CHAPTER I

INTRODUCTION

1.1 *Caesalpinia pulcherrima*

1.1.1 Botany of *Caesalpinia pulcherrima*

Common name	: Peacock flower, Barbados Pride, Dwarf Poinciana
Scienctific name	: *Caesalpinia pulcherrima* (Linn.) swartz
Synonym	: *Poinciana pulcherrima* (Linn.)
Family	: Fabaceae (Leguminosae) , subfamily Caesalpiniodae or Caesalpiniaceae

Barbados Pride or Dwarf Poinciana is related to the Flam boyant, and the generic name commorates the Italian philosopher, doctor and botanist Caesalpinus. The plant was placed earlier in the genus Poinciana, also known as "Flam boyant" in the tropics. It is one of the loveliest decorative shrubs in tropical gardens and originally came from the islands of the west Indies. This plant is a very beautiful and common free-flowering treclet with slightly throny stems, growing to a height of about 3 m. Besides the usual colour form, there are yellow– and red–flowered varieties. The young seeds can be eaten raw. It is a host plant of the common Grass Yellow Butterfly.[1,2] **(Fig. 1.1)**

Morphology[3]

Shrub or small tree, hieght 1-3 m, unarmed or nearly so, glabrous. *Stipules* a 2 mm, caducous. *Leaves* : rhachis 10–40 cm; pinnae 3–10 pairs; leaflets 6–12 pairs, opposite, petiolulate (1-2 mm), elliptic–oblong, 10–20 by 6–10 mm round–emarginate at the tip, unequal at the base. *Racemes* axillary and terminal. *Bracts* linear, 3–7 mm, caducous. *Pedicels* 3–7 cm. *Sepals* unequal, the lowest hooded larger. *Petals* red or yellow, up to 25 mm long, the standard smaller. *Stamens* exserted; filaments red, 5–6 cm, hairy in the basal part. *Ovary*10–12 ovulate; style 5–6 cm. *Pods* short stalked above the receptacle (2–5 mm), flattened, 7–12 by 1.5–2 cm. *Seeds* 8–10 mm. The morphology of *Caesalpinia pulcherrima* is shown in **Fig.1.2**[3]

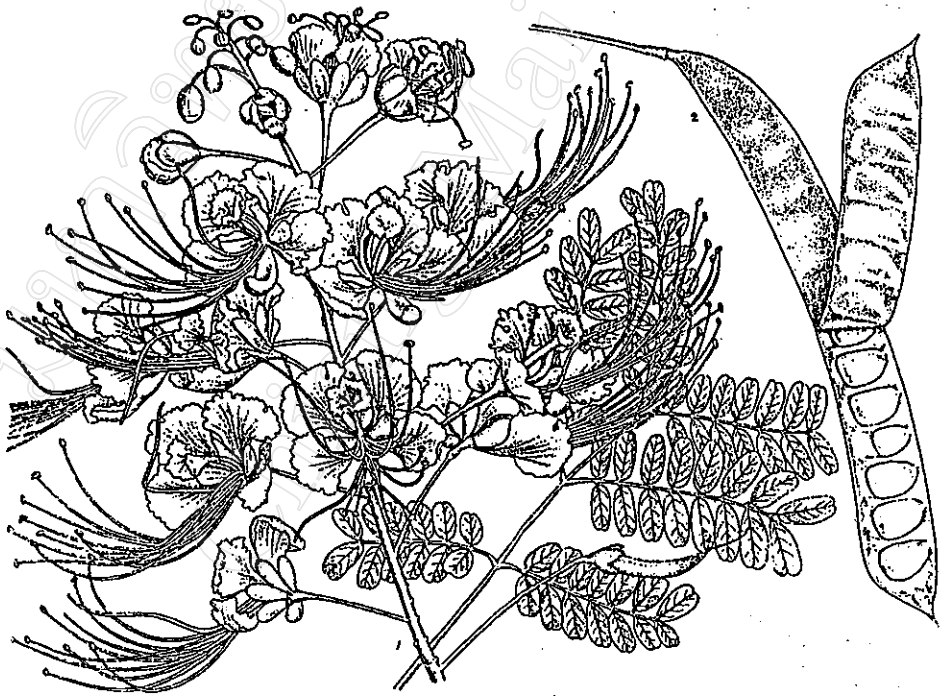


Figure 1.1 *Caesalpinia pulcherrima* (Jain, S.K. and DeFilipps, R.A., *Medicinal Plants of India*, Reference Publications, Michigan, United states of America, 1991, 211.)[4]

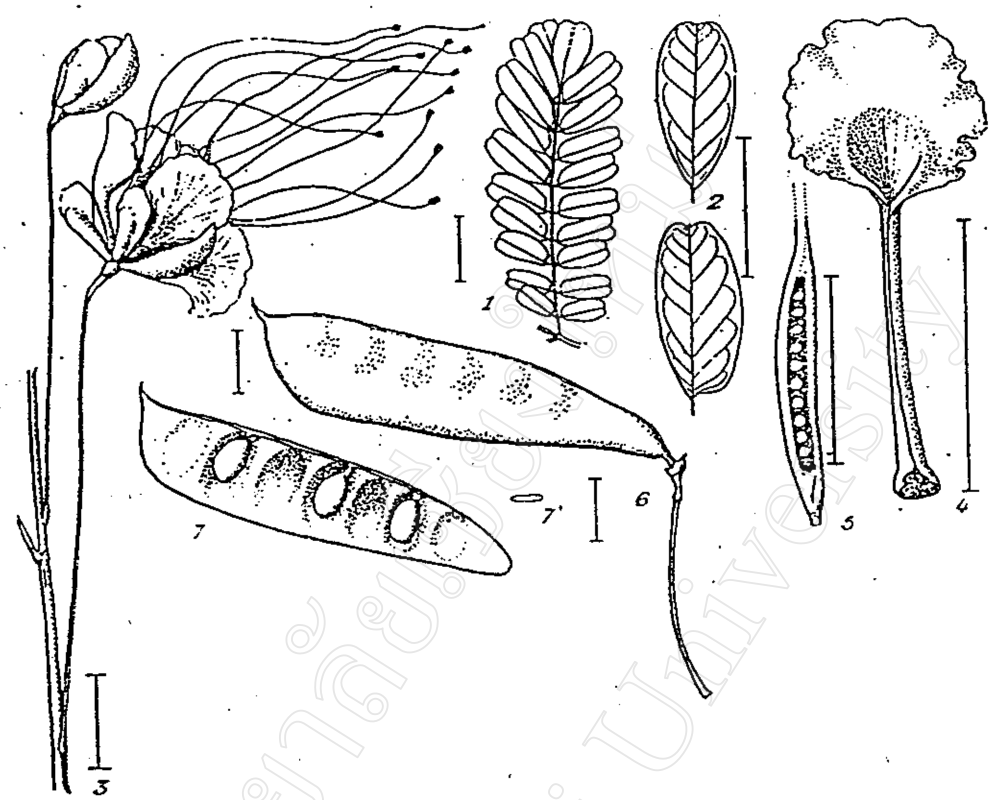


Figure 1.2 Morphology of *Caesalpinia pulcherrima* (The forest Herbarium, Royal Forest Department, *Flora of Thailand (part one; Legumonosae-caesalpinioideae)*, Bangkok, Thailand, 1984, 66.)[3]

Distribution[3]

The *Caesalpinia pulcherrima* is probably from south America and is widely cultivated through-out the tropics. In Thailand, it is widely cultivated as ornamental and medicinal.

Other Names[4]

Hindi	: Guletura
Andhra Pradesh	: Ratnagandhi
English	: Pride–of–Barbados, Peacock flower, Paradise flower
Bengal	: Krishnachura

Gujrat : Sandhesharo

Kerala : Set–Timandaram

Tamilnadu : Mayuram

Caesalpinia genus are climbers or shrubs or small trees, usually prickly. The large genus *Caesalpinia* (about 200 species) is pantropical, the greater part of the species occurring in south and central America, and about 30 species in Asia. Many species are known in Thailand. There are various Thai names of each species[3], as shown below;

- *Caesalpinia pulcherrima* (Linn.) swartz

  Nok Yung Thai, Hang Nok Yung Thai (General); Champho, Sompho, Sumpho (Northern); Khwang Yoi (Eastern); Nuat Maeo (Shan/Northern)

- *Caesalpinia sappan* (Linn.) (Sappan wood)

  Fang (General), Khwang (South–western); Nam Khong (Northern); Ngai (Karen /South–western)

- *Caesalpinia decapetala* (Roth.)

  Kam Chai (Central)

- *Caesalpinia mimosoides* (Lamk.)

  Phak Pu Ya; Nam Pu Ya (Northern); Phak Kha Ya (North-eastern); Phak Kat Ya (South–eastern); Cha Rueat (Peninsular)

- *Caesalpinia godefroyana* (O.) kuntze

  Fang Pik Kai, Fang Ka, Phong Ka (Eastern); Nam Huen Nam Han (South-western); Thao Ranap Nam (Central)

- *Caesalpinia parviflora* (Prain.)

  Wan Thalueng, Wua Thalueng (Eastern)

- *Caesalpinia orista* (Linn.)

  The Phi (Peninsular)

- *Caesalpinia bonduc* (Linn.)

  Ba Khi Haet, Ma Kaleng (Northern); Sawat (Central); Damat (Malay /Peninsular)

- *Caesalpinia major* (Medik.)

