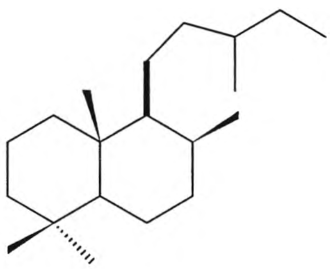
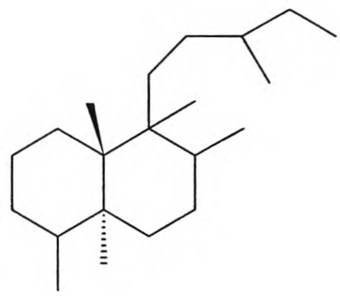
CHAPTER 1
INTRODUCTION

In Thailand, medicinal plants have widely been used as therapeutic drugs or herbal medicine. Since plant-derived chemicals (secondary metabolites) have involved in nature as biologically active compounds with particular effects on other organisms, some of them are useful to mankind as pharmaceuticals, fragrance, flavors, colors and stimulants. Therefore, medicinal plant still serve as a source for scientist to be developed into new and more active compounds.The plants in the Euphorbiaceae, which consist more than 5000 species in 800 genera[1], are of interest because they contain many attractive compounds and are always found to be novel compounds or show an excellent biological activities. This family are also known to produce various diterpenoids such as pimaranes, kaurane, jatrophane, lapdane, clerodane and lathyrane diterpenoids. Diterpenoid compounds consisted a large group of compounds derived from geranylgeranyl pyrophosphate which have twenty carbons with bi-, tri-, or tetra cyclic skeletons. (Skeleton structure of diterpenoids from this family are shown in **Figure 1.1**). They have recently reported for several studies on chemical constituent from many parts of *Gelonium multiflorum* which mostly constituents were diterpenoids. The miscellaneous rearranged pimaranes, isopimaranes (diterpene lactones) and kaurane diterpenoids comprise a group of the constituents of this plants. On biological activities of Euphurbiaceae family could be used as highly irritant, carcinogenic, tumour-promoting diterpenoids. Besides, some compounds have aroused interest because of their antitumor, antiwrithing, analgesic and phytotoxic activities. Particularly, the folkloric medicine of *G.multiflorum* in Thailand and India has been used as therapeutically for a long time. Dried seed of *G.multiflorum* from India is considered as a good tonic for gums and has been employed as a purgative in hepatic troubles[4]. In Thailand, many parts of *G.multiflorum* are supposed to treat several diseases[5], especially dried wood is used for cancer[6].

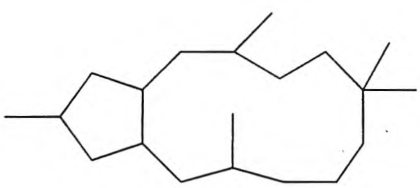
Thus, chemical constituents from *G.multiflorum* together with their pharmacological activities including clinical trials is still an important for further study.



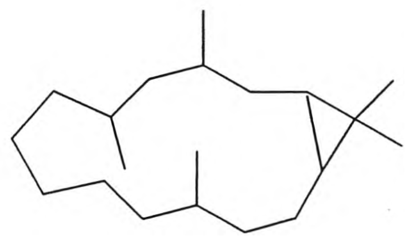
Lapdane



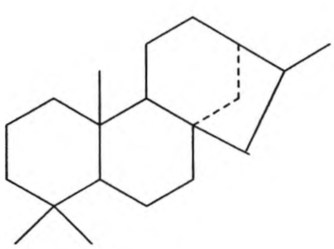
Clerodane



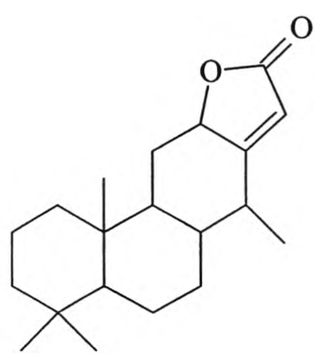
Jatrophane



Lathyrane



Kaurane



Diterpene lactone

Figure 1.1 The skeleton of some diterpenoids

1.1 Pharmacological Activities of *G.multiflorum*

The seed of *G.multiflorum* was extracted and isolated to afford "Gelonin[7]" which possessed a variety of intriguing.

