CHAPTER I

HISTORICAL INTRODUCTION

Thailand is rich in natural resources especially in plants. A tremendous number of plants furnish materials for medicine. Some of the plants are harmful or apparently of little or no values, while others seem to be useful. Plants are used in the form of crude drugs and the prescriptions were formulated in the form of multicomponent drugs. Little has been known about the active constituents of the crude drugs or their pharmacological activities. Attempts to have a thorough study of local medicinal plants have been initiated. Many of local medicinal plants are official in pharmacopoeiae, and are used in folk-medicine, including the plants in the family Vitidaceae.

The family Vitidaceae, according to the system of Engler (1), contains 12 genera with about 700 species, 350 species of which belong to the genus *Cissus*. It is widely distributed in the tropics of both hemispheres, from tropical East Africa, tropical Asia, throughout India, Ceylon, Malaya, and the Philippines (2).

In Thailand, the genus *Cissus* is widely distributed in all parts of the country. According to Craib (3) and Smitinand (4) at present there are about 22 species of *Cissus* and their scientific names are listed below as originally recorded :

*** *Cissus adnata* Roxb. (syn. *Vitis adnata* Wall.)

*** *C. aristolochiodes* Plachon

* *C. assamica* Craib

* *Cissus calcicola* Craib

** *C. carnosa* Lam. (syn. *Vitis trifolia* Wall.)

*** *C. convolvulacea* Planchon

* *C. Craibii* Gagnep.

*** *C. discolor* Blume (syn. *Vitis discolor* Dalz.)

* *C. dissecta* Craib

*** *C. hastata* Miq. (syn. *Vitis hastata* Miq.)

* *C. hexangularis* Thorel ex Planchon

* *C. Marcanii* Craib

*** *C. modeccoides* Planchon

*** *C. modeccoides* Planchon var. *kerrii* Craib

*** *C. quadrangularis* Linn. (syn. *Vitis quadrangularis* Wall.)

** *C. pedata* Lam. (syn. *Vitis pedata* Vahl ex Wall.)

*** *C. repanda* Vahl (syn. *Cissus rosea* Walp., *Vitis repanda* W.& Arn.)

*** *C. repens* Lam. (syn. *Vitis repens* Wall.)

*** *C. rheifolia* Planchon

* *C. Robinsonii* Ridl. (syn. *Vitis Robinsonii* Ridl.)

** *C. siamea* Planchon

* *C. siamica* Planchon

(* reported by Craib, ** reported by Smitinand, *** reported by both Craib and Smitinand)

The medicinal uses of the vitidaceous plants are well documented. The following pages contain a literature survey about the medicinal uses, poisonous properties and chemical constituents of the plants in the family Vitidaceae.

In 1876, Bernays (5) recorded the serious illness of two children who had swallowed the juice from the chewed leaves of Virginia

creeper, *Parthenocissus quinquefolia* (Linn.) Planchon [syn. *Ampelopsis hederacea* De Candolle, *Hedera quinquefolia* Linn., *Vitis hederacea* Wildenow, *V. quinquefolia* Lamark, and *Cissus quinquefolia* Persoon (6)] The symptoms presented were violent vomiting and purging, with considerable tenesmus followed by collapse and stupor for two hour ; this was followed by another period of vomiting and purging, and then the children recovered. Two years before this, Gorup-Besanez (7) had examined the juice of this plant and found large quantities of oxalic acid. In 1911, Holm (8) had shown the presence of calcium oxalate crystals in many parts of the plant. One year later, Warren (6) analyzed all the data and proposed that the toxic property of this plant might be due to the presence of free oxalic acid and calcium oxalate in the plant.

