CHAPTER I

INTRODUCTION

1. Information and significant of the problem.

The kingdom of plant has been represented an enormous reservoir of biologically valuable molecule to be discovered. Among the estimated 350,000 plant species, only a small percentage has been used for medicinal purposes and very few of the phytochemical, biological and pharmacological activities were investigated (1). The number of 21 from 151 lead substances of natural origin that have been modified to produce 1,300 drugs in the current British National Formulary (2). There are about 50 commercially anticancer drugs which have been approved by USFDA consisting of drugs based on natural origin in 1/3 of total approved agent.

In regard to the antineoplastic potentials of Rubiaceae, some evidence has already been presented that clearly demonstrates that anthraquinones inhibit the enzymatic activity of topoisomerase II. An example of antineoplastic anthraquinones that target topoisomerase II is mitoxantrone (Novatrone®), which is currently approved for clinical use in the United States (3). In the Pacific Rim, about 150 species of plants classified within the family Rubiaceae are medicinal, of which *Prismatomeris albidiflora*, *Knoxia valerianoides*, *Damnacanthus indicus*, and *Morinda umbellata* are known to produce anthraquinones. An interesting development from Rubiaceae would be to investigate its members for anthraquinones and assess them for topoisomerase inhibitors. The discovery of inhibitors of topoisomerase II of clinical antineoplastic value can be reasonably expected.

Camptothecin is a monoterpenoid quinoline alkaloid that is also known to occur in the subclass Rosidae: *Camptotheca acuminata* Decsne. (Family Nyssaceae, order Cornales), and *Nothapodytes fetida* (Wight.) Sleum. (Family Icacinaceae, order Celastrales); and the Asteridae: *Ophiorrhiza mungos* L. (Rubiaceae). Camptothecin was found to inhibit topoisomerase and to be active against experimental tumors.

However, initial clinical trials showed little response and severe cystitis, but more effective analogs were developed, such as irinotecan. Cancer chemotherapy alone, however, is not very effective in producing long-term survival or treating the most common solid tumors, and the need for new anticancer drugs is critical.

The family Rubiaceae consists of 650 genera and 10,500 species under worldwide distribution. Most species are herbaceous. There are about 55 genera in the Northern part of Thailand (4). The most-popular member of this family is coffee, known as a major economic and the most important crop. Various species of Cinchona is the source of quinine used as a cure against malaria. This family is characterized by the opposite leaves or whorled and stipulate; epipetalous stamen and the inferior ovary; flower bisexual, regular; calyx tube adnate to ovary; fruit capsular, baccate or drupaceous; embryo large, in rich endosperm (5).

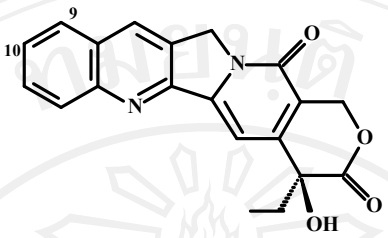
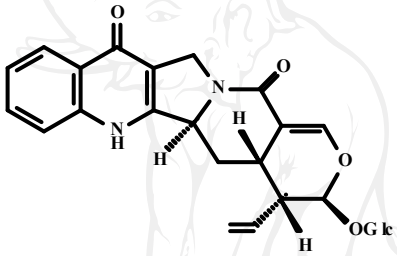
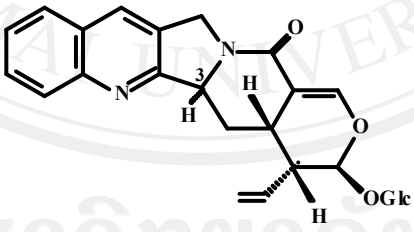
Ophiorrhiza L. (Rubiaceae, Ophiorrhizeae) is a predominantly herbaceous genus distributed from East of India to the West of Pacific and from South of China to North of Australia. Altogether more than 400 species have been described, though many of them probably superfluously. However systematic knowledge of this genus is still inadequate, recent regional revisions are available only for marginal parts of its area: the Pacific (6), China (7), and the Indian subcontinent (8). An Asiatic genus of possibly 150 species. The roughly 30-35 species recorded form Thailand are found in all parts of the country and normally grow in shady, moist to wet areas in both lowland and montane evergreen forest types but also in mixed deciduous and bamboo forests. Seed dispersal in *Ophiorrhiza* is comparable to in *Argostemma*. As in the latter, fruits always move into an upright position, even if the flowers were oriented differently (9).