Research in Gelonin from *G. multiflorum* was investigated and improved continuously because it gave the most interesting activities. It was found in strong protein synthesis inhibition to reticulocyte lysate[7]. The gelonin components were developed differently; M –195 –gelonin immunotoxin killed HL 60 cells[8], encoding gelonin inhibited protein synthesis *in vitro*[9,10] and lutropin-gelonin conjugate performed cytotoxic activity in mouse leyding tumor-cells-potentiation of the hormonotoxin activity by different drugs[11]. Besides, there were partial-purification of 2 proteins which sensitize ribosomes to gelonin-sensitization is not linked to phosphorylation of ribosomal-proteins[12]. Currently, the gelonin toxin is available for use in the construction of recombinant immunotoxins[13]. In addition, gelonin is more active in inhibiting endogenous protein synthesis by lysates of RSV-transformed or RAV-1-infected cells[14].

Interestingly, these results indicated that "Gelonin" possessed an important role as protein synthesis inhibitor.

Recently, GAP31 (Gelonium Anti-HIV protein 31 kDa), a protein isolated and purified to homogeneity from Euphorbiaceae himalaya seeds (*G.multiflorum)*,was found to inhibit HIV-1 infection and replication[15,16,17], suggesting that it might be a clinically important agent in the treatment of AIDS. Moreover, it also possessed anti-tumor activity against certain human tumor cell lines[18]. The ability of GAP31 to interrupt both DNA and RNA function may be related to its multiple antiviral actions[19]. The effect of GAP31 on the infection and replication of Herpes Simplex Virus (HSV) was studied. It might be useful for the therapy of herpesvirus infections[20].

Other biological activities from stems of *G. multiflorum* such as antipasmodic and hypotensive activities[21] as well as promising inhibition on tumor promoter-induced Epstein-Barr Virus (EBV) [22] were reported. In our laboratory, we discovered an attractive result of cytotoxicity against brine shrimp and antioxidant activity.

1.2 Chemical Constituents of *G.multiflorum*

Many parts of *G. multiflorum* have been reported to contain various kinds of natural compounds. Three triterpenes and several sugars were isolated from the barks extracted with petroleum ether and enzyme hemicellulase, respectively.[23, 24, 25] Earlier workers (1979) reported the presence of four fatty acids, caprylic acid, myristic acid, oleic acid and palmitic acid [26], from the seeds. Fourteen years latter, Binayak and Ajit isolated two new flavone diglycosides, gelomuloside A and gelomuloside B, and a known glycoside from n-butanol-soluble fraction[27] .The roots were extracted and isolated a new diterpene diol,ent-kaurene-3β-15β-diol.[28]

From the leaves of *G.multiflorum* , a new flavone glycoside was isolated and characterized as luteolin 7,4′-dimethyl ether 3′-glucoside by Nazneen and Nizam.[29] Moreover, Tarapatra et al. found ten diterpene lactones, gelomulide A,B,C,D,E,F,G,H,I and J, as minor constituents from this part and those structures were established on the basis of their ^{1}H NMR,^{13}C NMR and mass spectral evidence. Additionally, 2D NMR techniques and the X-ray crystallographic analysis were used to confirm the structure of gelomulide I and its absolute configuration.[30, 31, 32]

The chemical constituents of *G.multiflorum* were summarized in **Table 1.1** and **Figure 1.3.**

G. multiflorum was used as a folkloric medicine and found to contain such active components as gelonin and GAP31. *G. multiflorum* stems were of considerable interest since chemical constituents of this part have not been studied. So, it is an attractive reason for further investigation.

The target of this research can be summarized as follow. The stem extracts from *Gelonium multiflorum* A.Juss, especially extracts which have interesting activities, will be first separated by means of chromatography techniques, such as column chromatography (CC) and then purified by proper methods to afford chemical constituents. For structural elucidation all of them will be carried out by spectroscopy techniques. Finally, biological activities of the pure compounds will be investigated.

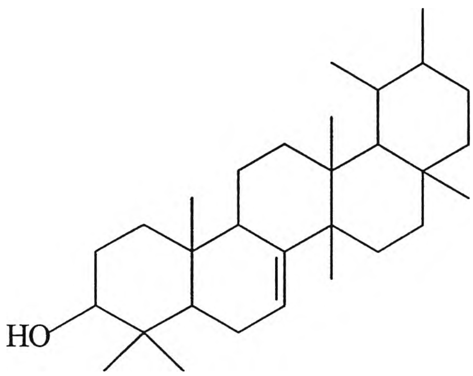
1.3 Botanical Aspect and Distribution

Gelonium multiflorum A.Juss or *Suregada multiflorum* Baill. in Thailand has been known as "Khanthongphayaabaat" or "Maak duuk"(central region) , "Khop naang nang" (Trang) , "Salotnam" (Chanthaburi) , "Khanthong" (Phichit) ;etc[33]. *G.multiflorum* is a shrubby tree. It is 4-15 metres high. Leaves are single leaf. The edges of leaves are smooth. Flowers have male and female in the different trees (diocious). Petals which are under the hypogynous have rather regular size, single level five merous. In the male flowers, the number of pollen is twice of the outer petals. On the other hand, the female flowers of the ovaries have three lobes, three cells and three stigmas. The fruit is round and fleshy. The plants in the Euphorbiaceae family are widely distributed over the earth and especially are abundant in tropical and sub-tropical parts of Asia and Africa. In Thailand, it is distributed generally in every region[5]. (**Figure1.2**)

