L Phenyalalnine
FP	Receptors activated by PGF
ft	Feet
g	Gram
GIT	Gastro Intestinal Tract
G-protein	GTP binding signal transducer protein receptors
H_1, H_2 and H_3	Histamin receptors 1, 2 and 3
HETEs	Hydroxyeicosatetraenoic acids
hIL_6	Human interleukin type 6
IA	Alkaloids from chloroform extract
IGE	Immunoglobulin E
IL-1, IL-2, IL6, IL8 and (IL)-1β	Interleukin types; 1, 2, 6, 8 and 1 beta
(INF) γ	Interferon gamma
in.	Inch
IP	Prostanoid receptors
IP_3	Inositol-1,4,5-trisphosphate
K^-	Potassium
L	Liter

LD_{50}	Lethal dose 50
LTs	Leukotrienes
LTB_4, LTC_4, $LTD_4$and LTE_4	Leukotriene B_4, C_4, D_4 and E_4
MC_T	Mast cells containing tryptase
MC_{TC}	Mast cells containing tryptase and chymase
mg/kg	Milligram per kilogram
mIL_5	Murine interleukine type 5
ml	Milliliter
mm	Millimeter
N	Normal
Na^+	Sodium
NA	Non-alkaloid fraction from chloroform extract
NF-□B	Transcription factor
nM	Nanomoler
nm	Nanometer
NO	Nitric oxide
NSAIDs	Non steroidal anti-inflammatory drugs
NSC	Non-selective calcium channel
p.o	Orally
PAF	Platelet activating factor
PGs	Prostaglandins
PGE_2, $PGF_{2\alpha}$ and PGH_2	Prostaglandin types E_2, $F_{2\alpha}$ and H_2
PGI_2	Prostaglandin types I_2 (Prostacyclin)
PIP_2	Phosphatidylinositol-5,4-bisphosphate
PKC	Protein kinase C

PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLD	Phospholipase D
PMNs	Polymorphonuclear leukocytes
R1	Alkaloids from. fraction D6
Rf	Refractive value
ROS	Reactive oxygen species
s.e.m	Standard error of mean
SP	Substance P
TNF-α	Tumor necrosis factor alpha
TP	Receptor activated by TX
TX	Thromboxane
UV	Ultraviolet
v/v/v	Volume per volume per volume
w/v	Weight per volume
WHO	World Health Organization
µL	Microliter
µM	Micromolar

PUPLICATIONS ASSOCIATED WITH THIS THESIS

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KESAN ANTIINFLAMASI, ANALGESIK DAN KAJIAN AWAL KIMIA DAUN *CRINUM ASIATICUM*

ABSTRAK

Tujuan penyelidikan ini adalah untuk mengkaji kesan antiinflamasi dan analgesik daun *Crinum asiaticum* dengan pemfraksian kimia berpandukan aktiviti sebagai satu percubaan untuk menentukan sebatian aktif dalam tumbuhan ini. Kesan antiinflamasi diuji menggunakan cara edema aruhan karagenan pada tapak kaki belakang mencit sedangkan kesan analgesik diuji dengan cara Randall dan Selitto (1957). Serbuk kering daun *C. asiaticum* diekstrak secara bersiri, mula-mula dengan pelarut eter petroleum, diikuti dengan kloroform dan akhir sekali dengan metanol. Kesan antiinflamasi dan analgesik terkuat ditunjukkan oleh ekstrak kloroform (250 mg/kg, p.o.). Ekstrak kloroform kemudiannya difraksikan menggunakan kromatografi turus menjadi 5 fraksi (*f1* – *f5*). Fraksi *f4* dan *f5* didapati memberikan kesan positif terhadap ujian antiinflamasi dan analgesik. Kombinasi fraksi *f4* dan *f5* difraksikan lagi dengan kromatografi turus menjadi 8 fraksi: D_1 – D_8. Keputusan percubaan mendapati dengan dos 250 mg/kg hanya fraksi D_6 merencat edema dengan ketara yang menyarankan sebatian yang memberikan kesan antiinflamasi terdapat dalam fraksi D_6. Hiperaktiviti (yang kadang-kadang membawa maut) dan diarea terjadi pada mencit yang dirawat dengan ekstrak kloroform, methanol dan fraksi D_1, D_2 dan D_3. Hiperaktiviti tidak berlaku pada mencit yang dirawat dengan fraksi D_4 - D_8. Analisa fitokimia awal

menggunakan kromatografi lapisan tipis dan semburan dengan reagen khusus menunjukkan fraksi *f4*, *f5* dan D_6 mengandungi alkaloid, antron, kumarin, triterpena, glikosida, flavonoid dan minyak pati. Pemfraksian ekstrak kloroform tumbuhan ini menjadi fraksi alkaloid (IA) dan bukan alkaloid (NA) mendapati kesan antiinflamasi terdapat dalam fraksi alkaloid yang disertai dengan kesan hiperaktiviti, sedangkan kesan analgesik terdapat pada fraksi bukan alkaloid. Kesimpulannya sebatian kimia yang mempunyai kesan analgesik dalam tumbuhan ini adalah berbeza dengan sebatian yang memberikan kesan antiinflamasi dan kesan sampingan hiperaktiviti dan diarea.