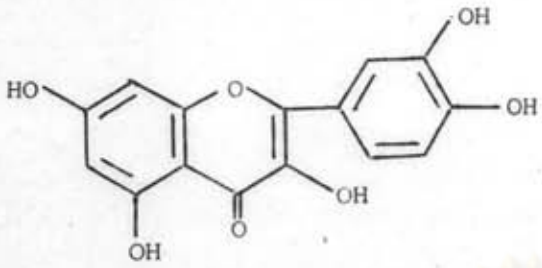
In 1937, Balley (9) reported the following native uses of East African plants. In Tanzania, a part of the root of *Cissus adenocaulis* Steud. was applied to the swollen limbs in an infectious disease called "Dulasi" and to abscesses and boils. The roots are also a remedy for the prevention of miscarriage. The leaf juice of *Cissus hildebrantii* Gilg. has been applied topically by the Shambala of Somalia to wounds caused by snakebite and poison arrow. The Swahilis used the root of *Cissus oliveri* Gilg. in the treatment of "Dulasi" ; the leaves of this plant were also used in constipation. The Masai used the root of *Rhoicissus erythroides* Planchon as a gonorrhoeal remedy and the plant juice is used as dressing for spear wounds.

The juice of the bulbous root of *Cissus cirrhosa* Thunb. has been used as an internal remedy and as an application to glandular swellings and to creeping sores (10).

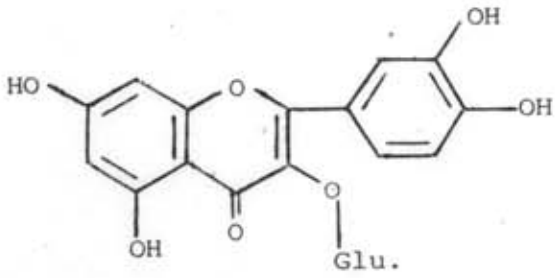
In Cambodia, the roots of *Vitis indica* W. & Arn. (syn. *Ampelocissus arnottiana* Planchon) are considered as pectoral and diuretic and in Konkan, the country folk used the roots as an alterative in the form of a decoction (11).

In India, the preperations of the powdered dry shoots of *Cissus quadrangularis* Linn. (syn. *Vitis quadrangularis* Wall.) has been used against digestive trouble (12). The leaves of this plant have been used in the treatment of internal ulceration and for external wounds ; the roots were used for myalgia, and the juice was used for earache. In Central Africa, decoction of this plant has been taken for blenorrhagia and to calm palpitations (13). This plant has also been known to have beneficial effects on bone healing for many centuries. The study of the effects of this plant in experimental animals was performed by Udupa *et al.* (14) in 1961. It was found that this plant did influence the healing of fractures and the effect of the drug was not due to the vitamin C content in the plant alone. This result was supported by the work of Singh and Udupa (15) and by Udupa and Prasad (16).

Further chemical characterization of this family has been limited primarily to flavonoids. Gorup-Besanez (7) reported the presence of pyrocatechin and oxalic acid in *P. quinquefolia* Planchon. The chemistry of vitidaceous plants became more interesting with the isolation of two flavonoids, quercetin and isoquercitrin from grapes (*Vitis vinifera* Linn.) which were reported in 1952 by Williams and Wender (17).



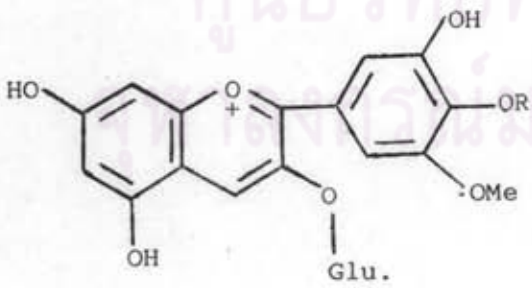
quercetin



isoquercitrin

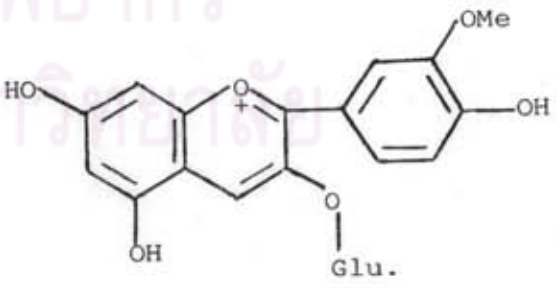
In 1967, Stern *et al.* (18) isolated methylanthranilate, as an essential component, from Grape of the Labrusca (*V. labrusca* Linn.)

