CHAPTER I

INTRODUCTION

Malaria has been recognized as an important parasitic disease of humans for centuries. It is the most important parasitic disease in tropical country including Thailand. Malaria causes about 350–500 million infections in humans and approximately 1.3–3 million deaths annually, his represents at least one death every 30 seconds. The vast majority of cases occur in children under the age of 5 years, pregnant women are also especially vulnerable. The death rate is expected to double in the next twenty years. Precise statistics are unknown because many cases occur in rural areas where people do not have access to hospitals and/or the means to afford health care. Consequently, many cases are undocumented.[1-3]

1.1 Life cycle of the Plasmodium parasite and its epidemiology

Malaria in humans is caused by a protozoan of the genus *Plasmodium* and the four subspecies, *falciparum, vivax, malariae*, and *ovale*. The species that causes the greatest illness and death in Thailand is *P. falciparum*. The disease is transmitted by the bites of mosquitoes of the genus *Anopheles,* of which the *Anopheles gambiae* complex (the most efficient) is responsible for the transmission of disease. In the human host the parasite is found primarily inside of the red blood cells (RBC). The parasite reproduces asexually inside of the RBC, and following this, the RBC breaks open releasing many new parasites (merozoites). These parasites then infect more RBC's, and this ultimately leads to the destruction of massive numbers of RBC's. The characteristic "chill and fever" (paroxysm) associated with malaria occurs when the parasites are released from the RBC's, and since the release of parasites is periodic, the paroxysms are periodic. For examples, the paroxysms associated with a tertian malaria (e.g., *P. vivax*) occur about ever 48 hours, and those associated with a quarten malaria (e.g., *P. malariae*) occur about every 72 hours. The malarial life cycle is shown in **Figure 1.1**.

Malaria remains one of the most lethal and widespread diseases due to the emergence of parasites resistance to most available antimalarial drug.[4] Resistance is thought to be acquired genetically by the parasite as a result of spontaneous mutations. Mutant clones are selected especially when the parasite is in an environment of sub-therapeutic levels of drug directed against it. According to pharmacology experts, this is especially the case when background immunity is weak and drug pressure high. Chloroquine is by far the most used antimalarial in conventional malaria therapy. It is an inexpensive and readily available drug in many endemic areas. However, owing to widespread drug resistance, the drug is becoming increasingly ineffective in many parts of the world. In India, the foci of chloroquine resistance have now spread. In a few countries like Thailand, chloroquine resistance is as high as 85 % among *P. falciparum* cases. Chloroquine is now getting increasingly replaced by sulphadoxine-pyrimethamine (S/P) combination, also known by the brand name Fansidar, as the second-line drug. Prior to 1978, S/P drugs resulted in cure rates of 80-90 % but since then failure rates have risen to more than 50-60 %. According to the World Health Organisation, resistance to other powerful drugs like mefloquine, halofantrine, pyronaridine and metakelfin have also been emerging and expanding since 1978. In India, reports of chloroquine resistant *P. vivax* too have appeared in recent years.