ABSTRACT

The aim of the study was to investigate the anti-inflammatory and analgesic activities of *Crinum asiaticum* leaves through activity guided chemical fractionation in an attempt to determine the active compound(s) in the plant. The anti-inflammatory activity was evaluated using the method of carrageenan induced hind paw oedema in mice while analgesic activity was evaluated using Randall and Selitto (1957) method. The dried pulverized *C. asiaticum* leaves were serially extracted with petroleum ether, followed by chloroform and lastly methanol. The strongest anti-inflammatory and analgesic activities were shown in the chloroform extract (250 mg/kg, *p.o*). The chloroform extract was then fractionated using column chromatography into 5 fractions (f_1-f_5). Fractions *f4* and *f5* screened positive for both anti-inflammatory and analgesic activities. Combination of *f4* and *f5* were fractionated again using column chromatography to afford 8 fractions: D_1 - D_8. The results show that, at the dose of 250 mg/kg only fraction D_6 significantly inhibited carrageenan induced oedema which suggest that the compound(s) with anti-inflammatory activity is in fraction D_6. Hyperactivity (which sometime lead to death) and diarrhoea were observed in mice treated with chloroform and methanol extracts as well as D_1, D_2 and D_3 fractions. Hyperactivity was absent in fractions D_4 - D_8 treated mice. Preliminary phytochemical analysis by thin layer chromatography using specific reagents showed that fractions *f4*, *f5* and D6 contained alkaloids, anthrones, coumarins, triterpenes, glycosides, flavonoids and essential oils. Fractionation of the chloroform extract of the plant into alkaloid (IA) and non-alkaloid

(NA) fractions found that the anti-inflammatory activity was in the alkaloid fraction and it was accompanied with hyperactivity while the analgesic activity was in the non-alkaloid fraction. This led us to conclude that the compound(s) responsible for analgesic activity is different from the compound(s) that caused anti-inflammatory activity and the side effects of hyperactivity and diarrhoea.

CHAPTER ONE: INTRODUCTION

1.1 Herbal medicine in history

Despite the impressive accomplishments of the modern pharmaceutical industry, a large number of diseases are yet to be effectively treated. Furthermore, iatrogenic disorders are a consistent accompaniment of modern drug therapy. As a result, there is an increasing need for the development of the so-called "alternative treatment by natural medicinal products", for which predictable pharmacological activity and therapeutic efficacy can be documented.

Herbal medicine is a branch of natural medicine which uses and values plants as potent allies in overcoming disease and maintaining health. Like other natural medicine therapies, it views humans as part of nature and therefore observes and works with Allah's gifts in the management of disease. Herbal medicine is mankind's oldest medicine as it has been used for thousands of years. It is still used as an alternative to or in conjunction with modern medicines, and is also the subject of much current scientific interest. Generally, it is found as the geranium on the windowsill, the thyme in the garden, and the grass in the meadow.

Search for chemotherapeutic agents from natural products has equipped modern medicine with a wide range of curative agents. Despite spectacular advances in

synthetic drugs they have retained their importance. Herbal products are less potent and in general cause less adverse reactions than modern drugs. Besides their use in primary health care may enable savings to be made on national health bills (Phillipson, 1979). Treatment with herbs is designed to resolve imbalances and restore the healthy function of the whole system as well as particular organs. Herbs can treat the underlying, sometimes subtle causes of disease, as well as manifestations as pain, inflammation or fatigue, and help to foster the positive natural healing force of the body (Viki, 1994).

The importance of traditional medicine as a valuable and readily available source for primary health care has been recognized by the World Health Organization (WHO), which supports the use of herbal remedies that have been proven to be safe and effective. In some Asian countries including China, Japan, Korea and Vietnam, medical practitioners are trained to prescribe both traditional medicines and modern medicines.

In Malaysia, there are four major groups of traditional medicines namely the Malay traditional medicines, Indian traditional medicines, Chinese traditional medicines and western traditional medicines. Each has its own distinctive group of commonly used herbal materials. In Malaysia despite the availability of hundreds of traditional medicines and the possession of an exceptional heritage of plants and herbs, clinical data needs to be built up to document efficacy of the country's plant and herbal potential. It is not enough that some of these herbs have been used for decades and may be for even centuries to treat several diseases.

Malaysia, like many other countries has a long history in the use of plants to treat inflammation, pain and many other related diseases. In spite of the development of more

potent and convenient modern medicines, some plants are still occasionally used to treat inflammatory related diseases especially in some rural areas. It suggests that some of these plants must have some merit to be used as a source of medicine; otherwise, they would not have survived the time and onslaught of modern medicine. Gastrointestinal (GIT) side effects are the worst unwanted effects of modern anti-inflammatory and analgesic drugs. Eventhough some anti-inflammatory drugs have now been developed with less GIT side effects (Vane, 1998a). In an attempt to find more potent anti-inflammatory drugs with minimum side effects a lot of research is now being conducted in many parts of the world including Malaysia using traditional medicinal herbs. Plants with anti-inflammatory and analgesic history were extracted and screened for their anti-inflammatory and analgesic activities using various screening models like carrageenan induced hind paw oedema in mice or rats. Work is also still going on to investigate and clarify the mechanism of action of the anti-inflammatory activity of the plants proven to have anti-inflammatory activity in the preliminary screening.

