

## CHAPTER IV CONCLUSION

In this research, the bark of *Bombax malabaricum* which was the plant in the Bombacaceae, were investigated for their chemical constituents because of their utilization as therapeutic drugs. From the bark of *Bombax malabaricum*, five chemical constituents were obtained from hexane, chloroform and n-Butanol extracts which were separated by using flash chromatography, they were :

I, a white solid, 0.04 g (0.06% wt. by wt. of hexane crude extract) was a mixture of seven long chain aliphatic hydrocarbons ( $C_nH_{2n+2}$ ), m.p 57-58 °C

II, a white needle-like crystals, 0.09 g (0.10% wt. by wt. of hexane crude extract and 0.02% wt. by wt. of chloroform crude extract) was  $\beta$ -sitosterol, m.p 137-138 °C,  $R_f = 0.72$  (silica gel : 5% MeOH in chloroform)

III, a white amorphous solid, 0.03 g (0.04% wt. by wt. of chloroform crude extract) was  $\beta$ -sitosteryl-3-O- $\beta$ -D-glucopyranoside, m.p 256-258 °C,  $R_f = 0.44$  (silica gel : 10% methanol in chloroform)

VI, a pale purple amorphous solid, 0.07 g (0.10% wt. by wt. of hexane crude extract and 0.10% wt. by wt. of chloroform crude extract) was Lupeol, m.p 215-216 °C, %  $R_f = 0.37$  (silica gel : 5% methanol in chloroform)

V, a yellow oil, 0.004 g ( $1.16 \times 10^{-2}$ % wt. by wt. of n-BuOH crude extract) was 2-hydroxy-2-methyl propanoic acid, ethyl ester, m.p 208-210 °C,  $R_f = 0.52$  (silica gel : 5% methanol in chloroform)

It was previously found that Lupeol isolated from this plant possessed biological activities. Lupeol is useful in therapeutic drugs. There were reports which revealed biological activities of Lupeol such as Poliomyetitis, Antimalignancy in Spragne rat (38), effect a fungus type Verticillium (39) and Lupeol acetate antisoreness (40).



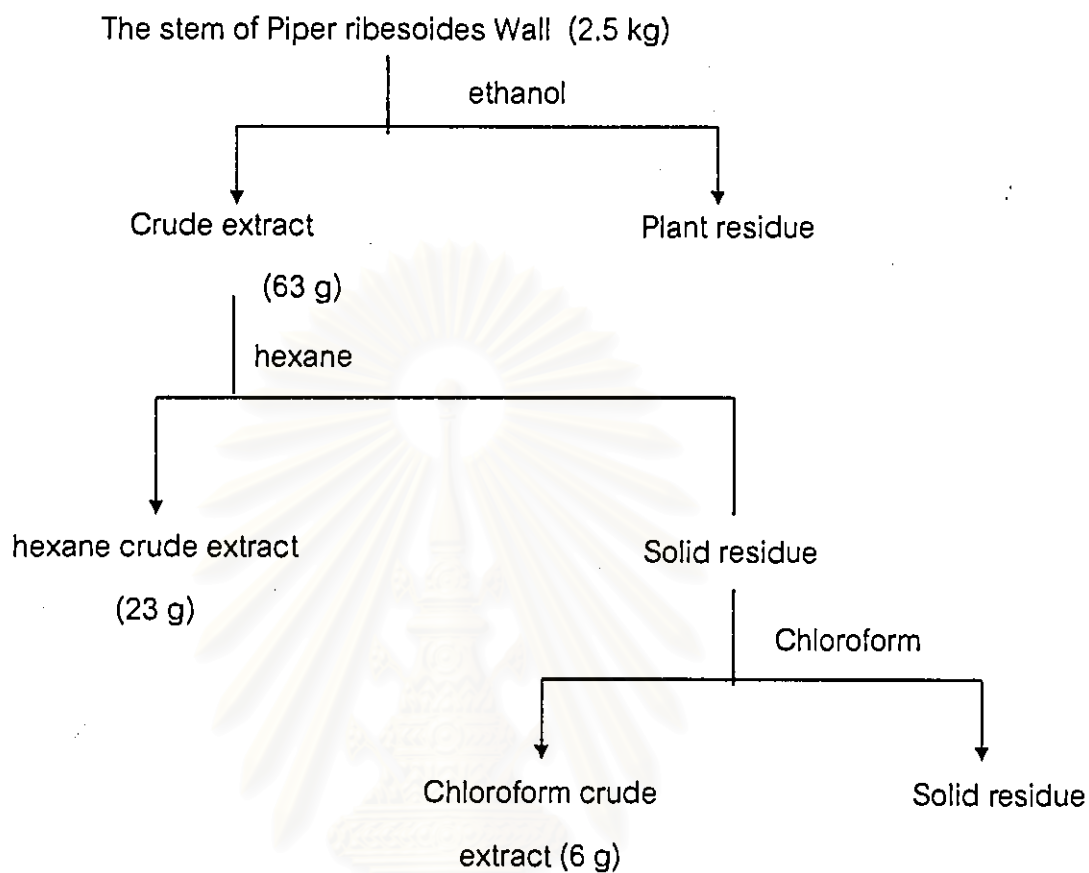
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From previous research, (31) *Piper* L. (Piperaceae) is a large genus of herbs, or somewhat woody climbers found in the warm, humid regions of the world. Several species furnish pepper and other spices. *Piper ribesoides* wall is known locally as "Ta Khaan" and is distributed in the forests of southern Thailand. The fruits and stems of *Piper ribesoides* are used in Thai folklore medicine as a carminative and a stimulant (2).