  Wat, Wiat, Kamchay (Peninsular)

- *Caesalpinia minax* (Hance.)

  Kham Phi Paeng, Khi Haet, Ma Dam (Northern)

- *Caesalpinia digyna* Rottler

  Kamchai (General); Nam Chai, Ba Ben, Khi Khak, Ma Nam Chai (Northern); Nam Han, Nam Daeng, Khi Raet (South-western); Ching Chai, Ngai, Hai, Hai Pun (Peninsular); Talumae, Suekipho (Karen/Northern)

- *Caesalpinia pubescens* (Desf.) Hattink

  Kadae (Peninsular)

- *Caesalpinia enneaphylla* (Roxb.)

  Khi Raet Yai, Kam Chai, Nam Chai (South-western)

- *Caesalpinia hymenocarpa* (Prain.) Hattink

  Nam Kong, Nam Chan (Northern); Nam Bang, Nam Chai (South-eastern)

- *Caesalpinia andamanica* (Prain.) Hattink

  Ngai Daeng, Ngai Yai, Sawat (Peninsular)

- *Caesalpinia furfuraceae* (Prain.) Hattink

  Nuam, Nguam, Pha Yuam, Nam Kong (Northern)

- *Caesalpinia cucullata* (Roxb.)

  Kam Chai, Nam Chan (Northern); Nam Kha (Northern-easern); Wan Thalueng Yai (Eastern); Nam Khang, Nam Chai (Central); Khi Raet (South-western)

1.1.2 Medicinal properties of *Caesalpinia pulcherrima*[1,4]

This plant, *Caesalpinia pulcherrima* has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes ;

stem and bark: an abortifacient, and ammenagogue

leaf : a stimulant and emmenagogue

flower : remedy fevers, bronchitis and asthma; pectoral, febrifuge

The flowers and leaves are supposed to be able to reduce fever by infusion, and in addition the leaves have purgative properties.

And *Caesalpinia pulcherrima* is known as medicinal in various regions[5], for example;

Taiwan : The flowers, leaves, and seeds are fabrifuge, stomachic, and diuretic.

Indo-China : It is said that the root may be posionous, it is regarded as astringent And anticholeric. The leaves, wood, and bark seem to be emmenagogue, and perhaps the leaves are abortient.

Indonesia : The leaves of yellow-flowered tree used to poultice a distended stomach, the bark of red-flowered tree is mentioned as remedy for diarrhea and the flowers are part of a mixture given to children subject to convulsions.

Philippines : The Filipions also know therapeutic use of the plant.

1.1.3 Review of Chemical Constituents of *Caesalpinia* genus

The chemical constituents revealed several types of compounds presented in plants of *Caesalpinia* genus. These could be classified into three main groups of chemical constituents as follow;

a. Terpenoids

As the structures of this group of compounds were elucidated, it became apparent that many of them could be regarded as being built up of isoprene or iso-pentene units linked together in various ways and with different types of ring closures, degrees of unsaturation, and functional groups.[6] Several types of terpenoids have been found in abundance in *Caesalpinaceae* plants, including diterpenoids, triterpenoids and sterols.

Diterpenoids

The diterpenoids are C_{20} compounds which may be formally regarded (with some exceptions) as a structure derived from four isoprenoid residues.[6] The diterpenoids could be classified as acyclic, monocyclic, bicyclic and tricyclic diterpenoids.[7] A huge number of new diterpenoids have been isolated from the genus. Cassane furanoditerpenoids, a type of diterpenoids, are usually distributed in *Caesalpinia* genus; *Caesalpinia minax*, *Caesalpinia bonduc, Caesalpinia major, Caesalpinia sappan, Caesalpinia pulcherrima, etc.* Some of which displayed interesting biological activity. The molecular skeleton of this kind of diterpenoid is constructed from the fusion of three cyclohexane rings and a furan ring.

In addition, most of furanoditerpenoids from the genus possess a C-5 hydroxyl group. The use of one- and two-dimensional NMR techniques, in particular, has helped elucidate the structure of new cassane-type diterpenoids effectively. The review of diterpenoids from *Caesalpinia* genus is shown in **Table 1.1**

Table 1.1 Diterpenoids from *Caesalpinia* genus

Plant	Plant part	Compounds	References
Caesalpinia pulcherrima (Linn.) swartz	bark	x-caesalpin (**1**)	8
	stem	pulcherrapin (**2**)	9
	root	vouacapen-5α-ol (**3**)	10
		6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol (**4**)	10
		8,9,11,14-didehydrovouacapen-5α-ol (**5**)	10
		pulcherrimin A (**6**)	11
		pulcherrimin B (**7**)	11
		pulcherrimin C (**8**)	11
		pulcherrimin D (**9**)	11
Caesalpinia bonduc or *Caesalpinia bounducella*	seed	α-caesalpin (**10**)	12-14
		β-caesalpin (**11**)	12-14
		δ-caesalpin (**12**)	12-14
		γ-caesalpin (**13**)	12
		ε-caesalpin (**14**)	15
		neocaesalpin A (**15**)	16
		neocaesalpin B (**16**)	17
		neocaesalpin C (**17**)	17
Caesalpinia bonduc	seed	neocaesalpin D (**18**)	17
	seed kernel	ζ-caesalpin (**19**)	18
	root	caesalpinin (**20**)	19

Table 1.1 Diterpenoids from *Caesalpinia* genus (cont.)

Plant	Plant part	Compounds	References
Caesalpinia bonduc	root	bounducellpin A (**21**)	20
		bounducellpin B (**22**)	20
		bounducellpin C (**23**)	20
		bounducellpin D (**24**)	20
		caesaldekarin C (**25**)	21
		caesaldekarin F (**26**)	21
		caesaldekarin G (**27**)	21
		caesaldekarin A (**28**)	22
		caesaldekarin H (**29**)	22
		demethylcaesaldekarin C (**30**)	22
		caesaldekarin I (**31**)	22
		caesaldekarin J (**32**)	22
		caesaldekarin K (**33**)	22
		caesaldekarin L (**34**)	22
Caesalpinia major	seed kernel	14-deoxy-ε-caesalpin (**35**)	23
DANDY		caesaldekarin A (**28**)	24
		caesaldekarin B (**36**)	24
		caesaldekarin C (**25**)	25
		caesaldekarin D (**37**)	25
		caesaldekarin E (**38**)	25
Caesalpinia minax	seed	caesalmin A (**39**)	26
Hance.		caesalmin B (**40**)	26

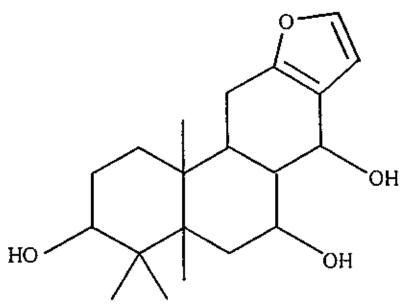
Table 1.1 Diterpenoids from *Caesalpinia* genus (cont.)

Plant	Plant part	Compounds	References
Caesalpinia minax Hance.	seed	caesalmin C (**41**)	27
		caesalmin D (**42**)	27
		caesalmin E (**43**)	27
		caesalmin F (**44**)	27
		caesalmin G (**45**)	27
Caesalpinia decapetala var. japonica or *Caesalpinia japonica*	root	caesaljapin (**46**)	28

Triterpenoids and sterols

There is a great increase in complexity on going from the diterprnoids to the C_{30} triterpenoids. Several tetracyclic triterpenoids are known, although the most important and widely distributed triterpenoids are pentacyclic. The steroid nucleus is the same as tetracyclic triterpenoids, the different is only some positions of methyl groups attached to the ring system. Most of triterpenoids and sterols that have been found in *Caesalpinia* plants are well known compounds such as;

- stigmasterol (from seed of *Caesalpinia minax*)[27]
- lup-20-(29)-en-3β-ol (from root of *Caesalpinia decapetala*)[28]
- campesterol, stigmasterol and β-sitosterol (from heartwood of *Caesalpinia sappan*) [29]
- viz-sitosterol (from stem bark of *Caesalpinia pulcherrima*) [30]



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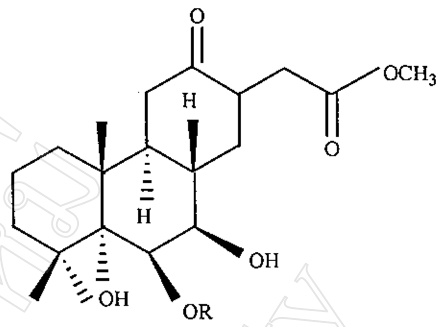
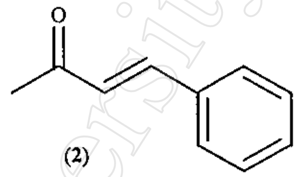


R = 

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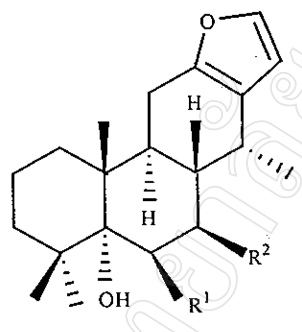
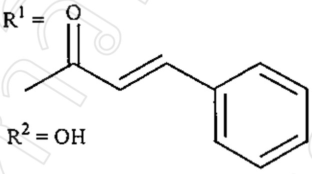
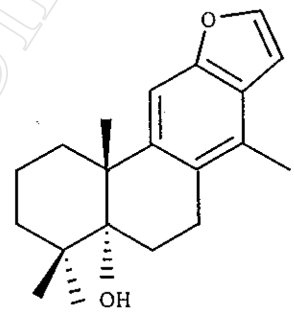