1.2 Herbal medicine in therapy

It is said that herbal medicine uses the whole plant. This doesn't mean that all parts of the plant are always used, but that the parts used –root, bark, leave, flower, or seed- are taken completely as they were provided. Ethnopharmacology, which is the area concerning the study of drugs based on cultural and traditional beliefs, has contributed directly and indirectly to many new drug discoveries (Gan, 1993). Several thousand new compounds isolated from natural sources are produced every year by the chemists as potent drugs and numerous techniques and models have been designed by pharmacologists to test these compounds in various animal species (Dhawan, 1982).

A variety of such products has gained wide acceptance in Japan, China, and some European countries as mild nontoxic medications. However, for many products the active ingredients and mechanism of action remain to be determined. The biologically active constituents of medicinal plants have been acting as the lead compounds in drugs development, and medicinal drugs developed by this process are supplied by chemical synthesis e.g. Transilast which has been synthesized on the basis of the structure of nondinoside, a constituent of *Nandina domestica*, which has anti-allergic effects (Sankawa, 1993).

In India, the ayurvedic system of medicine developed an extensive use of medicines from plants dating from at least 1000BC. More than 2000 plants have been investigated and the biological activity of many could be confirmed after fractionation of crude extracts of those plants (Dhawan, 1982). The developments of broad classes of compounds such as cocaine, morphine, quinine, atropine, ephedrine, codeine, emetine, caffeine, reserpine, vinblastine and vincristine are the results of the initial leads obtained from natural products (Mehrotra, 1982). In western medicine, many drugs originated from natural sources. The classical examples are that of digitoxin from foxglove; *Digitalis purpurea*, which was used in England in the 18th century for treatment of heart diseases; quinine, the major alkaloid from *Cinchona officinalis* the bark of which was used by the Incas to treat malaria; and reserpine from *Rauwolfia serpentina* whose ground roots were and are still used in India as a tranquillizer (Popli, 1982).

Biological activities of some Malaysian plants and their mechanisms of action have been studied and examined (Asmawi *et.al*, 1993). *Eupatorium odoratum* that is used as folk medicine in Malaysia has been reported to have biological activities like anti-

inflammatory, analgesic and antimicrobial activities (Wah, 1993). Essential oil extracted from the fresh leaves of *E. odoratum* showed antimicrobial activity (Wah, 1993). Artemisinin, a unique sesquiterpene endoperoxide, which is isolated from the Chinese plant *Artemisia annua*, is a very effective antimalarial agent with few side-effects (Klayman, 1985). Taxol has been discovered to be a promising antineoplastic agent (Ikram *et.al*, 1993). Ikram *et.al* (1993) also reported the antimicrobial activity of different alkaloids (isocorydine & obaberive) isolated from the Taiwanese traditional medicine, *Dehaasia triandra*. The antimalarial activity of this plant has been screened and the components responsible for the activity have been isolated and identified (Likhitwitayawuid *et.al*, 1993).

Traditional plants have been used for their anti-inflammatory and analgesic activities to heal wounds and control pains e.g. a poultice of crushed leaves and chalk of *E. odoratum* is used to cure wounds, control bleeding and also inflammation (Wah, 1993). *Erythraea centaurium*, which is known in Turkey as "Red Cantarone", has a widespread use in popular medicine for different types of inflamed wounds as a wound healer (Berkan *et.al*, 1991).

1.3 Herbal medicine as anti-inflammatory and analgesic substances

Anti-inflammatory and analgesic activities of hundreds of traditional medicines have been screened using different models. Four decades after being introduced by Winter *et.al* (1962) carrageenan-induced inflammation in rat/mice has been used extensively to screen anti-inflammatory activity and for activity-guided isolation of anti-inflammatory constituents. For example, 5-glutinen-3-one and friedelanol, both with anti-

inflammatory activity, had been isolated from rhizomes of *Polygonum bistorta* (Duwiejua *et.al*, 1999). In Indian ayurvedic medicine, plants have been used to treat many diseases. Three ayurvedic medicines which are claimed to possess anti-inflammatory, antispasmodic, anthelmintic and anti-asthmatic properties are *Zingiber officinale* (Sunthi), *Vitex negundo* (Nirgundi), and *Tinospora cordifolia* (Guduchi). Chattopadhyay *et.al* (1999) proved that these plants had anti-inflammatory activities and found that *Tinospora cordifolia* was more active than aspirin for acute inflammation. Extracts from leaves of *Cordia verbenacea* DC are used in Brazilian folk remedies as anti-inflammatory and cicatrizing agents and have also been credited with exerting beneficial effects on the alimentary tract (Serité *et.al*, 1990). The heartwood of *Dalbergia odorifera*, a traditional Chinese medicine, has been reported to inhibit leukotrienes (LTs) and prostaglandins (PGs) biosynthesis and to inhibit the release of β-glucuronidase and lysosome from rat neutrophils (Chan *et.al*, 1998). Osthol, a coumarine, the most active constituent of *Angelica pubescens f. biserrata* has been reported to inhibit inflammation through its inhibition of 5-lipoxygenase (5-LOX) and cyclo-oxygenase (COX) (Liu *et.al*, 1998). This coumarin also inhibits thromboxane (TOX) formation in platelets, phosphoinositides breakdown and acts as interleukin-1 (IL-1) blocker (Liu *et.al*, 1998).