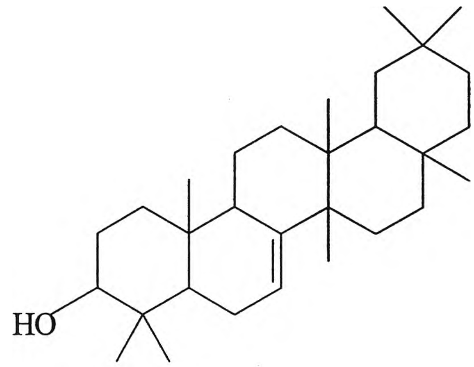
Figure 1.2 Flowers, fruits and leaves of *Gelonium multiflorum* A.Juss.

Table 1.1 Chemical constituents of *Gelonium multiflorum* A.Juss

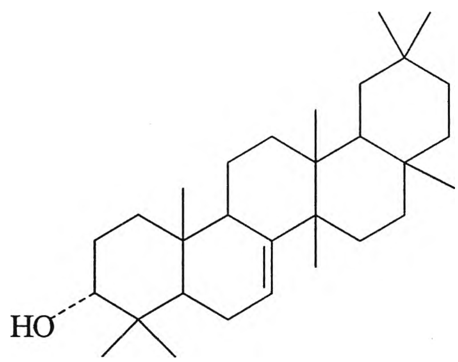
Year	Plant parts	Solvent extraction	Substances
1963	Bark	Benzene	bauerenol multiflorenol
1968	Bark	Enzyme hemicellulase, xylanase	glucose xylose arabinose
1969	Bark	Petroleum ether	bauerenol multiflorenol epimultiflorenol
1979	Seed	Methanol	caprylic acid myristic acid oleic acid palmitic acid
1987	Leaf	Methanol	luteolin-7,4′-dimethyl ether 3′-glucoside
1989	Leaf	Petrol,chloroform	gelomulides A,B,C,D,E,F multiflorenol jolkinolide B
1993	Seed	n-Butanol	7,4′-O-dimethylscutellarein-6-O-β-D-glucoside gelomuloside A gelomuloside B
1994	Root	Petrol	ent-kaurene-3β,15β-diol kanugin desmethoxy kanugin pinnatin
1998	Leaf	Petrol,chloroform	gelomulides B,D,E,G,H,I,J jolkinolide B bauerenol



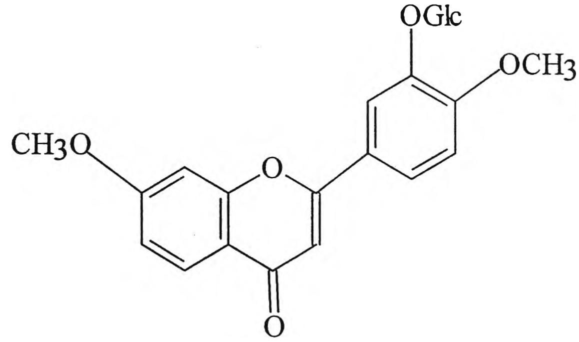
bauerenol



multiflorenol

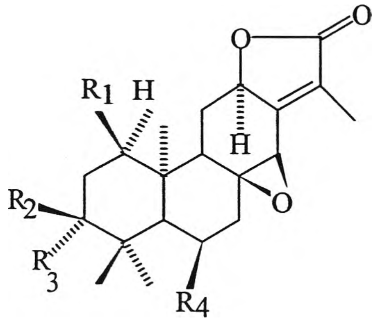


epimultiflorenol



luteolin 7,4′- dimethyl ether 3′- glucoside

Figure1.3 Compounds from *Gelonium multiflorum* A.Juss

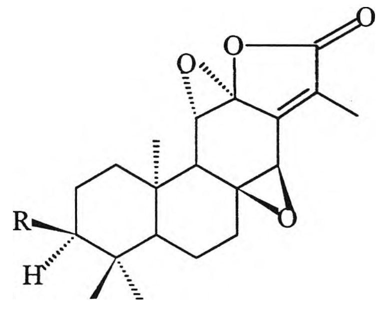


gelomulide A ($R_1 = R_3 = R_4 = H$, $R_2 =$ OAc)

gelomulide C ($R_1 = R_4 = H$, R_2,,$R_3 =$ O)