More studies were done by Koeppen and Basson (19) on the isolation of anthocyanin pigments from the skin of Barlinka Grape berries. The pigments were identified as oenin, mono-*p*-coumaryl oenin, the 3-glucoside of peonidin, petunidin, and delphinidin. This results were supported by the work of Somers (20).

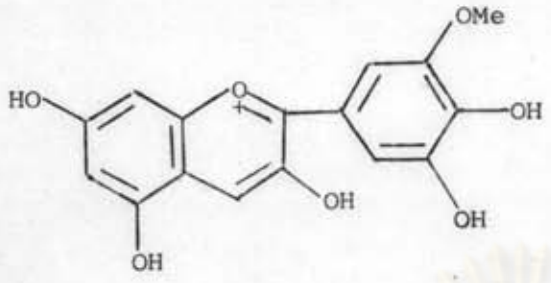


oenin, (R=H)

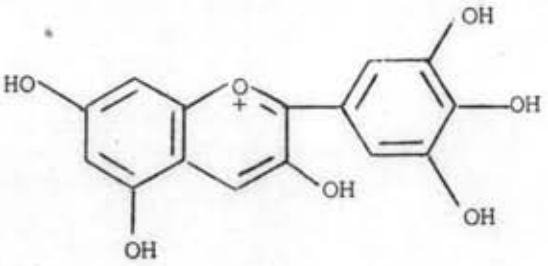
mono-*p*-coumaryl oenin, (R=*p*-coumaryl)



3-glucoside of peonidin

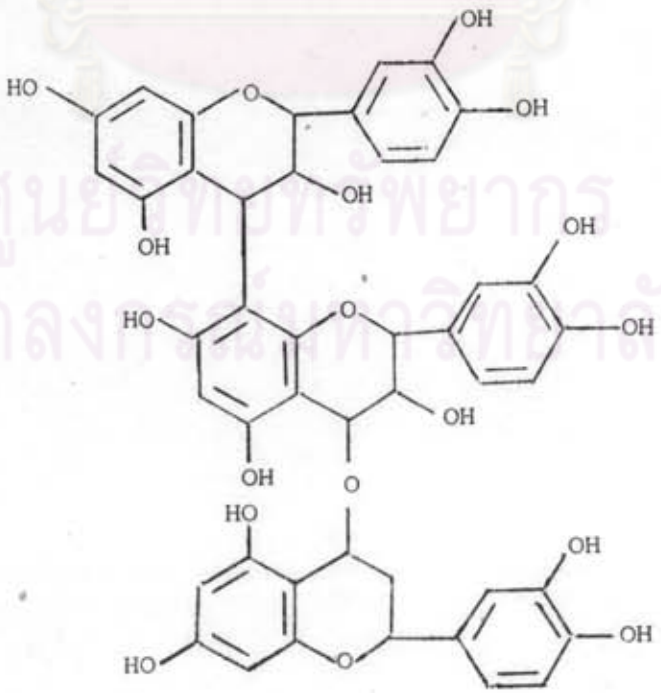


petunidin



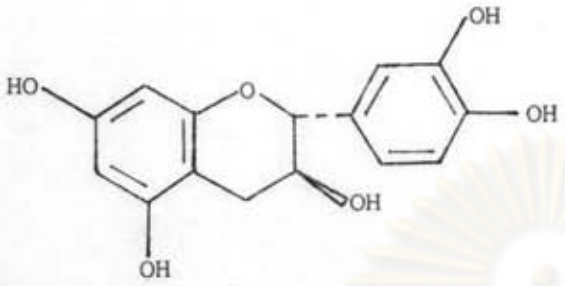
delphinidin

Bhatia *et al.*(21) isolated proanthocyanidin from the skins of white grapes. Based on the observations and the analytical values, the proanthocyanidin is considered to be a trimer of leucocyanidin having -C-C- and -C-O-C- linkages and having the structure below as the most probable.