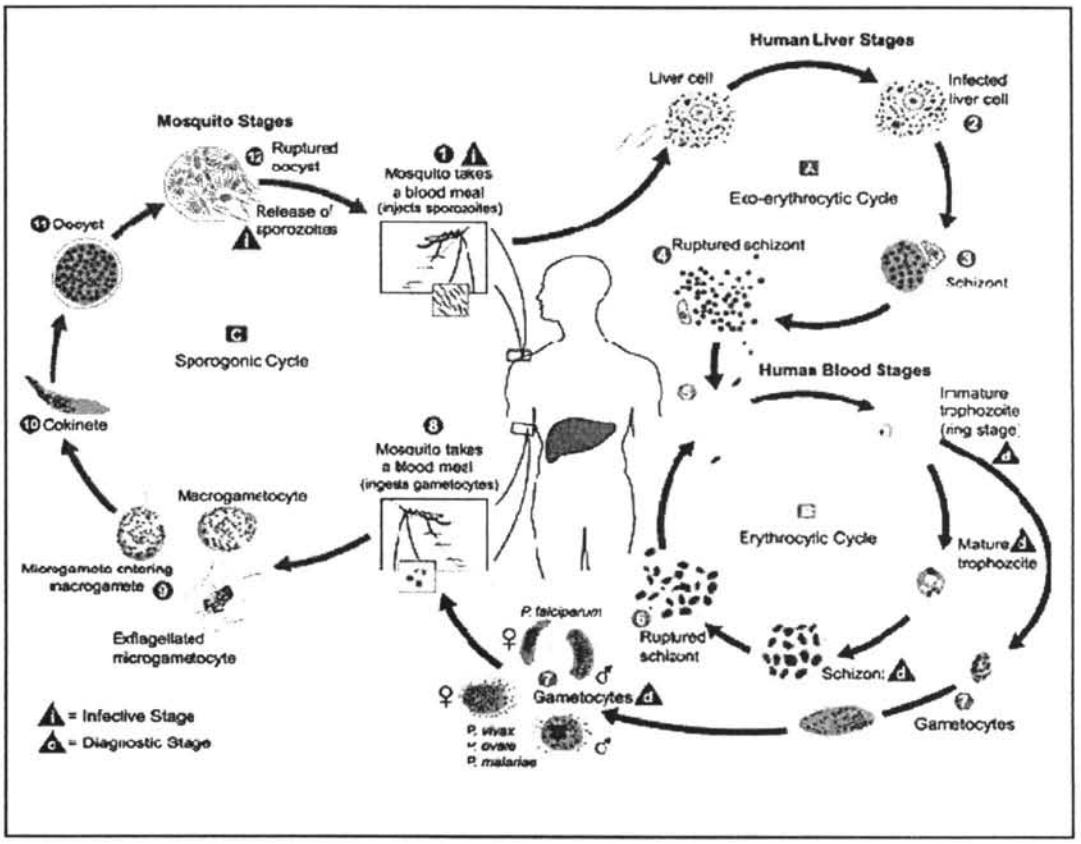


Figure 1.1 Life cycle of malaria

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating

zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle ❶.

1.2 Symptom

Malaria symptoms will occur at least seven to nine days after being bitten by an infected mosquito. Fever is the main symptom of malaria include shaking chills, headache, muscle aches, and tiredness. Nausea, vomiting, and diarrhea may also occur. The most severe manifestations are cerebral malaria (mainly in children and persons without previous immunity), anemia (mainly in children and pregnant women), and kidney and other organ dysfunction (e.g., respiratory distress syndrome). Persons repeatedly exposed to the disease acquire a considerable degree of clinical immunity, which is unstable and disappears after a year away from the endemic-disease environment. Immunity reappears after malarial bouts if the person returns to an endemic-disease zone. Most likely to die of malaria are persons without previous immunity, primarily children or persons from parts of the same country (e.g., high altitudes) where transmission is absent, or persons from more industrialized countries where the disease does not exist.

1.3 Medicinal plant and malarial activity

Approximately 80% of the world population still depends on traditional medicine as a source of the treatment of disease. Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. From a "scientific"

perspective, many herbal treatments are considered experimental. The reality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. The use of traditional and herbal seems to be the alternative choice of treatment in countries where malaria is endemic.[5-6] Local medicinal plants continue to be used in the treatment of malaria and update the evaluation of antimalarial activity of medicinal plants against *P. falciparum* is extensively studied.[7-9] Recently, interest in plants as potential sources of antiparasitic drugs was stimulated by the isolation of artemisinin from *Artemisia annua*, which was traditionally used in Chinese medicine. Artemisinin is considered as a potent antimalarial drug, even against chloroquine- and quinine- resistant *P. falciparum* and other malaria causing parasites.[10]

Herbal medicine has long been practiced by Thai people in all regions. In spite of this, there had been very little interaction among Thai traditional medical men. It is not surprising to find that many regions have developed their own systems of nomenclature, techniques and idiosyncrasy. For instance, the same species of herb may be known by different name in different districts.

It is most likely that early men learnt about the healing value of some herbs by observing habits of animal. It is well known that some carnivores eat particular plants to cure certain ailment. The principle of even the plants were probably tried, and the good results were passed on the succeeding generations. In the Eastern world, India and China are credited as being among the first to come up with traditional medical texts prescribing herbal medicines for numerous sicknesses.