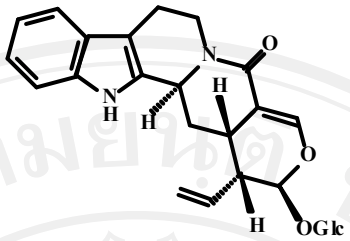
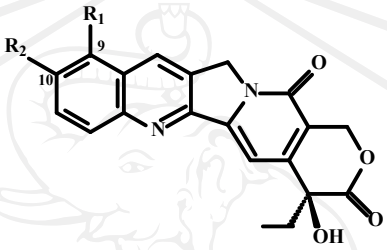
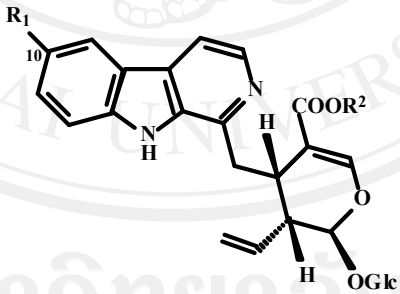
The genus *Ophiorrhiza* belongs to the family Rubiaceae, a small genus. Some species of *Ophiorrhiza* have been used in folk medicine as an antitussive, expectorant, analgesic, and for the treatment of amenorrhea and snakebite (11). Members of this genus are well known for their production of alkaloids, in particular camptothecin derivatives and β -carbolines (12-14). In order to discover new camptothecin-related alkaloids, investigations of the constituents of *Ophiorrhiza* plants that are distributed in Japan, that is, *Ophiorrhiza pumila* Champ., *Ophiorrhiza liukiuensis Hayata*, and *Ophiorrhiza japonica Bl.,* were carried out. As a result, it was found that *Ophiorrhiza*

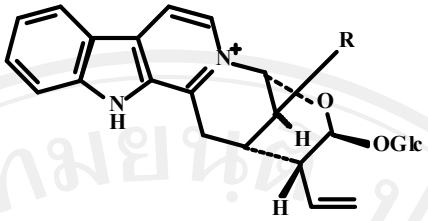
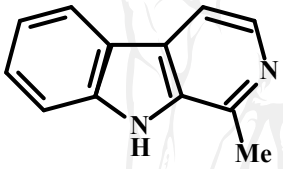
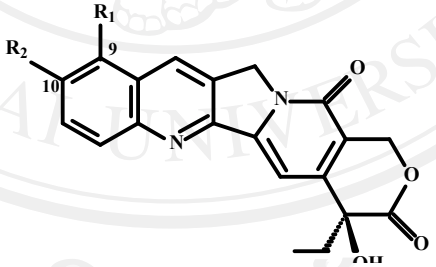
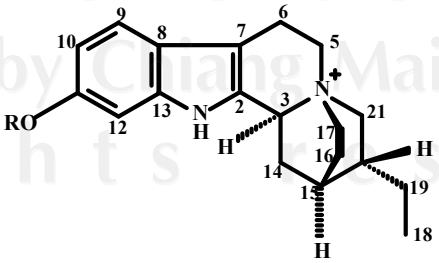
pumila produced camptothecin and its related alkaloids (12-13, 15-16), whereas *Ophiorrhiza japonica* produced β-carboline-type alkaloids and no camptothecin-related alkaloids (17-18). Another *Ophiorrhiza* plant, *Ophiorrhiza liukiuensis*, was found to produce both camptothecin- related alkaloids and β-carboline-type alkaloids (19). Recently the Camptothecin (20) was first isolated from *Camptotheca acuminate.* It is one of the most important alkaloids having inhibitory activities against tumor cells and HIV-I (12, 19, 21). Recently, camptothecin derivatives such as topotecan ® and irinotecan ® were used as clinical antitumor agents (12, 14, 20-22).