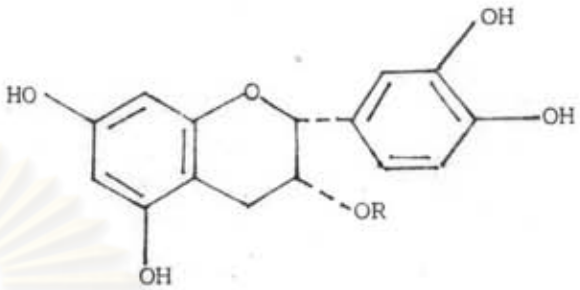


trimer of leucocyanidin

In the same year, Su and Singleton (22) investigated the seeds of grapes and the identification of several phenolic compounds were reported. They were identified as (+)catechin, (-)epicatechin, and epicatechin-3-gallate.



(+)catechin



(-)epicatechin, (R=H)

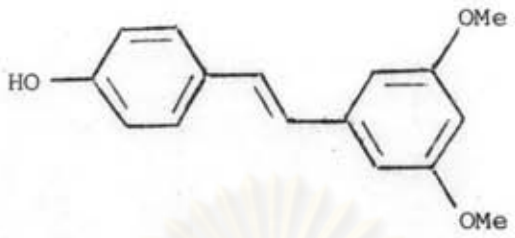
epicatechin-3-gallate,

(R=gallic acid)

In 1964, Sen (23) studied the active constituents of *Cissus quadrangularis* Linn. (syn. *Vitis quadrangularis* Wall.). It was reported that the plant contained a steroidal principle which was tentatively identified as a 3-ketosteroid. The steroidal principle gave a delayed positive response with the Liebermann-Burchard test and an immediate positive response with the Zimmermann test. Udupa *et al.* (24) studied the effect of this plant steroid on the rate of fracture repair and showed that this single component had not only maintained its beneficial effect on the rate of fracture healing, but also had certain distinct advantages over the use of a total extract of the herb. In 1966, Sen (25) reported the presence of two keto-steroids which have characteristic spectral data as previously reported (23). To fit the elemental analysis data, Sen suggested the possible empirical formulae of $C_{24}H_{45}O$ and $C_{23}H_{41}O$ for the two steroidal substances.

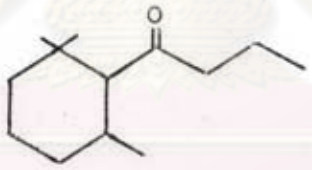
In 1979, Langcake *et al.* (26) isolated an inducible antifungal

compound from grapevine leaves (*V. vinifera* Linn.,cv Carbenet-Sanvignon) and identified as *trans*-pterostilbene (3,5-dimethoxy-4-hydroxy stilbene).



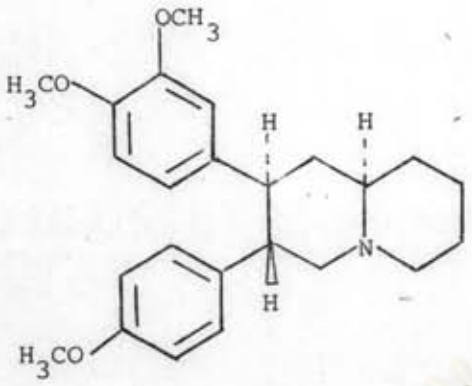
trans-pterostilbene

In 1981, Acree *et al.* (27) reported the presence of damascenone, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, in three species of grapes, *Vitis labruscana* (Baily), *V. vinifera* (Linn.), and *V. rotundifolia* (Michaux). It was found that *Vitis labruscana* (Baily) has the highest concentration of damascenone.