A series of diterpene derivatives, isolated from the plant *Endospermum diadenum* have been reported to inhibit inflammatory responses induced by different irritating agents either topically or systemically. The anti-inflammatory mechanisms work by blocking recruitment of neutrophils into inflammatory lesions, inhibition of degranulation and myeloperoxidase activity (Paya *et.al*, 1992). *Capparis spinosa*, a shrubby plant of rocky desert in Saudi Arabia used to treat inflammation, palsy, dropsy, gout and rheumatism

has been reported to significantly reduce carrageenan-induced oedema in rat paws (Mansour & Tariq, 1984). *Erythraea centaurium*, which is known in Turkey as "Red Cantarone" traditionally used for treatment of various inflammatory conditions, has been proved scientifically by Berkan *et.al* (1991) to have anti-inflammatory and antipyretic activities. The fresh fruit juice of *Eucballium elaterium* is traditionally used for the treatment of sinusitis in Turkey (Yesilada *et.al*, 1988). Cucurbitacin B, which has been isolated from this plant, has been reported by Yesilada *et.al* (1988) to has potent anti-inflammatory activity, in spite of high cytotoxicity at high doses. The aqueous extract of *Dichroa febrifuga* has been reported to suppress the endotoxin-induced inflammatory response by inhibiting the production of nitric oxide (NO) and the tumor necrosis factor alpha (TNF-α) supported its used as an anti-inflammatory drug (Kim *et.al*, 2000).

1.3.1 Alkaloids

Man has utilized alkaloids as medicines, poisons and magical potions from ancient times. The importance of alkaloids is established in the biological system and in important physiological and pharmacological activities, such as; analgesic, antimalarial, convulsion and anti-cancer activities. Different alkaloids have been isolated with anti-inflammatory activities. Fangchinoline and tetrandrin are the major alkaloids isolated from *Stephania tetrandrae*, a traditional medicine used in the treatment of inflammatory diseases in oriental countries including Korea (Choi *et.al*, 2000). These two alkaloids have been studied by Choi *et.al* (2000) for their anti-inflammatory activity. Tetrandrin was found to exert its activity through phospholipase-mediated release of arachidonic acid from the cell membrane similar to the action of corticosteroids (Di Rosa & Persica,

1979). It has been suggested that these alkaloids exhibit their anti-inflammatory activity through inhibition of 5-LOX (Choi *et.al*, 2000). Tetrandrine which was also found to inhibit the production of IL-1 by human monocytes has a potent inhibitory effect on murin intrleukin (mIL-5) and human interleukin (hIL-6), whereas fangchinoline inhibits production of IL-1 and TNF-α only (Seow, 1989 & Onai, 1995). Phytochemical studies on seven species of Malaysian Menispermaceae showed the presence of alkaloids as a main compound in all parts of the plant studied especially the leaves and stems (Aishah, 1984).

1.3.2 Coumarins

Coumarins are widely distributed in the plant kingdom. Some drugs used in folk medicine contain them as the major active compounds. The physiological activities of naturally occurring coumarins have been reviewed by Soine (1964). These pharmacological activities are such as, anticlotting (Suttie, 1987), hypotensive (Huang *et.al*, 1992), antitumor (Gawron & Glowniak, 1987), antinociceptive, anti-inflammatory and bronchodilator activities (Paya *et.al*, 1992 & Leal *et.al*, 2000). More than thirty constituents mostly coumarins such as simple coumarins (e.g. osthol), angular dihydrofurano coumarins (e.g. columbianedin and columbianetin acetate), coumarin glycosides (e.g. nodakenin), bergapten and umbelliferone have recently been isolated and identified from *angelica pubescens* f. biserrata (Liu *et.al*, 1998). Liu *et.al* (1998) reported some activities showed by those coumarins like anti-inflammatory, analgesic and calcium (Ca^{2+}) antagonistic activities. Anti-inflammatory, antinociceptive and bronchodilator activities of hydroalcoholic extracts from *Torresea cearensis*, *Justicia pectoralis*, *Eclipta alba*, *pterodon polygaliflorus* and *Hybanthus ipecacuanha* have been

reported (Leal *et.al*, 2000). These plants are used extensively in Brazil for respiratory tract diseases, and have in common the presence of coumarins as one of their chemical constituents. The effects of this plant on the key enzyme of inflammation have been studied. Low inhibitory activity on 5-LOX and inhibitory tendency on COX are exhibited by coumarin glycosides (Liu *et.al*, 1998).