Thailand abounds with medicinal herbs many of which have been successfully employed to cure the Thais of a wide rang of sicknesses. Some herbs with mild medicinal values such as ginger, fha-ta-lai-jone and lemon grass are skillfully

incorporated into traditional cuisine. Other medicinal herbs can be used as decorative plants.

The important of herbal medicine has been increasingly recognized by the public, and international as well as national organizations. Systematic research is being carried out by several local laboratories with the purpose of supplementing modern medicine.

1.4 Botanical Aspects and Distribution

1.4.1 *Andrographis paniculata*

Division:	Angiosperms
Class:	Dicotyledonae
Order:	Personales
Family:	Acanthaceae
Genus:	*Andrographis*

Andrographis paniculata Nees (Acanthaceae), the fha-ta-lai-jone of Thai is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in Asia literally "king of bitters". It grows erect to a height of 30-110 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals. **Figure 1.2**.

1.4.1.1 Ethnobotanical of *A. paniculata*

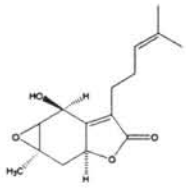
A. paniculata is used as a wonder drug in traditional as well as in tribal medicine in Thailand for multiple clinical applications. The therapeutic value of fha-ta-lai-jone is due to its mechanism of action which is perhaps by enzyme induction. The plant extract exhibits antityphoid and antifungal activities. Fha-ta-lai-jone is also reported to possess laxative, vulnerary, antipyretic, antiperiodic, antimalarial,[11] antihepatitic, antithrombogenic, anti-inflammatory,[12] antisnakevenom, expectorant, depurative, soporific, anthelmintic, antibacterial, antiviral,[13-14] immuno-stimulant,[15-16] hepatoprotective,[17] antithrombotic,[18] anticancer,[19] hypoglycemic,[20]

hypotensive[21] and useful in hyperdispsia, buring sensation, wounds, ulcers, chronic fever, intermittent fevers, inflammations, cough, bronchitis, skin diseases, leprosy, colic, flatulence, diarrhoea, dysentery, haemorrhoids and antipyretic properties to mention a few, besides its general use as an immunostimulant agent.

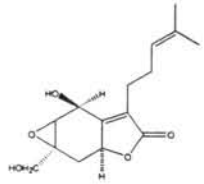
Figure 1.2 *Andrographis paniculata* Nees.

1.4.1.2 Previous studies in chemical constituents of *A. paniculata*

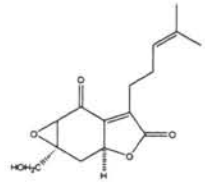
From the literature surveys, *A. paniculata* has been widely studied. Many lactones and flavonoids have been isolated and characterized [12,29] in **Figure 1.3**.



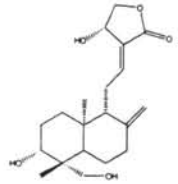
Paniculides A



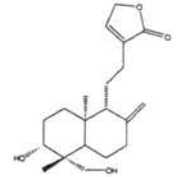
Paniculides B



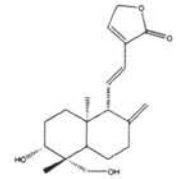
Paniculides C



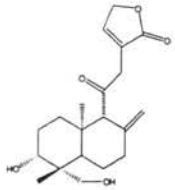
Andrographolide



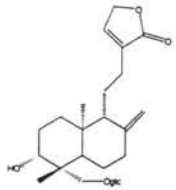
14-Deoxy andrographolide



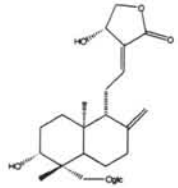
14-deoxy-11,12-di dehydroandrographolide