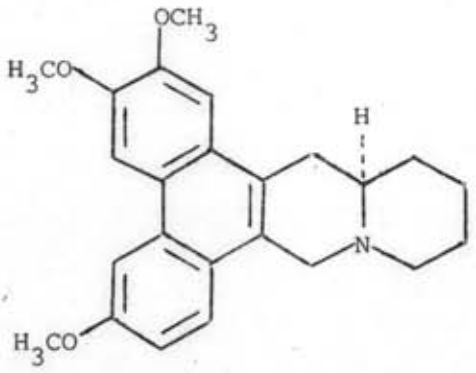


damascenone

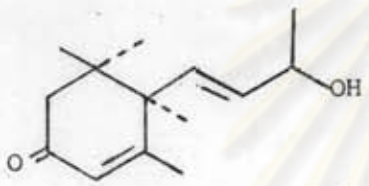
In 1981, Saifah *et al.* (28) worked on the plant known as "Ka Ya Wong" (*Cissus rheifolia* Planchon) which is found in the northeastern part of Thailand, and reported the isolation of two alkaloids, two terpenoids, and one flavonoid from the leaves. The alkaloids were identified as kayawongine and cryptopleurine. The alkaloid kayawongine is a new natural product. The terpenoids were identified as vomifoliol and romalea-allene. The C-glucoside flavonoid vitexin was also characterized.



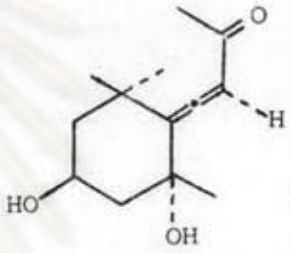
kayawongine



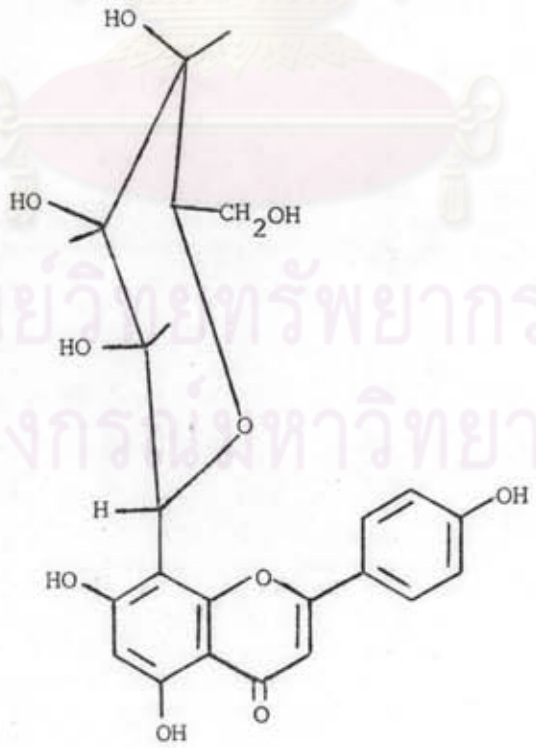
cryptopleurine



vomifoliol

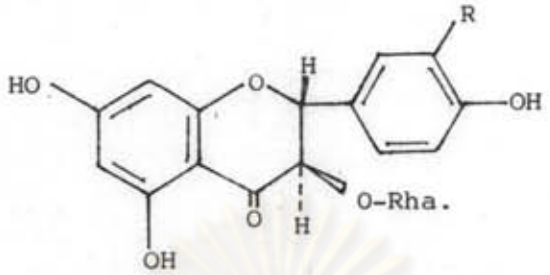


romalea-allene



vitexin

In 1983, Trousdale and Singleton (29) isolated the flavanonol glycosides from the skin of white grape (*Vitis vinifera* Linn.). The glycosides were identified as astilbin and engeletin.



astilbin, (R=OH)

engeletin, (R=H)

In the same year, Bhutani *et al.* (30) isolated two new tetracyclic triterpenoids along with sitosterol, δ -amyrin and δ-amyron from *Cissus quadrangularis* Linn. (syn. *Vitis quadrangularis* Wall.). The tetracyclic triterpenoids were identified as 3β, 21β-Dihydroxy-7-onocerene and 3β, 21β-Dihydroxy-7-onocerene.