1.3.3 Flavonoids

Flavonoids are polyphenolic compounds that occur commonly in plants. They occur as aglycons, glycosides and methylated derivatives. More than 4000 flavonoids to date have been isolated and identified (Duags *et.al*, 2000). Besides that flavonoids have been reported to inhibit the activities of several enzymes, including COX and LOX, monooxygenase, phospholipase A_2 (PLA_2) and protein kinases (PK) (Laughton *et.al*, 1991). Flavonoids have been used to treat several diseases like inflammation, diabetes mellitus, cancer, viral infections and stomach/duodenal ulcer (Havsteen, 1983 & Bose *et.al*, 1973). The biological activities of the flavonoids are thought to be the result of their antioxidant activities, where the inhibition of the enzymes by flavonoids could be attributed to their ability to react with reactive oxygen species (ROS) formed at or near the reaction center (Duags *et. al*, 2000). More than twenty flavonoids have been isolated and identified for their anti-inflammatory and anti-allergic activities from *Dalbergia odorifera* (Chan *et.al*, 1998). This study showed that some flavonoids e.g. cearoin and 3′-O-methylviolanone possess different inhibitory action mechanisms on superoxide anion formation. The 5-hydroxy-3, 6,7,3', 4'-pentamethoxyflavone (Artemetin, purified flavonoid) isolated from *Cordia verbenacea* have been reported to have anti-inflammatory activity on various animal models with a low toxicity (Serité *et.al*, 1990).

Studies on mechanism of action of this flavonoid revealed that artemetin markedly reduced vascular permeability and inhibited enzymes like LOX and COX. Flavonoids have been reported to induce the biosynthesis of lymphocyte interferon (Havsteen, 1983). The mechanism by which flavonoids relieve pain is thought to be like aspirin, through inhibition of PGs synthesis, but without side-effects. Moreover inhibition of PLA_2 may also be attributed to its analgesic activity (Havsteen, 1983). It is found that LD_{50} value of flavonoids in rats in the range of 2-10g per rat. Despite that the problem is their poor absorption in the gastrointestinal tract and also that they are easily degraded by the intestinal microflora (Havsteen, 1983).

1.3.4 Terpenoids and sesquiterpenes

One of the most abundant naturally occurring compounds in plants is terpenoids. The anti-inflammatory activity of triterpenes and sesquiterpenes is quite promising. Glycerhetinis acid, a triterpene isolated from *Glycerrhiza glabra* has been reported to have anti-inflammatory activity (Wagner, 1989). A registered product made of a sitosterol fraction of *Hypoxis rooperi*, showed a significant cortison like action on arachidonic acid release, resulting in a concomitant drop in COX and LOX products (Pegel & Walker, 1984). Several sesquiterpene lactone-producing plants, such as *Eupatorium formosanum*, have been used as anti-inflammatory herbal remedies as well as antipyretic drugs (Hall *et.al*, 1979). Studies on the mechanism of action of sesquiterpene lactones showed that α -methylene-γ-lactone and cyclopentenone moieties were required for inhibitory activities (Hall *et.al*, 1979). Studies showed that these anti-inflammatory agents are potent inhibitors of neutrophil migration, lysosomal rupture, enzymatic activity and also PGs synthesis, which were linked to elevated cyclic

adenosine monophosphate (cAMP) levels (Hall *et.al*, 1980). Rupiolin B and 11,13-dehydrodeacetylmatricarin isolated from *Achillea setacea* have been found to have anti-inflammatory activity in the croton oil ear test (Zitterl-Eglseer *et.al*, 1991). On the other hand, studies done by Rüngeler *et.al* (1998), proved that sesquiterpene lactones isolated from *Tithonia diversifolia* do not influence the enzyme of arachidonic acid pathways, but inhibit the activation of transcription factor (NF- κB), thereby reducing the synthesis of inflammatory mediators such as cytokines and chemokines and subsequently reducing the degree of inflammation. Moreover, germacranolides, which are sesquiterpene lactones that are isolated from *Mikania* species, exert their anti-inflammatory activities through suppressing the activity of PLA_2 at μM concentrations (Ysrael & Croft, 1990).

1.4 The plant (*Crinum asiaticum*)

1.4.1 Botanical aspects

Family:	Amaryllidaceae
Species:	*Crinum asiaticum*
Part used:	Leaves.

Crinum, Linn. a rather large genus of bulbous herbs of the family Amaryllidaceae, is found throughout the tropics and subtropics, mostly along the coast. Many have been brought into cultivation for the beauty of their flowers, and exist in gardens along with hybrids that have been raised between them. *Crinum asiaticum*, Linn. (Family: Amaryllidaceae) which is known in the Malay Peninsula as *Bunga tembaga suasa* (copper and gold flower), is a tuberous herb, with light green leaves up to about 5 ft. long and 6 in. wide (Henderson, 1954). In Southeastern Asia, Malaysia and Polynesia it occurs on the coasts. Most, if not all, species are poisonous in varying degrees (Burkill, 1966). Medicinally it has been used since early times as a potent folk medicine in the treatment of injury and inflammations. Its lotion can be applied anywhere on the body (Burkill, 1966).

Figure 1.1, Plant *Crinum asiaticum* bearing flowers.