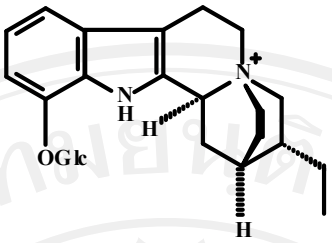
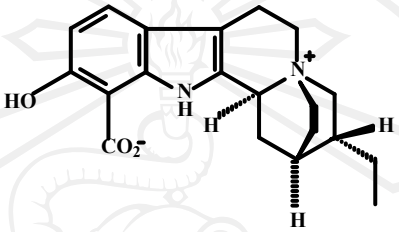
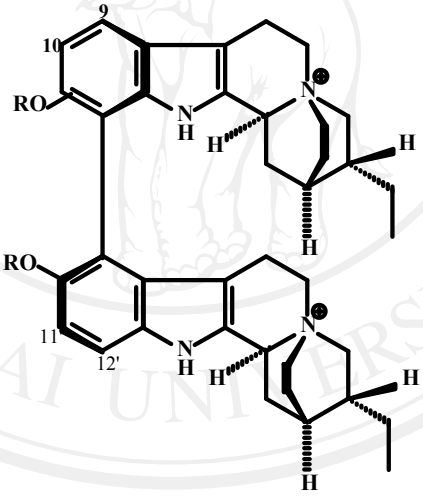
The aim of this study was to investigate the chemical constituents and biological activity of some *Ophiorrhiza* genus growing in Northern part of Thailand which may play an important role in drug development.

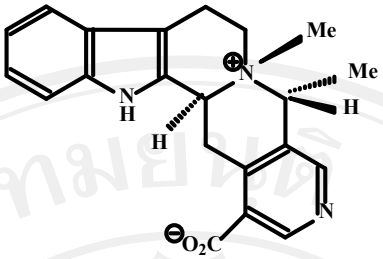
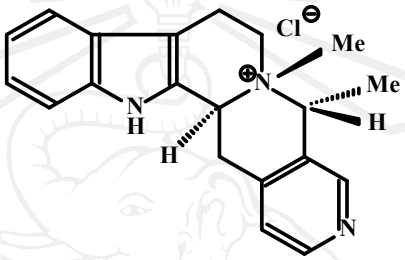
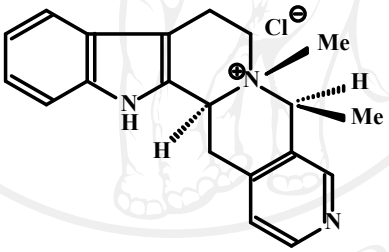
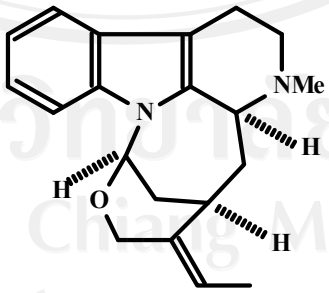
2. List of compound from *Ophiorrhiza* genus.