(3) $R^1 = R^2 = H$

(4) R^1 = 

$R^2 = OH$



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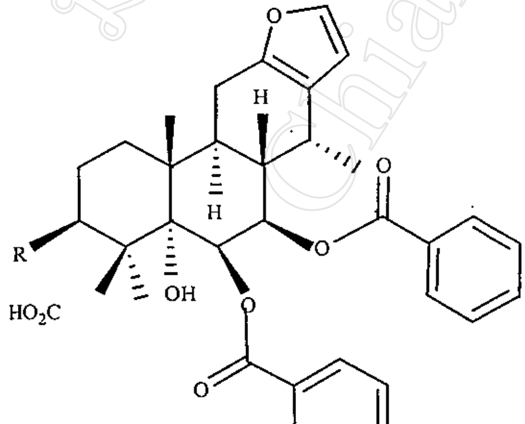
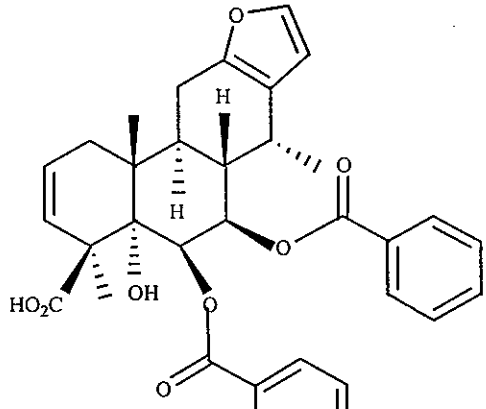


(6) R = OH

(7) R = H

(8) R = CH_3CO_2



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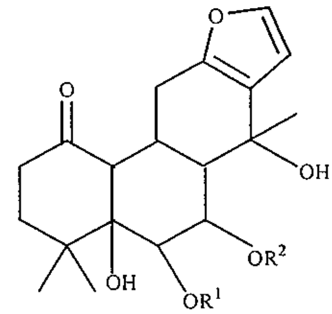
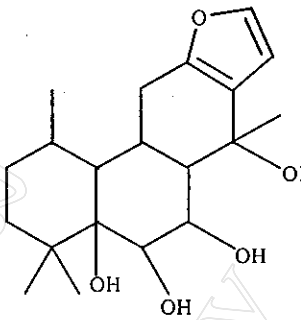


(10) $R^1 = R^2 = Ac$
(11) $R^1 = R^2 = H$



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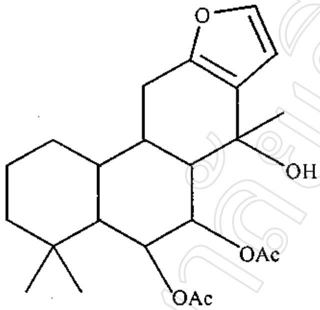

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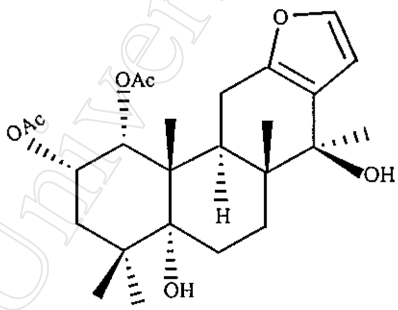

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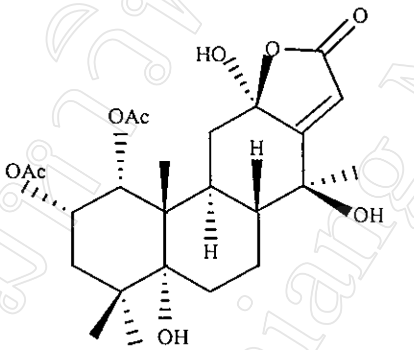

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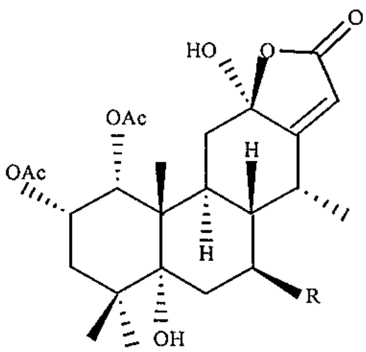
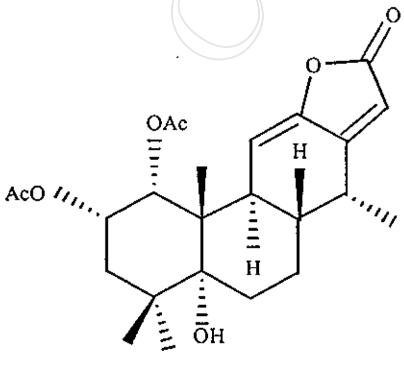


(16) R = H
(17) R = OH



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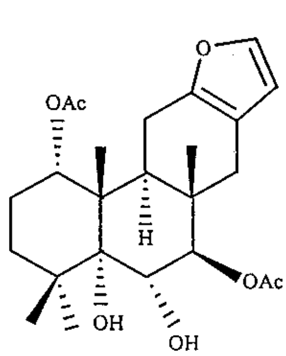

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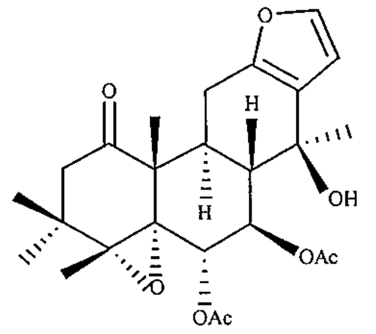

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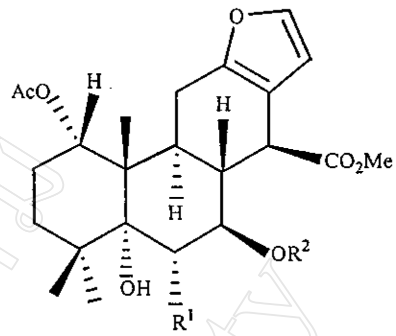
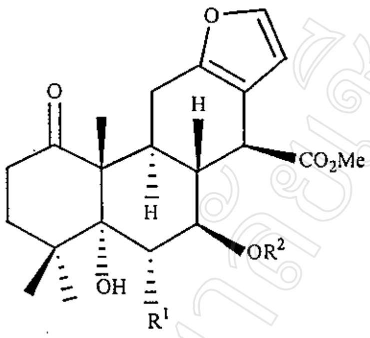
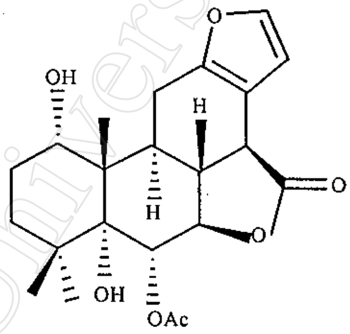


(21) R^1 = OAC, R^2 = H

(23) $R^1 = R^2$ = H



(22) R^1 = OAC, R^2 = H



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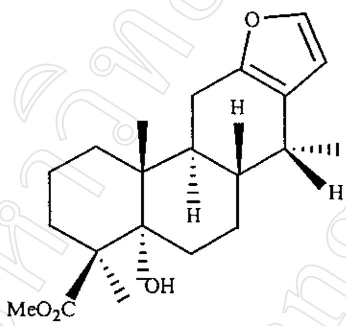

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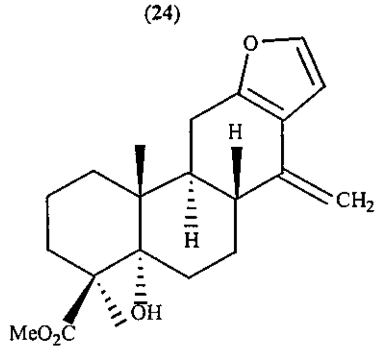

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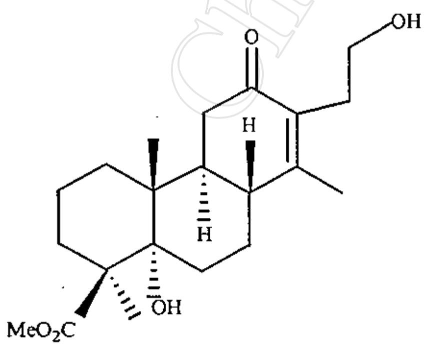

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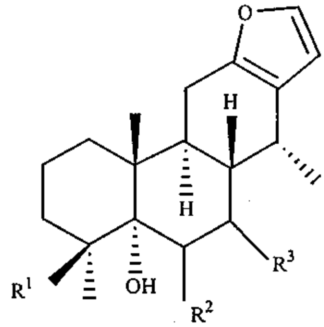
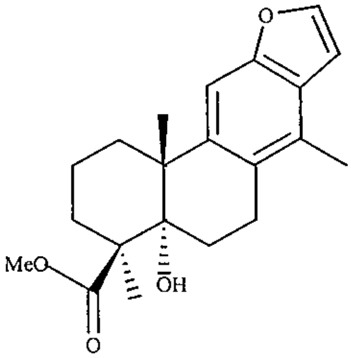