1.4.2 Phytochemistry

A wide range of plant species belonging to the genus *Crinum* has been phytochemically investigated. Among the species that have been studied are *C. asiaticum, C. bulbispermum*, and *C. latifolium* which are the subject of many phytochemical studies (Ghosal *et.al*, 1985a). Although research has been carried out on the chemical components in the genus *Crinum*, their pharmacological properties are generally not well known. *Crinum* alkaloids have been much studied scientifically since early times (Ghosal *et.al*, 1985a). Alkaloids are found in almost all genera of the subfamily Amaryllidaceae (William & Wildman, 1970).

In spite of the lack of pharmacological works on *Crinum* alkaloids, some of these alkaloids have been reported to have interesting biological activities such as analgesic

and anti-tumor activities (Ghosal *et.al*, 1984 and Cozanitis *et.al*, 1983). Review done by Ghosal *et.al* (1985a), showed that a lot of alkaloids have been isolated and identified chemically from *Crinum* species especially *C. asiaticum*. These alkaloids are discussed below.

1.4.3 Amaryllidaceae alkaloids

The study of Amaryllidaceae alkaloids began with the isolation of lycorine from *Narcissus pseudonarcissus* in 1877. Since that time, about 150 species belonging to 36 genera of this family were examined for alkaloids. Over 100 structurally different alkaloids, comprising 12 distinct ring types have so far been isolated from different parts and at different periods of vegetation of Amaryllidaceae species (sub-family, Amaryllidiodeae) (Ghosal *et.al*, 1985a). The genus Crinum is a true representative of the family as it exhibits all the chief chemical traits of the Amaryllidaceae. Lycorine is the most common glucoalkaloid of the family and the first one to be isolated. Lycorine and most of the Amaryllidaceae alkaloids have been isolated from fruits, bulbs and roots of *Crinum* plants including *C. asiaticum* (El- Moghazi & Ali, 1976; Ghosal *et.al*, 1985a & Ghosal *et.al*, 1981). Ghosal and his colleagues did very significant work on the Amaryllidaceae alkaloids especially the alkaloidal constituents of *C. asiaticum*.

Eventhough a lot of alkaloids have been isolated from *C. asiaticum*, most of these alkaloids have been extracted and isolated from fruits bulbs and roots. For example, ungeremine, criasbetaine, lycoriside, crinamine have been isolated from the fruits (Ghosal *et.al*, 1985d and Ghosal *et.al*, 1986); and crinasiadine and crinasiatine from the bulbs of different *Crinum* species (Ghosal *et al.* 1985c). This plant is still subjected to

systemic anti-inflammatory and antiphlogestic evaluation to support its use. Indeed few pharmacological studies have been reported (Ghosal *et.al*, 1985b & Samud *et.al*, 1999). The list of most of the alkaloids that have been isolated from *C. asiaticum* are shown in (Table1.1) with their reported biological activities.

Table 1.1, Alkaloids isolated from *C. asiaticum* with their biological activities.

Alkaloid	Occurrence	Biological effect	Reference
Ambelline	scape portion		Ghosal *et.al*, 1985a
Belladine			
Criasbetaine	fruits	Anti-tumor	Ghosal *et.al*, 1986
Crinamine			
Crinamine-6-OH			
Crinasiadine	bulbs	Bacteriostatic Tumor inhibitor	Ghosal *et.al*, 1985a
Crinasiatine	bulbs	Bacteriostatic Tumor inhibitor	Ghosal *et.al*, 1985a
Crinine			
Galanthamine-*N*-demethyl		Analgesic Anticholinergic Cholinesterase activity	Abd El Hafiz *et.al*, 1991
Galanthamine-*O*,*N*-diacetyl			
Haemanthamine			
Haemanthidine			
Hamayane			
Lycorine	scape portion	Anti-tumor Anti-viral	Ghosal *et.al*, 1985a Abd El Hafiz *et.al*, 1991
Lycorine-1-O-glucoside	roots	Immunostimulator	Chattopadhyay 1999
Lycoriside	fruits, bulbs and roots		Ghosal *et.al*, 1985a
O-Methylnorbelladine			
Palmilycorine	fruits, bulbs and roots		Ghosal *et.al*, 1985a
Pratorine (= hippadine)			
Pratorinine			
Pseudolycorine			
Pseudolycorine-1-*O*-β-D-glucoside			
Ungeremine	fruits	Anti-tumor	Ghosal *et.al*, 1986

1.4.4 Ethnopharmacology

C. asiaticum has long been used in folk medicine to treat diseases. As a folk medicine, this plant has been used in so many countries. In the Malay Peninsula, a poultice of bounded leaves was used to treat fever and swellings (Burkill, 1966). In Indonesia, since early times, they were used as an antidote for wounds from poisoned arrows, poisonous bites or stings, and also after eating poisonous food, e.g. certain fish (Perry and Lily, 1980). In India, Watt (Dict. 1889) has recorded its emetic effects. The bruised hot leaves, coated with caster oil or mustard oil, are applied to the inflamed ends of toes and fingers, inflamed joints and sprains. The warm leaf juice with the addition of a little common salts is used for ear complains. These leaves have an interesting history and activity. The leaves can be pounded alone or with various ingredients; another way to make a poultice is to oil the broader leaves and heat them until they wilt. Moreover, in Indonesia they rub the affected part with bounded leaves to stimulate perspiration; also, oiled and heated leaves are used to relieve headache and local pain (Perry and Lily, 1980). Dutch soldiers, who were wounded by poisoned arrows, swallowed its juice and chewed its roots for its antidote activity after which they poulticed the wound with chewed roots (Burkill, 1966).