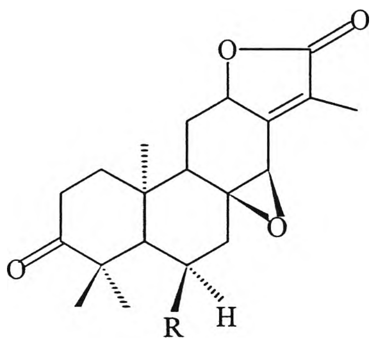
gelomulide F ($R_1 =$ OAc , R_2,$R_3 =$ O ,$R_4 = H$)

gelomulide G ($R_1 = R_3 = H$, $R_2 = R_4 =$ OAc)



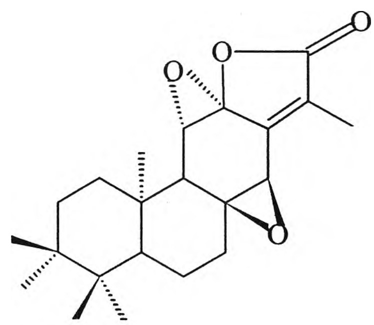
gelomulide B (R = OAc)

gelomulide J (R = OH)

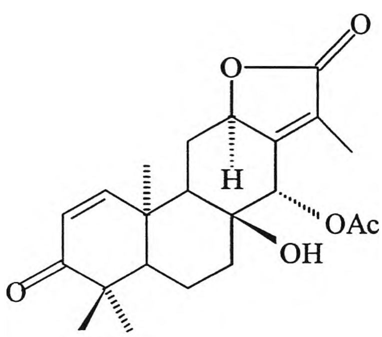


gelomulide D (R = H)

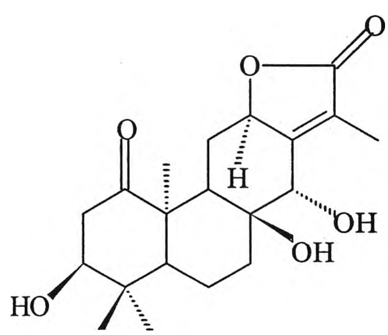
gelomulide E (R = OAc)



jolkinolide B

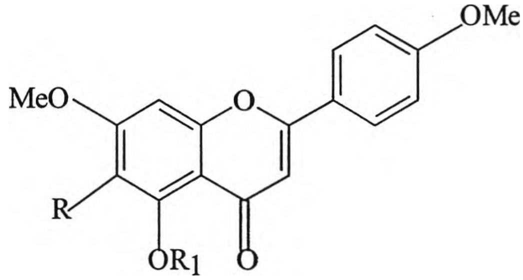


gelomulide H



gelomulide I

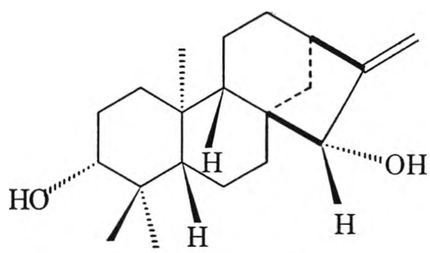
Figure 1.3 (Cont.) Compounds from *Gelonium multiflorum* A.Juss



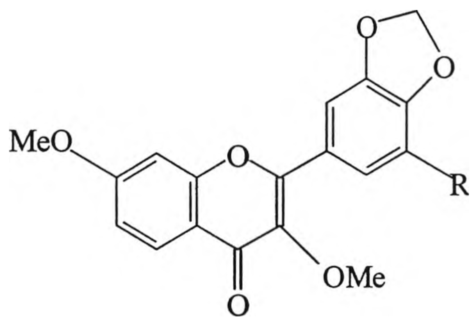
7,4′-O-dimethylscutellarein-6-O-β-D-glucoside (R = -Glc , R_1= H)

gelomuloside A (R = -Glc^2-Rha , R_1= H)

gelomuloside B (R = -Glc^2-Xyl , R_1= H)

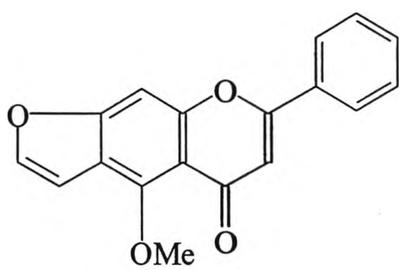


ent-kaurene-3β-15β-diol



kanugin (R = OMe)

desmethoxy kanugin (R = H)



pinnatin

Figure 1.3 (Cont.) Compounds from *Gelonium multiflorum*