(28) R^1 = Me, R^2 = β-OAc, R^3 = H

(29) R^1 = CH_2OAc, R^2, R^3 = H

(30) R^1 = COOH, R^2, R^3 = H

(31) R^1 = CH_2OH, R^2 = H, R^3 = β-OH



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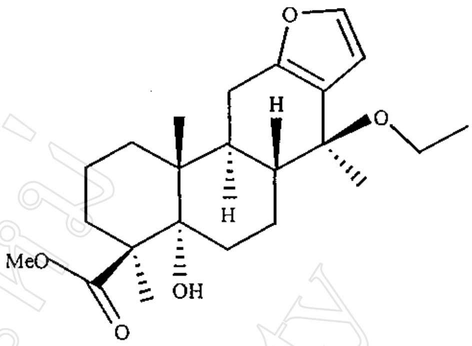

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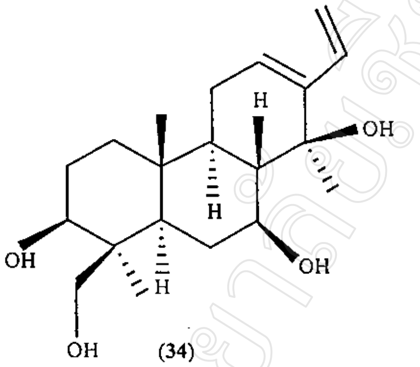

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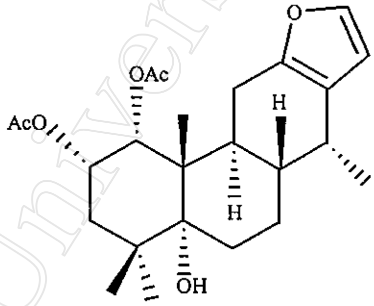

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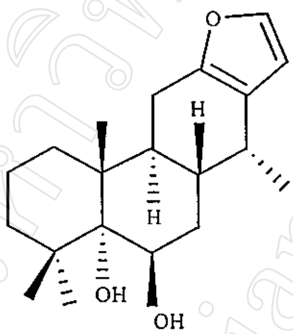

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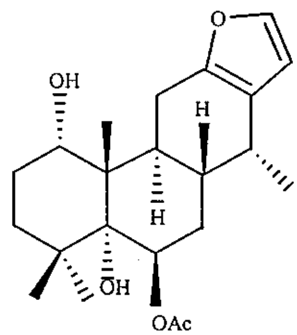

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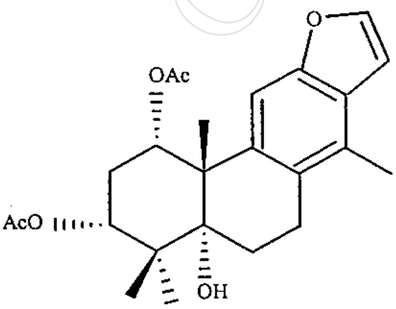

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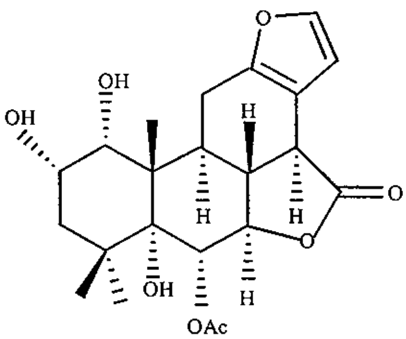

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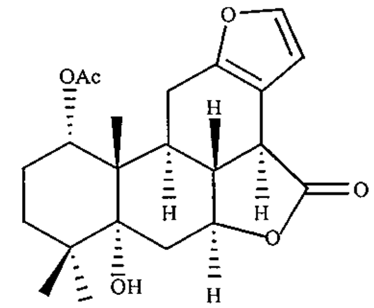

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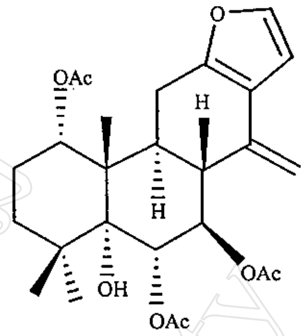

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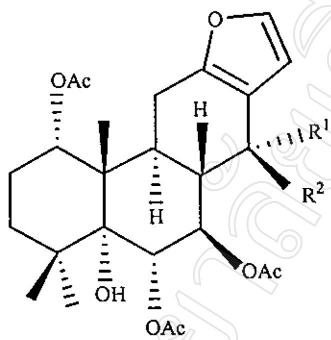
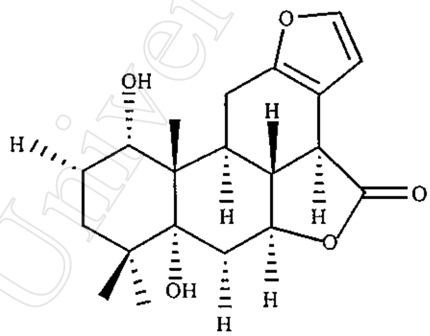


(42) $R^1 = OH, R^2 = Me$
(43) $R^1 = Me, R^2 = OH$
(44) $R^1 = Me, R^2 = OMe$



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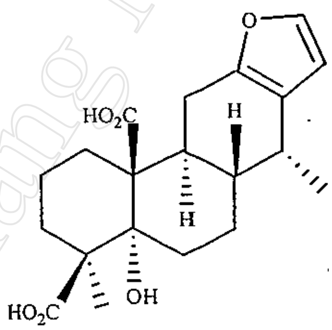
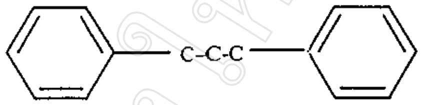


(46)

b. Flavonoids

The flavonoids group may be described as a series of C_6-C_3-C_6 compounds, *i.e.* their carbon skeleton consists of two C_6 groups (substituted benzene rings) connected by three-carbon aliphatic chain ;



The flavonoids have been classified by their molecular skeletons in several types; flavones, isoflavones, flavonols, flavanones, flavanonols, leucoanthocyanins, anthocyanins, catechins, chalcones, dihydrochalcones, aurones and xanthones. *Caesalpinia* plants, especially *Caesalpinia sappan,* are rich sources of these homoisoflavonoids and chalcones. The homoisoflavonoids, whose main skeleton consists of sixteen carbon, differ from that of fifteen carbon in the isoflavonoidal skeleton. The review of flavonoids from *Caesalpinia* genus is shown in **Table 1.2.**

Table 1.2 Flavonoids from *Caesalpinia* genus

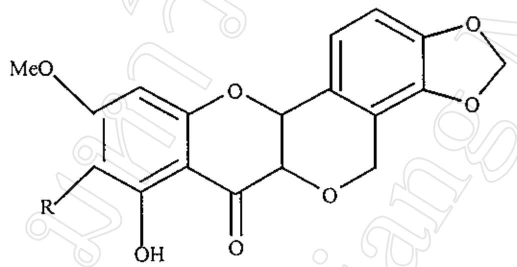
Plant	Plant part	Compounds	References
Caesalpinia pulcherrima	stem	bonducellin (**47**)	31
		8-methoxybonducellin (**48**)	31
		pulcherrimin (**49**)	31
		6-methoxypulcherrimin (**50**)	31
Caesalpinia sappan	heartwood	7-hydroxy-3-(4′-hydroxybenzyl-idene)-chroman-4-one (**51**)	32
		3,7-dihydroxy-3-(4′-hydroxy benzyl)-chroman-4-one (**52**)	32

Table 1.2 Flavonoids from *Caesalpinia* genus (cont.)

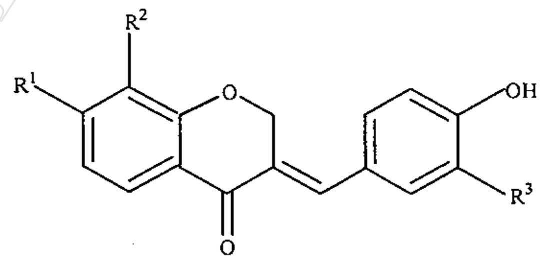
Plant	Plant part	Compounds	References
Caesalpinia sappan	heartwood	3,4,7-trihydroxy-3-(4′-hydroxy benzyl)-chroman (**53**)	32
		8-methoxybonducellin (**48**)	32
		sappanol (**54**)	33
		episappanol (**55**)	33
		3′-deoxysappanol (**56**)	33
		3′-*O*-methylsappanol (**57**)	33
		3′-*O*-methylepisappanol (**58**)	33
		4-*O*-methylsappanol (**59**)	34
		4-*O*-methylepisappanol (**60**)	34
		sappanone B (**61**)	34
		3-deoxysappanone B (**62**)	34
		3′-deoxysappanone B (**63**)	34
		sappanone A (**64**)	35
Caesalpinia japonica sieb *et* Zucc	wood	3′-deoxy-4-*O*-methylsappanol or (3,7-dihydroxy-3-(4-hydroxy benzyl)-4-methoxychroman (**65**)	36
		sappanol (**54**)	36
		episappanol (**55**)	36
		4-*O*-methylsappanol (**59**)	36
		4-*O*-methylepisappanol (**60**)	36

Table 1.2 Flavonoids from *Caesalpinia* genus (cont.)