This plant, especially its leaves, is extensively used in the rural areas where it commonly grows. Chemical investigation started long time ago to isolate the chemical constituents of this plant though not much work has been carried out on the leaves of this plant. Most, if not all, the chemical constituents isolated from this plant are alkaloids. Still activity-guided isolation of chemical constituents of this plant especially leaves has not been found in the previous work on this plant. The question arises whether alkaloids are responsible for the biological activities e.g. anti-inflammatory and

antipyretic activities, of this plant especially in the leaf part. This has yet to be ascertained. In this study, we proved that alkaloids-containing extracts from the leaves of this plant possess anti-inflammatory and analgesic activities. The biological activities of alkaloids isolated and identified from different parts of this plant are listed in Table.1.1.

1.4.5 Important of alkaloids in biological systems

From ancient times man have utilized alkaloids as medicines, poisonous, and magical potions. Only recently we have gained precise knowledge about the chemical structures of many of these interesting compounds. The term *alkaloid*, or "alkali-like," was first proposed by the pharmacist W. Meissner, in 1819. It is usually applied to basic, nitrogen-containing compounds of plant origin. Examples of well-known alkaloids are *morphine* (*opium poppy*), *nicotine* (tobacco), *quinine* (cinchona bark), *reserpine* (*rauwolfia*), and *strychnine* (*strychnos vomica*) (William & Wildman, 1970). The importance of these alkaloids and other alkaloids in the biological system are very well known. These activities include analgesic, antimalarial, convulsion and anti-cancer activities.

Alkaloids isolated from Amaryllidaceae have been reported to exhibit different pharmacological and microbiological effects such as antiviral, antitumor, and anticholinergic (Abd El Hafiz *et.al*, 1991). The anti-viral activity of lycorine, the most widespread Amaryllidaceae alkaloid, has been the subject of several recent reports (Ghosal *et.al*, 1985a). Pseudolycorine and pretazettine are active against several leukemias. Ungeremine and criasbetaine, two alkaloids which were isolated from *C.*

asiaticum, showed significant anti-tumor activity (Ghosal *et.al*, 1986). On the other hand, galanthamine possesses analgesic, anticholinergic, and cholinesterase activities, and has been used in the treatment of myasthenia gravis, myopathy, and also of Alzheimer's disease and other diseases of the nervous system (Abd El Hafiz *et.al*, 1991). Lycorine-1-O-glucoside has been reported to be a potential immunostimulatory agent (Ghosal *et.al*, 1984). 1,2-β-Epoxyambelline, a new immuno-stimulant alkaloid from *C. latifolium*, has been isolated and chemically identified (Ghosal *et.al*, 1984). It has been showed that a number of Amaryllidaceae alkaloids caused a transient fall in blood pressure in laboratory animals in high doses. Indeed with regard to the cytotoxicity of many Amaryllidaceae alkaloids there is still more investigation needed to study the biological activities of the others. Alkaloids from *C. asiaticum* need to be identified pharmacologically in order to discover the function of alkaloids in their biological activities especially anti-inflammatory and analgesic activity.

1.5 Inflammation

1.5.1 Definition and introduction

Inflammation may be considered as a protective mechanism that on occasions becomes deregulated and leads to a chronic inflammatory state. It can occurs in all tissues and organs in the body in response to a variety of stimuli that may be mechanical, chemical, or infective. The symptoms of inflammation: heat, redness, pain, and swelling, were described by Celsius (30 B.C.–A.D). Three phases are involved in inflammation (Vogel & Vogel, 1997). The first phase of inflammation is caused by an increase in vascular permeability, resulting in the exudation of fluid from the blood into the interstitial space; the second phase by infiltration of leukocytes from the blood into the tissues and the third phase by granuloma formation .A variety of chemical mediators, such as histamine (H), 5-hydroxytryptamine (5-HT), bradykinin (BK), and the PGs family of compounds and other arachidonic acid metabolites e.g. LTs, have been implicated in the production of symptoms of inflammation. Arrays of physiological substances, also called autacoids, are involved in the process of inflammation and repair. A clear differentiation exists between the events associated with acute inflammation and those associated with the chronic inflammatory state, although in both cases a number of different stimuli are capable of producing an essentially common series of events.