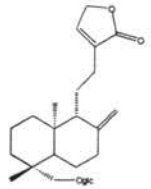
14-deoxy-11-oxo andrographolide



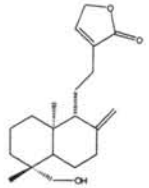
Deoxyandrographolide -19β-D-glucoside



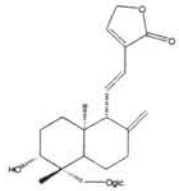
Andrographoside



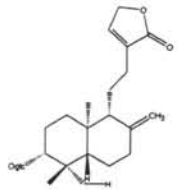
Neoandrographolide



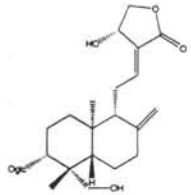
Andrograpanin



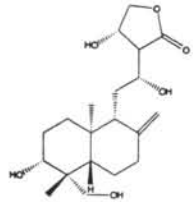
14-deoxy-11,12-di dehydroandrographiside



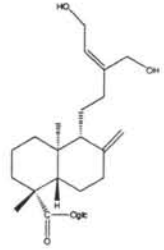
3-*O*-β-D-glucopyranosyl-14,19-dideoxyandrographolide



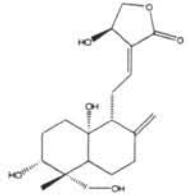
3-*O*- β-D-glucopyranosyl-andrographolide



12*S*-hydroxyandrographolide

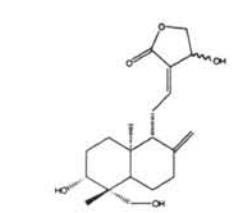


andrographatoside

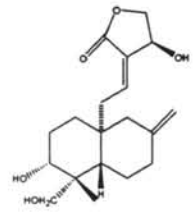


14-*epi*-andrographolide

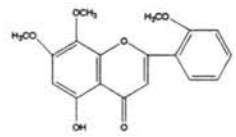
Figure 1.3 The chemical constituents of *A .paniculata* Nees.



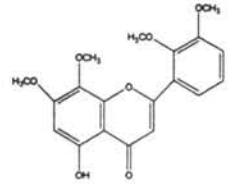
Isoandrographolide



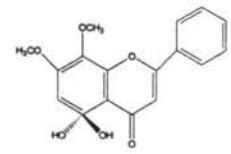
Andropanolide



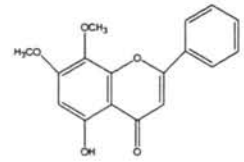
Andrographin



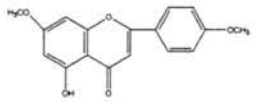
5-Hydroxy-
2',3',7,8-tetramethoxyflavone



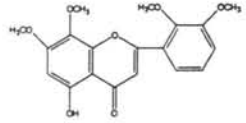
(*dl*)-5-Hydroxy-
7,8-dimethoxyflavone



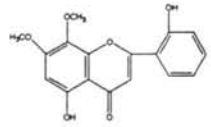
5-Hydroxy-7,8-
dimethoxyflavone



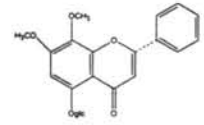
Apigenin-4',7'-
di-*o*-methyl ether



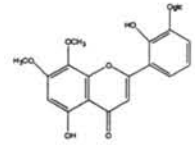
Mono-*o*-methyl-wightin



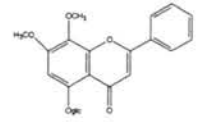
Panicolin



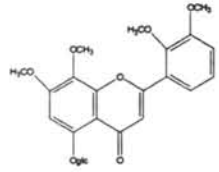
Andrographidine A



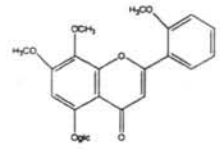
Andrographidine B



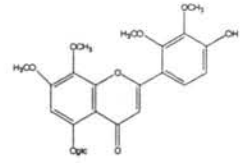
Andrographidine C



Andrographidine D



Andrographidine E



Andrographidine F

Figure 1.3 The chemical constituents of *A .paniculata* Nees. (continue)

1.4.2 *Caesalpinia bonduc*

Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Fabaceae
Genus:	*Caesalpinia*

Caesalpinia bonduc (L.) Roxb. (Fabaceae) is widely distributed throughout the tropical and subtropical regions of Southeast Asia. In Thailand, it is commonly known as "swat". It is a woody scrambling shrub, to c. 5m high. Leaves 20-40 cm long with large bipinnate leaves that have sharp recurved hooks on the underside. **Figure 1.4**.