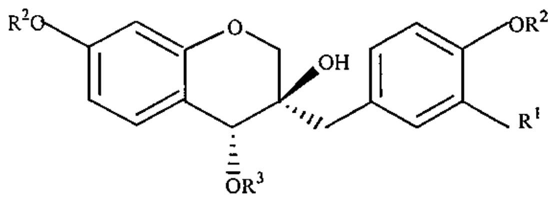
Plant	Plant part	Compounds	References
Caesalpinia japonica sieb *et* Zucc	wood	sappanone A (**64**)	36
		sappanone B (**61**)	36
Caesalpinia pulcherrima	stem	4′-methylisoliquiritigenin (**66**)	31
Caesalpinia sappan	heartwood	4,4′-dihydroxy-2′-methoxy-chalcone (**67**)	32
Caesalpinia japonica sieb *et* Zucc	wood	3-deoxysappanochalcone (**68**)	36, 28
		sappanchalcone (**69**)	36, 28
		isoliquiritigenin (**70**)	36, 28
		butein (**71**)	36, 28



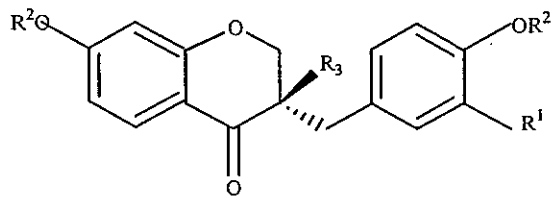
(**49**) R = H
(**50**) R = OMe



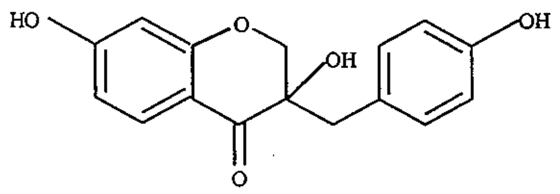
(**47**) R^1 = OMe, R^2 = H, R^3 = H
(**48**) R^1, R^2 = OMe, R^3 = H
(**51**) R^1 = OH, R^2 = H, R^3 = H
(**64**) R^1 = OH, R^2 = H, R^3 = OH



(**55**) R^1 = OH, R^2,R^3 = H
(**58**) R^1 = OMe, R^2,R^3 = H
(**60**) R^1 = OH, R^2 = H, R^3 = Me



(**61**) R^1,R^3 = OH, R^3 = H
(**62**) R^1 = OH, R^2,R^3 = H
(**63**) R^1 = OH, R^2 = Me, R^3 = OH



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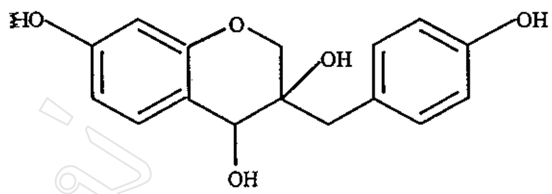

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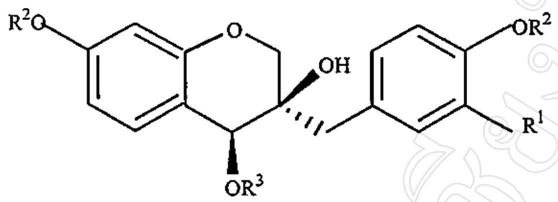
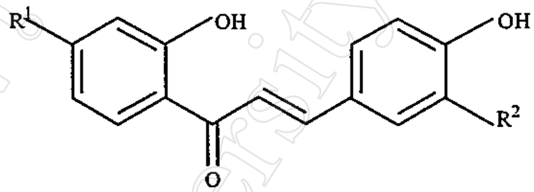


(54) R^1 = OH, R^1,R^3 = H, R^4 = OH
(56) R^1 = R^2 = R^3 = H, R^4 = OH
(57) R^1 = OMe, R^2,R^3 = H, R^4 = OH
(59) R^1 = OH, R^2 = H, R^3 = Me, R^4 = OH
(65) R^1 = H, R^2 = Me, R^3 = H, R^4 = H



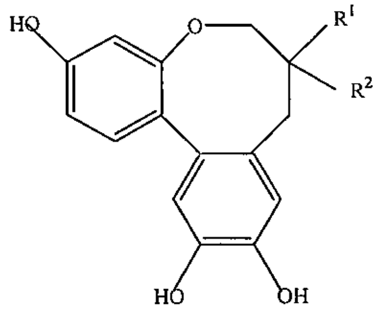
(66) R^1 = OMe, R^2 = H
(67) R^1 = OMe, R^2 = H
(68) R^1 = OMe, R^2 = H
(69) R^1 = OMe, R^2 = OH
(70) R^1 = OH, R^2 = H
(71) R^1 = OH, R^2 = OH

c. Miscellaneous Compounds

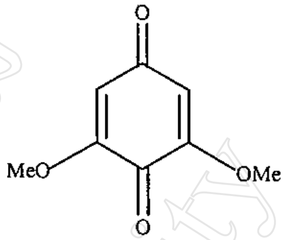
Caesalpinia plants also contain miscellaneous compounds from several parts of plant such as dibenzoxocin derivatives **(72-76)** , brazillins and their derivatives **(78-82)**. In addition, there are quinone **(77)**, elligitannin and some acids in stem of *Caesalpinia pulcherrima.* Aromatic **(83-85)** and lactone **(86)** compounds have been isolated from heartwood of *Caesalpinia sappan.* The review of this group is showed in **Table 1.3.**

Table 1.3 Miscellaneous Compounds from *Caesalpinia* genus

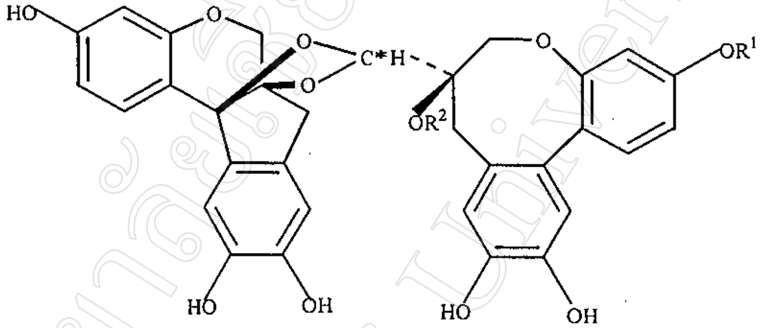
Plant	Plant part	Compounds	References
Caesalpinia japonica sieb *et* Zucc	wood	protosapanin A (**72**)	37
		protosapanin B or 7,8-dihydro-10-methoxy-3,7,11- trihydroxy-6H-dibenz[b,d]-oxocin-7- methanol(**73**)	38
		protosapanin C (**74**)	38
		protosappan E-1 (**75**)	37, 38, 29
		protosappan E-2 (**76**)	37, 38, 29
Caesalpinia pulcherrima	stem	2,6-dimethoxybenzoquinone (**77**)	31
		ellagitannins	30
		sebacic acid	30
		quercimeritrin	30
		prodelphindin	30
		gallic acid	30
		ellagic acid	30
Caesalpinia sappan	heartwood	brazillin (**78**)	35, 36, 29
		brazillin derivatives (**79-82**)	35,40
		brazilein (**83**)	29, 41
		caesalpin J (**84**)	42, 43
Caesalpinia sappan	heartwood	caesalpin P (**85**)	42
		brazilide (**86**)	44
		quercetin	32
		rhamnetin	32



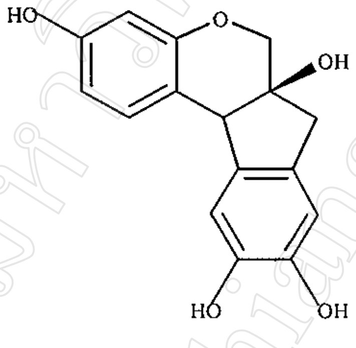
(72) $R^1, R^2 = O$
(73) $R^1 = CH_2OH, R^2 = OH$
(74) $R^1 = CHO, R^2 = OH$



(77)



(75) $R^1, R^2 = H$ (*S-configuration)
(76) $R^1, R^2 = H$ (*R-configuration)



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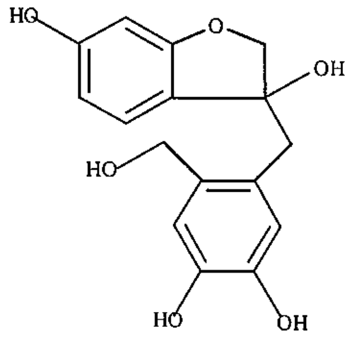

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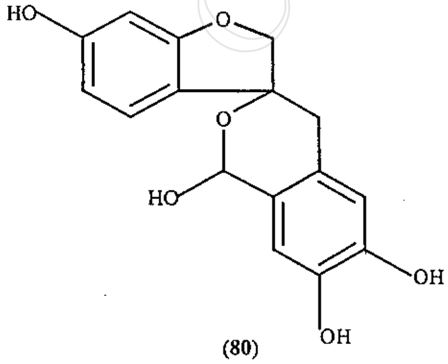