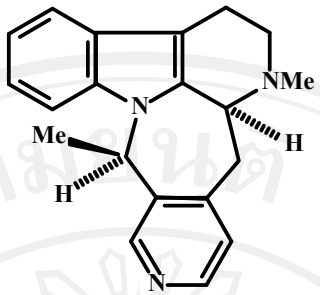
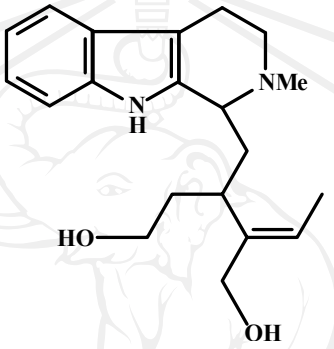
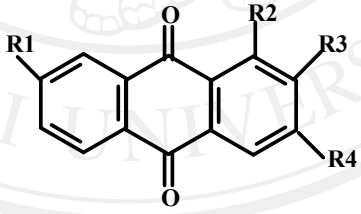
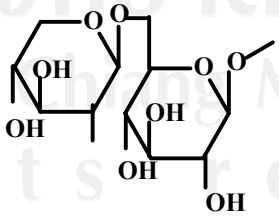
Compound name	Structure	Species	Ref.
Camptothecin		*O. mongos* *O. filistipula* *O. pumila* Champ. *O. liukiuensis* *O. kuroiwai* *O. japonica*	[12] [19] [21] [22] [23] [24] [25]
Pumiloside		*O. pumila* Champ. *O. liukiuensis* *O. japonica*	[12] [13] [15] [16] [19] [25]
(3*S*)-Deoxypumiloside **(3*R*)-Deoxypumiloside**		*O. pumila* Champ. *O. liukiuensis* *O. japonica*	[12] [13] [15] [16] [19] [25]

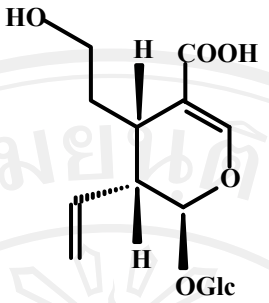
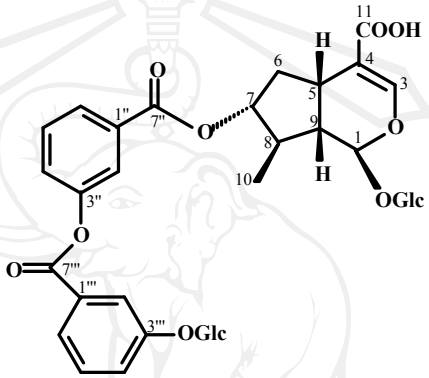
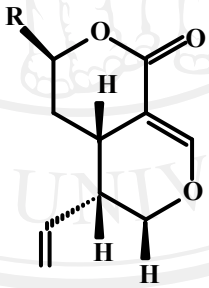
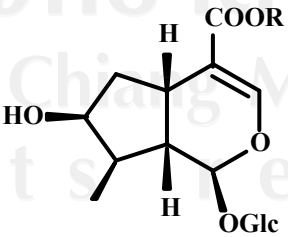
Compound name	Structure	Species	Ref.
Strictosamide		*O. pumila* Champ. *O. liukiuensis* *O. japonica*	[12] [13] [15] [16] [19] [25]
9-Methoxycamptothecin R^1= OMe R^2= H **Chaboside** R^1= OMe R^2= OGlc		*O. pumila* Champ. *O. liukiuensis* *O. japonica*	[12] [13] [15] [16] [19] [25]
Lyalosidic acid R^1= H R^2= H **Lyaloside** R^1= H R^2= Me **10-Hydroxylyalosidic acid** R^1= OH R^2= H		*O. japonica* *O. liukiuensis*	[17] [18] [19] [25]

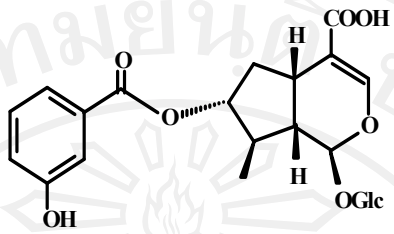
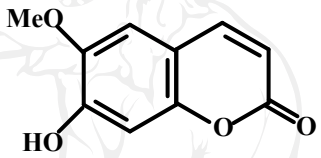
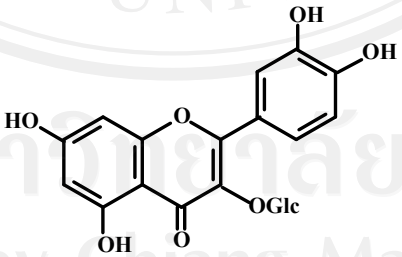
Compound name	Structure	Species	Ref.
Ophiorine A R= COO^-, 16H-α **Ophiorine B** R= COO^-, 16H-β **Ophiorine A methyl ester** R= COOMe, 16H-α **Ophiorine B methyl ester** R= COOMe, 16H-β		*O. japonica* *O. liukiuensis*	[17] [18] [19] [25]
Harman		*O. japonica* *O. liukiuensis*	[17] [18] [19] [25]
10-Methoxy champtothecin R^1= H R^2= OMe		*O. liukiuensis*	[25]
Ophiorrhizine		*O. blumeana* Korth. *O.bracteata* Bl. *O. major* Ridl	[26] [27] [29]