Several phytochemical screening studies of plants had done including samples of vitidaceous plants. In 1960, Arthur and Cheung (31) performed phytochemical studies of the Hong Kong medicinal plants, and reported the presence of alkaloids in leaves and stems of *Ampelopsis brevipedunculata* Maxim.ex Trautv., *A. cantoniensis* Seem., and *Vitis flexuosa* Thunb. In the same year, Kiang *et al.* (32) performed phytochemical survey of Malaya Plants for alkaloids. The positive tests for alkaloids were shown in 11 species from 2 genera of the family Vitidaceae, and recorded as follows :

Plant	Part of the Plant
Pterisanthes cissoides Blume	whole plant
Vitis angustifolia Wall.	leaves, stem, buds
V. curtissii Wall.	leaves, stem, flowers
V. furcata Ridl.	leaves, stem
V. glaberrima Wall.	whole plant
V. kunstleri Wall.	leaves, stem, fruit
V. lawsoni Ridl.	leaves, stem, fruit
V. lanceolaria Wall.	leaves, stem, fruit
V. mollissima Wall.	roots, fruit, stem
V. novemfolia Wall.	leaves, stem, roots
V. peduncularis Wall.	leaves, roots, fruit

In 1962, more than 3000 seed samples from a wide spectrum of the plant kingdom were screened by Earle and Jones (33). It was found that seeds of *Parthenocissus quinquefolia* Planchon and *Vitis vulpina* Linn. gave positive test for alkaloids. The result of *P. quinquefolia* Planchon was confirmed by the same investigators (34) and from this work the presence of a tannin was detected in *P. tricuspida* var. *vitchii* Rehd. In 1967, Persinos and Quimby (35) performed phytochemical screening on Nigerian plants for alkaloids, saponins, and tannins ; the presence of tannins and saponins were detected in a root sample of *Cissus hochstetteri* Planchon.

In 1971, Kapoor *et al.* (36) performed the phytochemical survey of Indian Plants for alkaloids, flavonoids, and saponins. The presence of flavonoids were detected in stem-bark and twigs of *Parthenocissus himalayanee* Planchon. Desai *et al.* (37) performed

phytochemical studies of some Indian plants in 1975, and found that the presence of stigmasterol was detected in *Vitis canarensis* Dalz. A recent study of New Guinea plants by Hartley *et al.* (38) showed negative test for alkaloids in 7 species from 4 genera of the family Vitidaceae.

The plant used in this investigation was identified as *Cissus quadrangularis* Linn. (syn. *Vitis quadrangularis* Wall.) known in Thai as Phet sang Khaat, เพ็ชรสังฆาต, San cha Khuat, สันชะควด (Bangkok) ; Saamroito, สามร้อยต่อ (Prachuap Khiri Khan) ; Khankho, ขันข้อ (Ratchaburi) (4). In Thailand, the plant has been used as a carminative, anti-hemorrhoid, and to promote fracture bone healing (39). Although this plant had been studied by Sen *et al* (23) in 1964, but Sen has not published again on the steroids of this plant, and to date no cititation of these three papers has appeared in the scientific literatures. In Sen's paper, there was some obvious misinterpretation of the spectral data of these steroids. Not only are these proposed formulae incapable of existance due to the odd number of hydrogen atoms, but all of his earlier speculations on the presence of both keto and hydroxyl functions are clearly impossible since each molecule contain only one oxygen atom. The structure of the chemical which promotes bone healing in *Cissus quadrangularis* Linn. remains to be identified. It is the purpose of this investigation to purify and identify the chemical constituents of *Cissus quadrangularis* Linn. and to prove the nature of the structures. Moreover, the chemical characterization of this plant may provide valuable information in the field of chemo-taxonomy.