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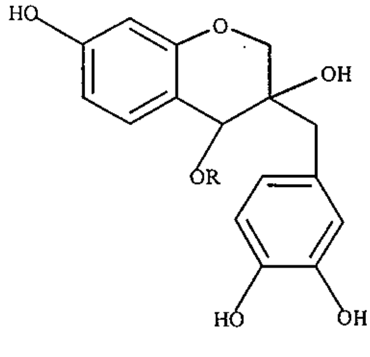
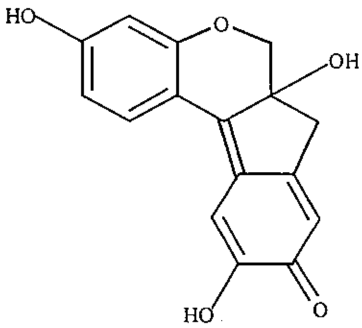


(81) R = H
(82) R = Me



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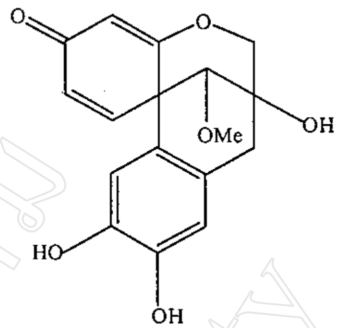

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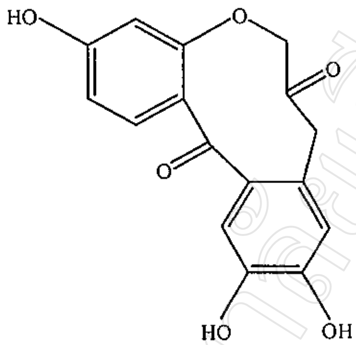

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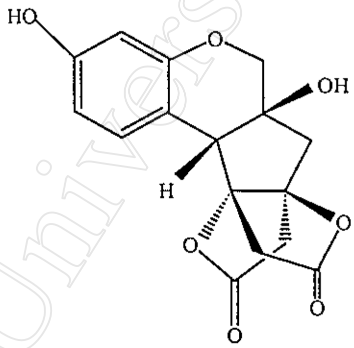

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1.1. 4 Review of Bioactive Compounds from *Caesalpinia* genus

According to earlier studies of the chemical constituents, it was found that some of which displayed biological activity. There were various types of bioactive compounds that isolated from *Caesalpinia* plants as shown below;

- **DNA repair-deficient yeast mutant Compounds** [11]

In 1997, Patil *et al.* reported isolation and structure determination of four novel dibenzoate diterpenes, pulcherrimins A,B,C and D (**6-9**) from MeOH/CH_2Cl_2 extract of the root of *Caesalpinia pulcherrima*. Pulcherrimins A and B (**6,7**) were found to be active as DNA repair-deficient yeast mutant.

- **Antiviral Compounds** [27]

In 2001, Jiang *et al.* has reported the finding of antiviral agents from seed of *Caesalpinia minax* Hance. The study had led to the isolation of five new cassane furanoditerpenoids, namely, caesalmins C (**41**), D (**42**), E (**43**), F (**44**) and G (**45**), along with stigmasterol. All of furanoditerpenoids (**41-45**) showed significant *in vitro* activity against the Para 3 (Parainfluenza virus type 3), with IC_{50} values ranging between 7.8 and 14.8 μg/ml Activity of caesalmins C, D, E and F, tetracyclic furanoditerpenoids, was better than the furanoditerpenoid lactones, caesalmins G, whereas stigmasterol showed only moderate activity.

- **Cytotoxic Compounds**

In 1983[31], McPherson *et al.* had isolated two new peltogynoids, pulcherrimin (**49**) and 6-methoxypulcherrimin (**50**) and two homoisoflavonoids, bonducellin (**47**) and a new 8-methoxybonducellin (**48**) from stem of *Caesalpinia pulcherrima*. In addition, two known compounds, 2,6-dimethoxybenzoquinone (**77**) and 4′-methylisoliquiritigenin (**66**) were isolated and were found to be cytotoxic in the KB test system *in vitro*. (ED_{50} of 2.8 and 3.2 μg/ml, respectively)

In 1986[10], three new furanoditerpenoids; vouacapen-5α-ol (**3**), 6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol (**4**) and 8,9,11,14-didehydrovouacapen-5α-ol (**5**) were isolated from the same source. It was found that only one of which namely 6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol (**4**) possessed cytotoxic activity. This compound displayed ED_{50} values of 1.8 and 3.5 μg/ml in the KB and P-388 *in vitro* test systems, respectively.

- **Antipercholesteremic Active Compounds** [40]

In 1985, Fuke *et al.* reported the isolation of two new aromatic compounds, which were structurally related to brazillin (**78**), from methanol extract of heartwood of *Caesalpinia sappan*. It was found that both compounds were effective for hypercholesteremia.

- **Anticomplementary Active Compounds** [29]

In 1998, Oh *et al.* has presented the anticomplementary activity of constituents from the heartwood of *Caesalpinia sappan*. The sterol mixture (campeaterol 11.2%, stigmaterol 18.9% and β-sitosterol 69.9%) showed strong activity with IC_{50} 0.7 ± 0.02 μg/ml of hemolysis on the classical pathway. Brazillien (**83**), brazillin (**78**), protosappanins A (**72**), B(**73**) and E(**74-75**) from the same source also displayed activity. The anticomplementary activity of protosappanin E (IC_{50} 88 ± 6 μM) was most potent followed by brazilien (IC_{50} 279 ± 5 μM), brazilin (IC_{50} 863 ± 57 μM), protosappanin B (IC_{50} 1351 ± 135 μM) and protosappanin A (IC_{50} > 2 mM).

1.2 *Acronychia pedunculata*

Acronychia comprises 47 species occurring from Sri Lanka and India to Indo-China, south-western China, Thailand, the whole of the Malesian archipelago, east to the Solomon Islands, New Caledonia and Loard Howe Island, and south to eastern and southern Australia. The wood of *Acronychia* is used for house building, utility furniture, flooring, lining, pan-elling, mouldings, turnery, carving and tool handles. It also produces a good quality charcoal and has been used as firewood.[45]

1.2.1 Botany of *Acronychia pedunculata*

Scienetific name : *Acronychia pedunculata* (Linn.) Miq.

Symnonym : *Acronychia arborea* Blume,
Acronychia laurifolia Blume,
Acronychia resinosa J.R. Forster ex crevost & Lemarie

Family : Rutaceae

Morphology[46]

A small tree, with pale smooth bark; younger branchlets glabrous to finely puberulent. *Leaves* simple; petiole up to 5 cm long; leaf-blade elliptic to suboblong or slightly obovate, at base usually cuneate, at apex obtusely acuminate; lateral nerves 3-7 pairs; texture thin-coriaceous; blades 3.5-24 cm long, 2-8 cm wide. *Inflorescences* mostly 4-24 cm, axes glabrous or nearly so. *Flowers* greenish-white, mostly 8-11 (rarely to 13) mm long, occasionally smaller, on glabrous or finely puberulent pedicels up to 12 mm long. *Sepals* deltoid, about 1 mm long. *Petals* usually pubescent within. Disc 1-2 mm wide, glabrous or nearly so. *Ovary* densely pubescent, hairs pale or tawny; style pubescent only at base. *Friuts* cream to tawny, drying brown, subglobose to angularly subconic, rarely somewhat 4-lobed, commonly

6-12 mm broad, the apex short apiculate. *Endocarp* thin, corneous. *Seeds* 3-7 mm long. **(Fig.1.3)**

Figure. 1.3 *Acronychia pedunculata* (World Health Oraganization Regional office for the Western pacific and Institute of Materia Medica, *Medicinal Plants in Viet Nam*, series No.3, Viet Nam, 1990, 16.)[46]

Vernacular Names [45,47]

English : Clawflowered Laurel

Indonesia : Jejerukan (sun-danese), Kayu semidra, Sariah (Javanese)

Malaysia : Ketiak, Memali, Tentgkorak Biawak (Peninsular)

Philippines : Uto (Fillipino)

Cambodia : Kramol, Panol

Loas : Cavi, Mak Thao Sang

Viet Nam : Bung, Dai

Thailand : Ka Uam (Northern), south-western, Kra Bueang Thuai (Central), Yaa Krong (Peninsular)

Distribution[45,47]

Acronychia peunculata grows wild in the midlands and the mountainous regions from Sri Lanka and India to Nepal, Burma (Myanmar), Indo-china, southen China, Taiwan and Thailand towards Peninsular Malaysia, Sumatra, Java, Borneo, the Philipines, sulawesi (Kabaena Island) and papua New Guinea (Western District).

Ecology[47]

A common tree is cheifly in the moist region which come from sea-level up to 1600 m Flowering occurs mostly in the February to April.

Part used[46]

The roots, twigs, stem bark and leaves are collected throughout the year. The plants are pulled up, stripped of rootlets, carefully washed and sliced. Selected leaves, not worm-eaten or withered, are sun-dried or heat-dried. The stem bark is used only externally.