1.5.2 Acute inflammation

Acute inflammation is the initial response to tissue injury; it is mediated by the release of autacoids and usually precedes the development of the immune response. The acute inflammatory response consists of three main vascular effects: (i) vasodilatation and increased blood flow, (ii) increased vascular permeability, and (iii) leukocyte migration into the injured tissues. This phase of inflammation is characterized by a greater monocyte migration from the blood to the inflammatory site directed by chemoattractants, with considerable intercellular interaction between this monocyte-macrophage population and lymphocyte of the T series. Molecular mechanisms of granulocyte and monocyte extravasation into inflammation sites have generated a lot of studies, which led to a general consensus expressed by the so-called 'multistep paradigm' (Figure 1.2) (Butcher, 1991 & Springer, 1994). This describes the overall process of extravasation as a three step mechanism consisting of (i) rolling of leukocytes on the endothelium as long as a few seconds which is enough time to allow chemoattractants to operate their activation functions, (ii) activation of integrins, which are important for the interaction of leukocytes with the endothelium, (iii) firm adhesion and extravasation. Each of these steps involves different membrane receptors on both the migrating and the endothelial cell side, and implies the active role of the soluble mediators (Febbri *et.al*, 1999). Febbri *et.al* (1999) reviews regulation of lymphocyte traffics by adhesion molecules. Nitric oxide (NO) has been found to exacerbate hydrogen peroxide-mediated endothelial-neutrophil adhesion by the formation of an iron-dependent oxidant like hydroxyl radical (Okayama *et.al*, 1998a). It has been demonstrated that the neutrophil adhesion induced by NO plus peroxide involves P-selectin mobilization to the cell-surface and endothelial platelet activating factor (PAF)

synthesis (Okayama *et.al*, 1998b). PAF plays a significant role in inflammation. It has been shown to induce chemotaxis of polymorphonuclear leukocyte (PMNs) and to promote their aggregation and secretion of lysosomal enzymes (Jouvin-March *et.al*, 1982). PMNs migration are involved in acute inflammation. This migration is directed by chemical gradients (chemotaxis). The activation of PMNs by chemoattractants via a receptor-mediated mechanism involves a series of signal transduction leading to the release of protein kinase C (PKC). Activation of PKC contributes to the pathogenesis of a number of inflammatory disease states (Chang *et.al*, 2000). It has been shown that activation of PKC leads to the expression and release of several inflammatory mediators including products of arachidonic acid metabolism and cytokines and activation of a number of inflammatory cells such as platelets, neutrophils, macrophages, lymphocytes and fibroblasts.

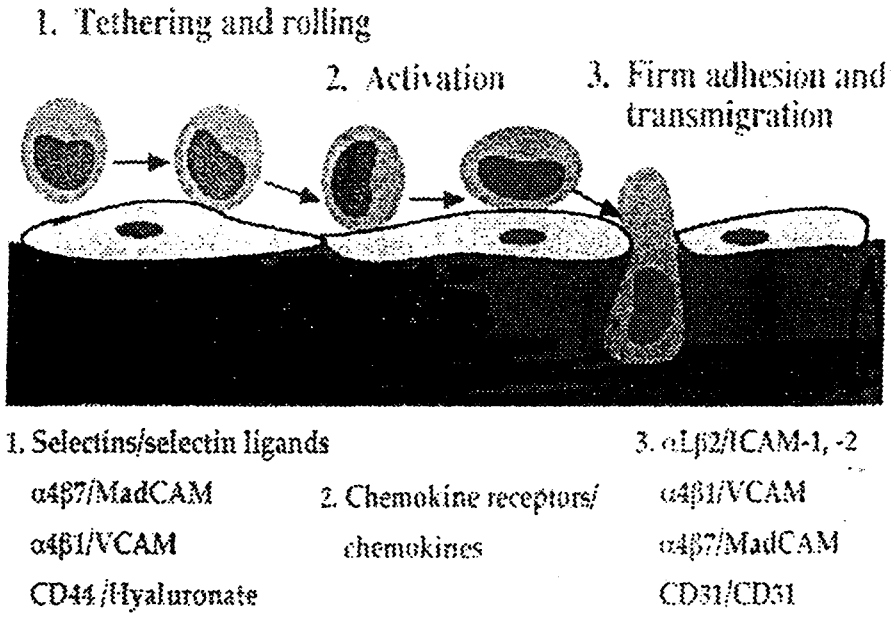

Figure 1.2, Molecular mechanisms of granulocyte and monocyte extravasation into inflammation sites, "multistep paradigm", (Febbri *et.al*, 1999).

1.5.3 Chronic inflammation

Chronic inflammation involves the release of a number of mediators that are not prominent in the acute response. The chronic state is characterized by marked infiltration of mononuclear cells of phagocytic and nonphagocytic types, that is, macrophages and lymphocytes, respectively. The observed results are stimulation of antibody formation, the presence of circulating antibody, and the formation of immune complexes. These various aspects of the activated immune system cause the complement cascade to produce complement activating factor a (C_5a), a major chemoattractant, which can then attract additional cells in order to facilitate and amplify the ongoing inflammatory response. Although the exact mechanism/s involved in switching acute inflammation towards a more chronic lesion are still not clearly defined, three mechanisms have been suggested (Lombardino, 1985). It is mostly suggested that the presence of an initial inflammatory stimuli that is incompletely phagocyted by phagocytes or the presence of antigen (the so called 'self protein') that is incompletely degraded will lead to the development of chronic inflammation.