Compound name	Structure	Species	Ref.
Bracteatine		*O. bracteata* Bl *O. major* Ridl	[27] [29]
Ophiorrhizine-12-carboxylate		*O. bracteata* Bl *O. bracteata* Korth.	[27]
Blumeanine 7R= H **Blumeanine di acetate** 7R= Ac		*O. bracteata* Bl *O. bracteata* Korth	[27]

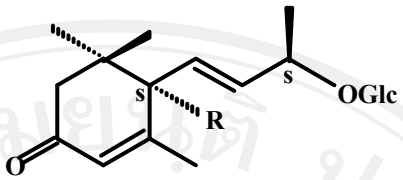
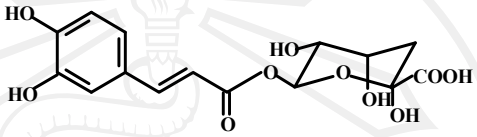
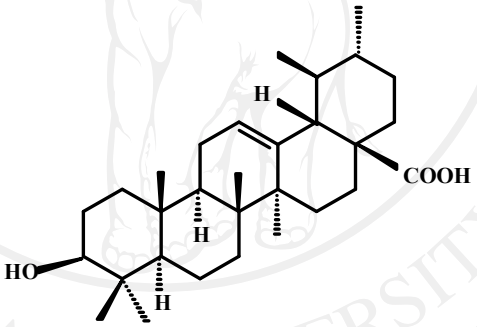
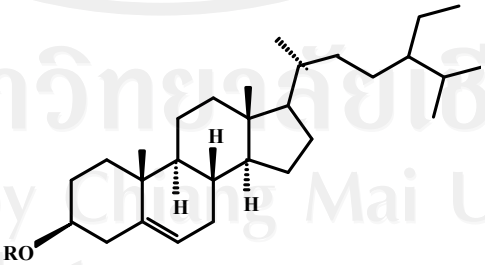
Compound name	Structure	Species	Ref.
Isomalidine-16-carboxylate		*O.* cf. *communis*	[28]
Isomalindine		*O.* cf. *communis* *O.* cf. *ferruninea*	[28] [30]
Malindine		*O.* cf. *ferruninea*	[28]
Dihydrocycloakage-rine		*O.* cf. *ferruninea*	[30]

Compound name	Structure	Species	Ref.
Mostuenine or 3,14-dihydrodecus-sine		*O.* cf. *ferruninea*	[30]
Tetrahydrocycloakage rine		*O.* cf. *ferruninea*	[30]
Ophiohayatone-A R_1= OH R_2= H R_3= OH R_4= CH_2OCH_3 **Ophiohayatone-B** R_1= H R_2= OH R_3= CH_2OH R_4= *O*-primeverose **Ophiohayatone-C** R_1= H R_2= H R_3= COOH R_4= OH	 ***O*-primeverose =** 	*O. hayatana* Ohwi.	[31]

Compound name	Structure	Species	Ref.
Demethylsecologanol		*O. luikiuensis*	[19]
3‴-*O*- glucosylsenburiside II		*O. luikiuensis*	[19]
Sweroside R= H ***epi*- Vogeloside** R= OMe		*O. luikiuensis*	[19]
Loganic acid R= H **Loganin** R= Me		*O. luikiuensis*	[19]

Compound name	Structure	Species	Ref.
Swertiaside A		*O. luikiuensis*	[19]
Scopoletin		*O. luikiuensis*	[19]
Hyperin		*O. luikiuensis*	[19]

Compound name	Structure	Species	Ref.