1.2.2 Medicinal Properties of *Acronychia pedunculata*

root [46] : The roots are utilized in the therapy of rheumatism, lumbago, pain in the limbs, post-partum blood stasis, furunculosis, impetigo and snake-bite. The dosage is 8 to 20 g per day, in the form of a decoction or elixir. The torrefied roots or leaves are effective as astomachic for dyspepsia in parturients in a daily dose of 6 to 12 g as decoction. A poultice made of heated leaves and a wash with a decoction of the trunk bark are useful for furunculosis and impetigo.

stem[47] : Wood is applied as anodyne, styptic, for broken bones, and severe wounds. The wood is good for poles and goldsmith's charcoal (worthington).

plant[48] : The plant has been used clinically to stop bleeding and pain (component of an effective coronary tablet, containing also *Carthamus*, *Ligustioum* and *Saliva* for treatig heart.

bark[47,49]: Applied as an external application to scores and ulcers (trimen)

leaf[47] : The leaves are scented. The odour is released when they are broken; it is rather like turpentine.

And *Acronychia pedunculata* is known as medicinal in various regions[5], for example; *China*, the wood is used as an anodyne and styptic against severe wounds and fractured bones, *Indo-China*; resin from roots is rubbed on as an embrocation to treat rheumatism and the bark and leaves are employed against scabies and colic. *Indonesia;* the roots and leaves were brought as medicinal.

1.2.3 Review of Chemical Constituents of *Acronychia* genus

Acronychia plants contain several types of chemical constituents. The compounds which had been reviewed are classified into three main groups as follows;

a. Alkaloids

All alkaloids contain nitrogen, frequently in a heterocyclic ring, and many are basic as their names indicate.[6] The large quantities of alkaloids were obtained, as chemical constituents of plants in the genus. Especially, *Acronychia baueri* is a rich source of various types of alkaloids, such as acridone, quinoline and furoquinoline alkaloids. *Acronychia pedunculata* have been reported that they contain quinoline and furoquinoline alkaloids, whose molecular skeleton differs from the quinoline alkaloid in that they have furan ring fused to the quinoline structure. For examples, the furoquinoline alkaloids, kokusaginine and evolitrin has been examined 0.1 % yield in leaves and 0.05 % yield in timber of *Acronychia laurifolia*, respectively. The review of these alkaloids is shown in **Table 1.4.**

Table 1.4 Alkaloids from *Acronychia* genus

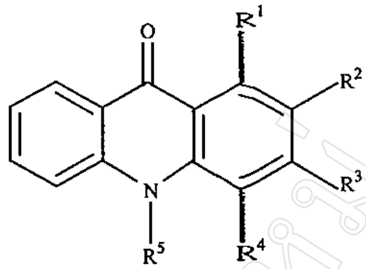
Plant	Plant part	Compounds	References
		<u>Acridone alkaloids</u>	
Acronychia baueri	leaf	1,3-dimethoxy-10-methyl acridone **(87)**	50
		xanthevodine **(88)**	50
		melicopine **(89)**	50
		melicopidine **(90)**	50
		melicopicine **(91)**	50

Table 1.4 Alkaloids from *Acronychia* genus (cont.)

Plant	Plant part	Compounds	References
Acronychia baueri	bark	normelicopin (**92**)	50
		normelicopidine (**93**)	50
		melicopine (**89**)	50
		1,2,3-trimethoxy-10-methyl acridone (**94**)	50
		1,3,4-trimethoxy-10-methyl acridone (**95**)	50
		melicopidine (**90**)	50
		normelicopicine (**96**)	50
		melicopicine (**91**)	50
Acronychia haplophylla	leaf	2,3-dimethoxy-1-hydroxyl methyl acridone (**97**)	50
		<u>Prenylacridone alkaloids</u>	
Acronychia baueri	bark	acronycine (**98**)	50 ,51
		des-*N*-methylacronycine (**99**)	50 ,51
		noracronycine (**100**)	50 ,51
	leaf	acronidine (**101**)	50 ,51
Acronychia haplophylla	bark	acrophylline (**102**)	50 ,51
		arophyllidine (**103**)	50 ,51
	leaf	acrophylline (**102**)	50
		acrophyllidine (**103**)	50
		<u>Quinoline alkaloids</u>	
Acronychia baueri	leaf	1,2-dimethyl-quinol-4-one (**104**)	50

Table 1.4 Alkaloids from *Acronychia* genus (cont.)

Plant	Plant part	Compounds	References
Acronychia pedunculata	root	2,3-methylenedioxy-4-7-dimethyl quinoline **(105)**	52
		γ- fagarine	52
		maculosidine	52
		Furoquinoline alkaloids	
Acronychia pedunculata	leaf	kokusaginine **(106)**	50, 53
	timber	evolitrine **(107)**	50, 53
	root	kokusaginine **(106)**	52
		evolitrine **(107)**	52
		skimmianine **(108)**	52
Acronychia baueri	bark	acronycidine **(109)**	50
	leaf	kokusaginine **(106)**	50
		skimmianine **(108)**	50
		acronycidine **(109)**	50



(87) $R^1,R^3 = OCH_3$, $R^2 = H$, $R^4 = H$, $R^5 = CH_3$

(88) $R^1,R^4 = OCH_3$, $R^2,R^3 = -OCH_2O-$, $R^5 = H$

(89) $R^1,R^2 = OCH_3$, $R^3,R^4 = -OCH_2O-$, $R^5 = CH_3$

(90) $R^1,R^4 = OCH_3$, $R^2,R^3 = -OCH_2O-$, $R^5 = CH_3$

(91) $R^1,R^2,R^3,R^4 = OCH_3$, $R^5 = CH_3$

(92) $R^1 = OH$, $R^2 = OCH_3$, $R^3,R^4 = -OCH_2O-$, $R^5 = CH_3$

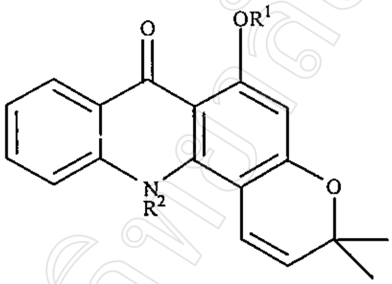
(93) $R^1 = OH$, $R^2,R^3 = -OCH_2O-$, $R^4 = OCH_3$, $R^5 = CH_3$

(94) $R^1,R^2,R^3 = OCH_3$, $R^4 = H$, $R^5 = CH_3$

(95) $R^1,R^3,R^4 = OCH_3$, $R^2 = H$, $R^5 = CH_3$

(96) $R^1 = OH$, $R^2,R^3,R^4 = OCH_3$, $R^5 = CH_3$

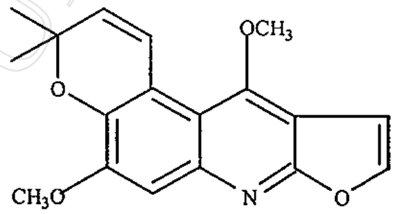
(97) $R^1 = OH$, R^2,R^3, $R^4 = H$, $R^5 = CH_3$



(98) R^1, $R^2 = CH_3$

(99) $R^1 = CH_3$, $R^2 = H$

(100) $R^1 = H$, $R^2 = CH_3$



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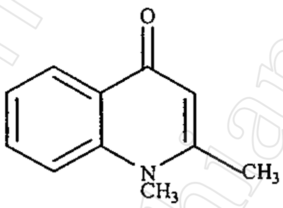

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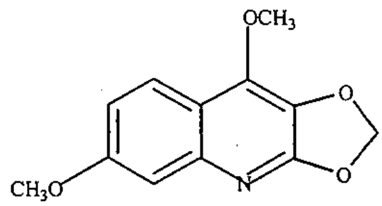

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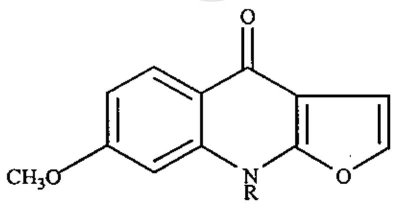
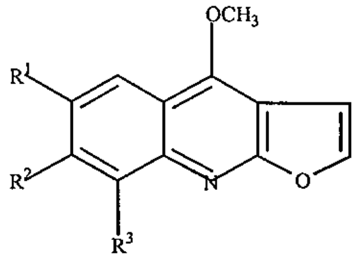


(102) $R = CH_2CH{=}C(CH_3)_2$

(103) $R = CH_2CH_2C(OH)(CH_3)_2$



(106) $R^1, R^2 = OCH_3$, $R^3 = H$

(107) $R^1 = H$, $R^2 = OCH_3$, $R^3 = H$

(108) $R^1 = H$, $R^2, R^3 = OCH_3$

(109) $R^1, R^2, R^3 = H$

b. Phenolic Compounds

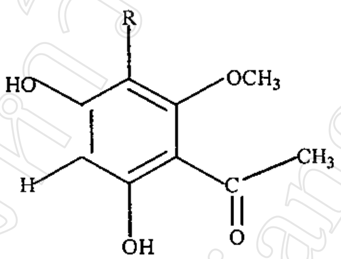
Acronychia genus of the family Rutaceae has been found to contain phenolic compounds from several parts of the plants. Acronylin and acrovestone are well known compounds isolated from bark, stem and root bark of *Acronychia pedunculata*. Some information of substances found in the plants referred to aryl ketones, that mostly have acetophenone core structure. X-ray analysis and spectroscopic method have been used for both structure identification and elucidation of novel compounds. The report of phenolic compounds is shown in **Table 1.5.**

Table 1.5. Phenolic compounds from *Acronychia* genus

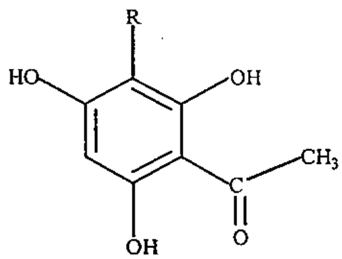
Plant	Plant part	Compounds	References
Acronychia pedunculata	bark	acronylin (**110**)	53, 54
	root bark	6-demethylacronylin (**111**)	50, 53
	fruit	1,1-di-[2′,4′,6′-trihydroxy-3′-(1″-oxo ethanyl)-5′-(3″-methylbut-2″-enyl) phenyl]-3-butane (**112**)	55
	root bark	acrovestone (**113**)	56, 57
		1-[2′,4′-dihydroxy-3′,5′-di-(3″-methylbut-2″-enyl)-6′-methoxy] phenyl ethanone (**114**)	56
			56
	stem bark	acroveatone (**113**)	57
	leaf	1-[2′,4′-dihydroxy-3′-(3″-methylbut-2″-enyl)-5′-(1‴-ethoxy-3‴- methylbutyl) -6′-methoxy]phenyl ethanone (**115**)	58

Table 1.5. Phenolic compounds from *Acronychia* genus (cont.)