1.4.2.1 Ethnobotanical of *C. bonduc*

C. bonduc is a popula traditional medicinal plant. Swat is also reported to have antipyretic, antidiuretic, anthelmintic and antibacterial, anti-inflammatory,[23] anthelminitic and antimalarial drug,[24] antianaphylactic and antidiarrhoeal, antiviral,[25] antiasthmatic, antiemetic and antiestrogenic properties. A decoction of the roots has been used as a tonic and for the treatment of rheumatism and backache. Its seed kernels have been used as an antimalarial, anti-inflammatory, anticancer and antitussive agent.[26]

Figure 1.4 *Caesalpinia bonduc* (L.) Roxb.

1.4.2.2 Previous studies in chemical constituents of *C. bonduc*

From the literature surveys, *C. bonduc* has been widely studied. Many diterpenoid has been isolated and characterized [27-31] in **Figure 1.5**.

From the attractively antimalarial activity of chemical constituents of *A. paniculata* and *C. bonduc* were selected for further investigation of phytochemical constituents and their effects on malaria. The goals of this research can be summarized as following:

1. To extract and isolate the compounds from *A. paniculata* and *C. bonduc*.
2. To elucidate the structures of the isolated compounds.
3. To determine the antimalarial activity and cytotoxicity of the isolated compounds.