***(6S,9R)*-Rseoside** R= OH ***(6R,7E,9R)*-9-hydroxy megastima-4,7-dien-3-one-9-*O*-*β*-D-glucoside** R= H		*O. luikiuensis*	[19]
Chlorogenic acid		*O. luikiuensis*	[19]
Ursolic acid		*O. luikiuensis*	[19]
β- Sitosterol R= H **Daucosterol** R= Glc		*O. luikiuensis*	[19]

3. Characteristic of *Ophiorrhiza* species

3.1. ***Ophioriza trichocarpon*** Blume (Syn. *O. trichocarpa* Bl., *O. hispidula* Wall., *O.hispidula* G.Don and *O. villosa* Kurz). is a herb. Its stem is erect branched. The **leaves** are simple, opposite, ovate acute or acuminate, glabrous or with scattered short 0.1-0.3 mm. appressed hairs above, nerves densely pubescent beneath; stipules filiform from a subulate base, caducous. **Inflorescence** terminal dichotomous cyme**,** small, sessile or shortly peduncled, dense-flowered tomentose, bract minute (appressed to branches, < 2 mm. long), appressed to branch, usually homostylous. **Flower** buds rounded or slightly angular in the upper part and at the top, ***calyx-teeth*** short 5 lobes, subulate, valvate in bud; ***corolla*** 0.6 mm, puberulous, tube narrow; ***stamen*** 5, inserted on the corolla tube, anther linear; ***disk*** very large, 2-lobed; ***ovary*** 2-loculed, ovule many in each locule, basal placentation; ***style*** filiform; ***stigma*** positioned at the anthers level in the middle part of the corolla tube. **Fruits** capsules coriaceous, compressed, obcordate,pubescent; ***peduncle*** up to 3 cm. long, pedicels short, to 1.5 mm (9-10).

Figure 1 *Ophiorrhiza trichocarpon* Blume. Queen Silikit Botanic Gaden. Mea rim district Chiang Mai province, Thailand.

3.2. *Ophioriza rugosa* Wall. (Syn. *O. prostrata* Don). is a slender herb with rooting below. Its stem is above pubescent or tomentose. The **leaves** are 2.5-6.3 cm. ovate-lanceolate, acuminate at both end with minute scattered hairs above and pubescent or tomentose on leaf-nerves beneath; stipule with 1 or more long points. **Inflorescence** cymes few-fid tomentose or glabrate rather contracted, bracteoles few filiform caduceus or ebracteolated; ***calyx-teeth*** subulate; ***corolla*** glabrous or pubescent 0.6-0.8 cm. tubular, lobes short keeled on the back. **Fruits** capsule glabrous (9-10).

Figure 2 *Ophiorrhiza rugosa* Well. Queen Silikit Botanic Gaden. Mea rim district Chiang Mai province, Thailand.

3.3. *Ophiorrhiza* aff. *nutans* Cl. *ex* Hk. f. is a herb. Its stem is creeping below then erect. The **leaves** are 7.5-12.6 cm. elliptic-lanceolate, acuminate glabrous above or with scattered hairs, nerves pubescent beneath, lurid when dry; stipules large subulate-lanceolate ciliate entire or 2-fid. **Inflorescence** cymes, 2.5-5.5 cm. diameter, flat-topped, rough with crisped hairs, contracted peduncle very stout suberect; bracteoles large, half as long as the flowers, lanceolate or linear-oblong, ciliate; ***calyx-teeth*** ovate, acute, glabrous or hispid; ***corolla*** white, 1.6 cm. long, villous within, tubular glabrous, lobes short dilated at the base, narrowing upward. **Fruits** capsule hispid (10).

Figure 3 *Ophiorrhiza* aff. *nutans* Cl. *ex* Hk. f. Doitung mountain forest Maephaloung district, Chiang Rai Province