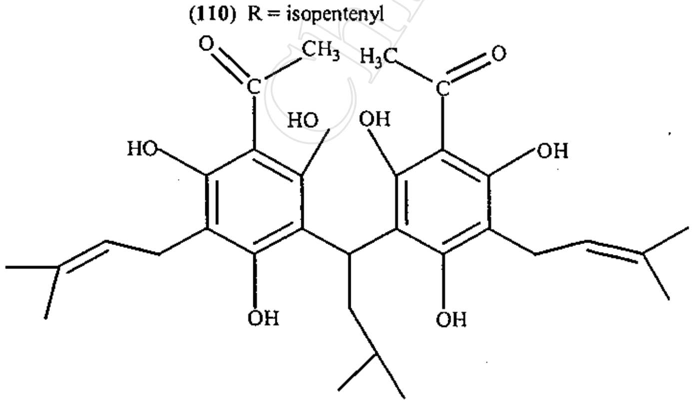
Plant	Plant part	Compounds	References
Acronychia pedunculata	leaf	1-[2′,4′-dihydroxy-3′,5′-di-(3″-methylbut-2″-enyl)- 6′-methoxy] phenyl ethanone (**114**)	58
Acronychia porteri	leaf	5,3′-dihydroxy-3,6,7,8,4′-penta methoxy-flavone (**116**)	59
		5-hydroxy-3,6,7,8,3′,4′-hexa methoxy-flavone (**117**)	58
		3,5-dihydroxy-6,7,8,3′,4′-penta methoxy-flavone (**118**)	58



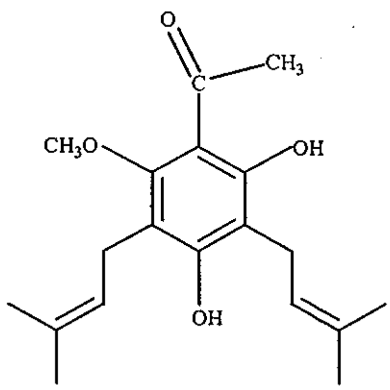
(110) R = isopentenyl



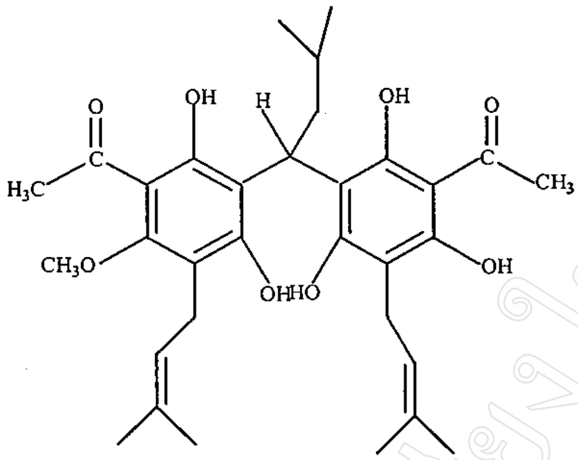
(111) R = isopentenyl



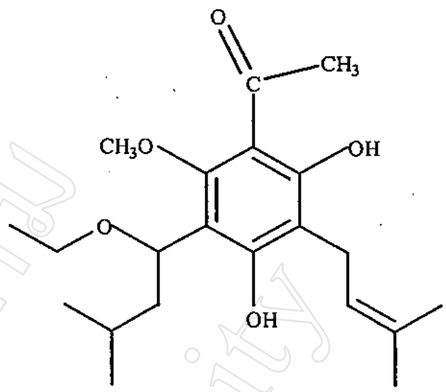
(112)



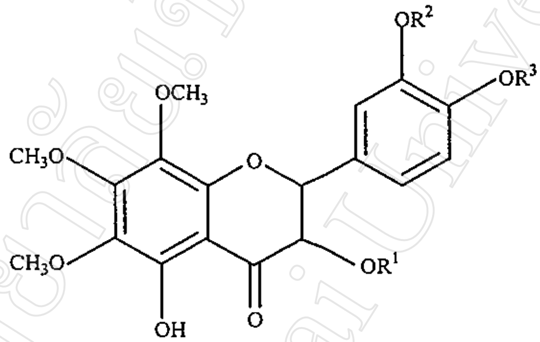
(114)



(113)



(115)



(116) $R^1, R^3 = H, R^2 = H$
(117) $R^1, R^2, R^3 = CH_3$
(118) $R^1 = H, R^2, R^3 = CH_3$

2.3.3 Miscellaneous Compounds

Although the alkaloids and phenolic compounds are abundance in *Acronychia* plants, they also constitute other compounds such as triterpenoid, lignan, coumarin *etc.* From the review of chemical constituents in *Acronychia pedunculata*, many parts of this plant have been studied. For examples, α-pinene and limonene[50,60], main components of essential oil in plants, have been isolated from the leaves. β-Sitosterol was also found in the heartwood[50,60]. In addition, potassium oxalate[50,61] and bauerenol[61] were obtained from the stem bark together with the coumarin bergapten and triterpenoid β-amyrin from the root bark.[56] Bauerenol was also found in *Acronychia baueri*.[61,62]

2.4 Review of Bioactive Compounds from *Acronychia* genus

- **Cytotoxic Compounds**

Funayama *et al.*[51] have reported cytotoxic activity of alkaloids acronycine (**98**), isolated from *Acronychia baueri* and their synthesized derivatives. It was found that acridone alkaloid; acronycine and 1,2-dihydroacronycine, a semisynthesized compound, were weakly active, but neither noracronycine nor its semisynthesized derivatives, dihydroacronycine was cytotoxic against KB test system *in vitro*. They concluded that *O*-methyl group of acridone alkaloid is essential for the activity.

Therefore, syntheses of more acronycine derivatives were carried out for testing of antitumor activity *in vitro* on other type of cancer cell line such as P-388 leukemia[63], L-1210 cells[64] *etc.*

Wu *et al.*[57] have studied cytotoxic compounds by bioassay-directed fractionation of the plant extract. They isolated acrovestone (**113**), a known aryl ketone, from stem and root bark of *Acronychia pedunculata*. This compound was shown for the first time to be a cytotoxic agent in human KB tissue culture assay (100% inhibition at 0.5 μg/ml) and cytotoxic activity against A-459, P-388 and L-1210 cell with ED_{50} values of 0.98, 3.28 and 2.95 μg/ml, respectively.

Cui *et al.*[52] presented bioassay-directed fractionation of root extract of *Acronychia pedunculata* using the KB-V1^{+} human tumor cell line and this work led to the isolation of six quinoline alkaloids, one novel and five known compounds. Alkaloids evolitrine (**107**), skimmiamine (**108**), kokusaginine (**106**) and maculosidine showed weak cytotoxic activity when evaluated against apanel of human cancer cell lines, while novel one, 2,3-methylenedioxy-4,7-dimethylquinoloine (**105**), γ-fagarine, sesamolin and yangambin were inactive.

Lichius *et al.*[59] have reported cytotoxic activity on KB (human nasopharyngeal carcinoma cells) of flavonols from leaves of *Acronychia porteri*. Three flavonols were obtained, namely 5,3′-dihydroxy-3,6,7,8,4′-penta methoxy-flavone (**116**), 5-hydroxy-3, 6,7,8,3′,4′-hexamethoxy-flavone (**117**) and 3,5-dihydroxy-6,7,8,3′,4′-penta methoxy-flavone (**118**). All of these flavonols showed cytotoxic activity against KB cells. The first compound was the most active with IC_{50} values of 0.04 μg/ml followed by the compound (**118**) (IC_{50} 0.1 μg/ml) and compound (**117**) (IC_{50} 6 μg/ml).

- **Antimitotic Compounds**

Lichius *et al.*[59] have isolated bioactive compounds from dried leaves of *Zieridium pseudobtusi* folium and leaves of *Acronychia porteri*. They obtained three flavonols from *Acronychia proteri* that only one of them namely 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxy-flavone (**116**) showed inhibition of tubulin assembly into microtubles (IC_{50} 12 μM).

This compound was, however, found to be inactive when evaluated *in vivo* against early-stage subcutaneous pancreatic ductal adenocarcinoma O3 in B6D2F1 female mice.