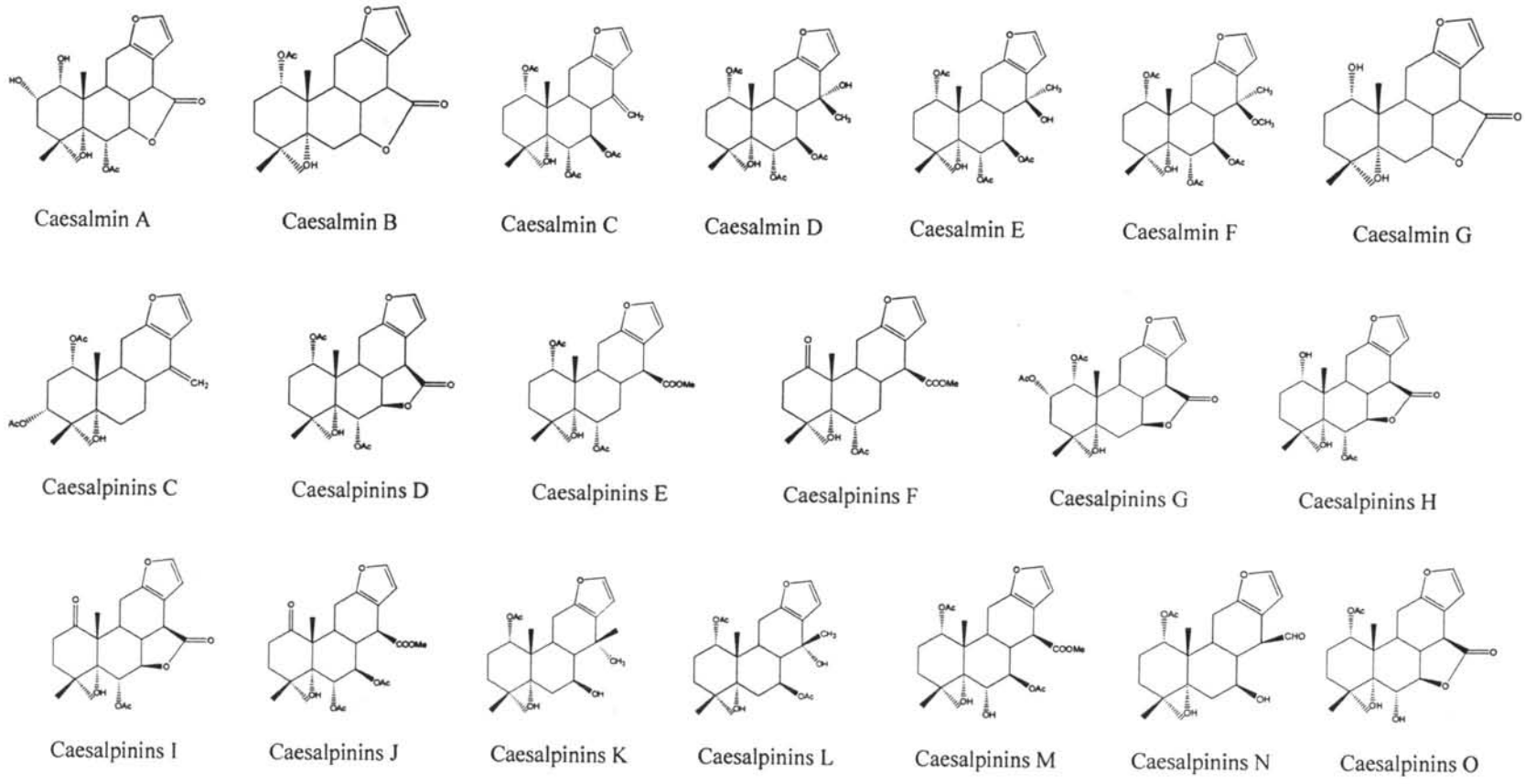


Figure 1.5 The chemical constituents of *C. bonduc*

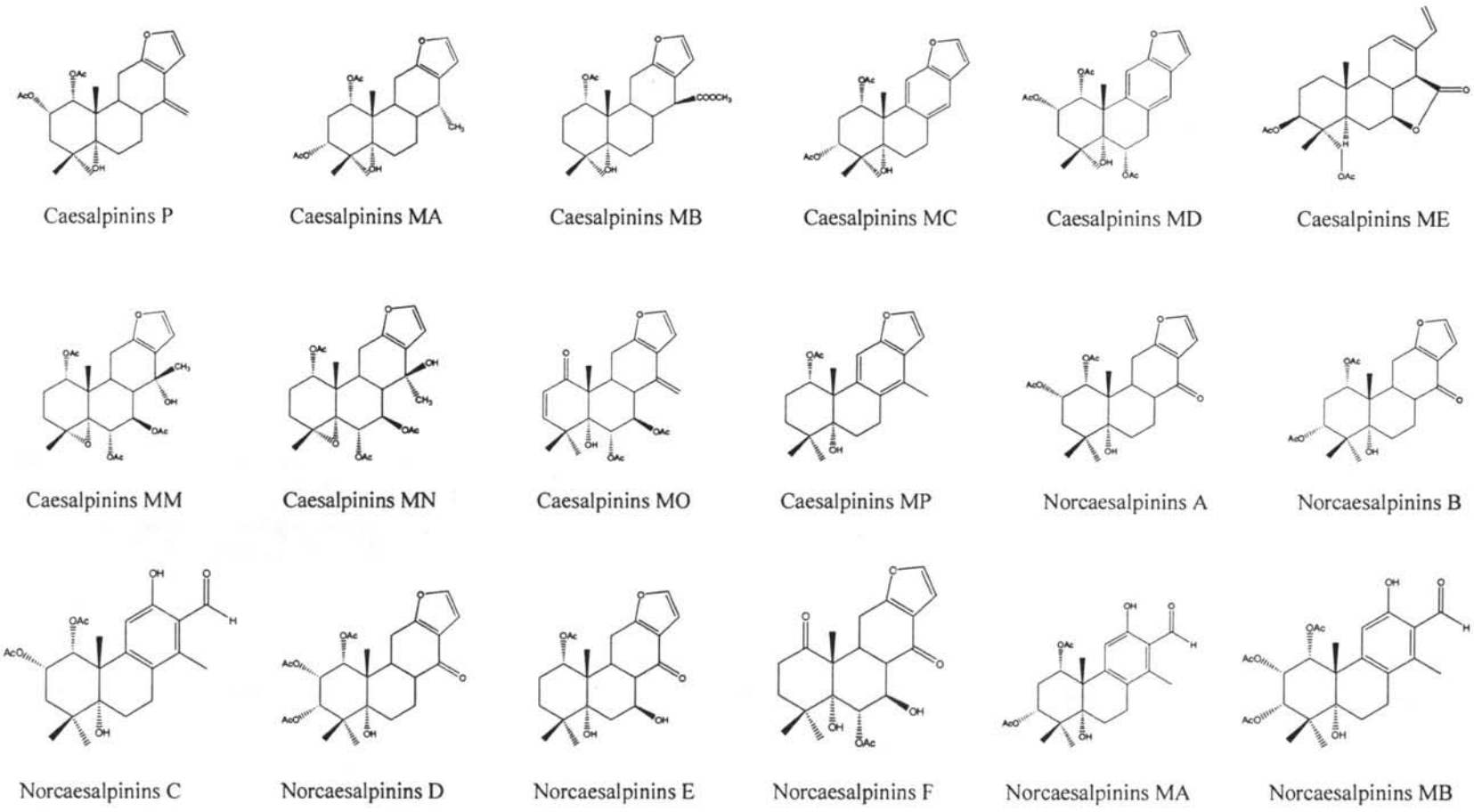


Figure 1.5 The chemical constituents of *C. bonduc* (continue)

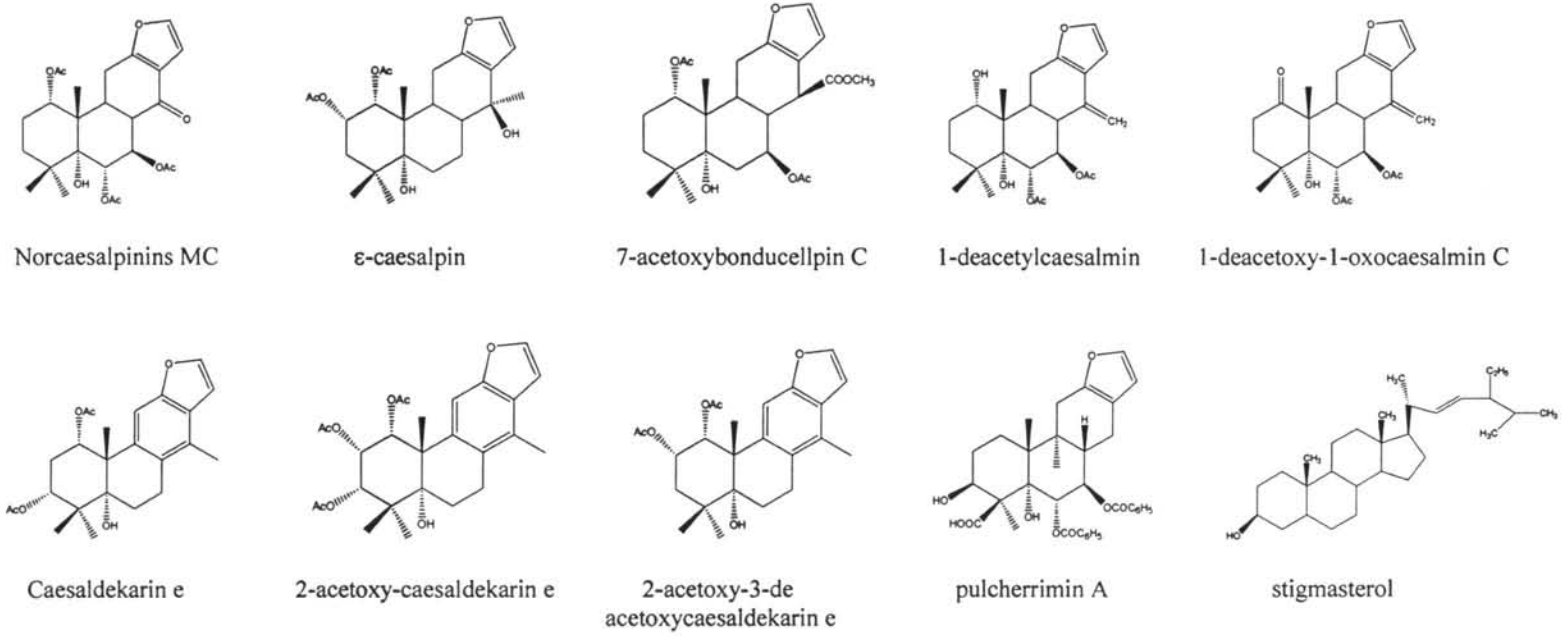


Figure 1.5 The chemical constituents of *C. bonduc* (continue)