The stems of *Piper ribesoides* wall. were collected from Trung Province, Thailand, in June, 1997 and were authenticated by comparison with herbarium specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and cooperatives, Thailand. A voucher specimen of plant materials is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

The stems of *Piper ribesoides* Wall. (2500 g) were dried-air and soaked in 10 litres of ethanol for 5 days at room temperature. The process was repeated for 3 times. The solution was filtered and the combined filtrate was evaporated to give the ethanol extract as dark brown resinous material weighed 63.30 g (2.53% wt. by wt. of the stem of *Piper ribesoides* Wall.).

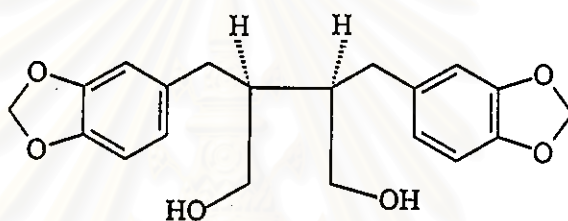
The crude extract was re-extracted by hexane until the solution was colorless. The filtered solution was concentrated to afford the hexane crude extract as dark green oil gave 23 g (0.93% wt. by wt. of the stem of *Piper ribesoides* Wall.). After extraction with hexane, the residue was further processed by chloroform, respectively. The chloroform crude extract as dark brown oil gave 6 g (0.25% wt. of the stem of *Piper ribesoides* Wall.).

Scheme 2 Extraction of the stem of *Piper ribesoides* Wall.

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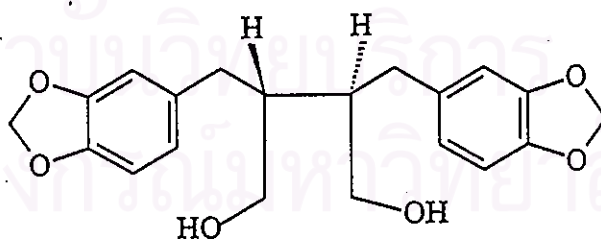
One compound, interesting chemical constituent of the stem of *Piper ribesoides* Wall. was isolated from chloroform crude extracts by silica gel column chromatography 2,3-Bis (1,3-benzodioxol-5-ylmethyl) -1,4-butanediol. (Dihydrocubebin) (41).

The conformation of this compound was then studied by  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , Mass spectrum and elemental analysis.



Cis-form (I)

or



Trans-form (II)

The structure of Cis, Trans 2,3-Bis (1,3-benzodioxol-5-ylmethyl)-1,4-butanediol

Table 3.14 The IR absorption band assignments of Compound VI

Wavenumber (cm <sup>-1</sup> )	Band type	Assignments
3445	strong, broad	O-H stretching vibration of O-H
2975,2856	strong	C-H stretching vibration of -CH <sub>3</sub> ,-CH <sub>2</sub> -
1650	weak	C=C stretching vibration
1075,1125	strong	C-O stretching vibration
825	strong	C-H out of plane bending vibration

This compound was bright brown solid and melting was 112-113°C

The IR spectrum (Fig. 26) indicated an important absorption band of hydroxy group at 3445 cm<sup>-1</sup> and absorption band of C-O at 1075-1125 cm<sup>-1</sup>.

The <sup>1</sup>H-NMR spectrum (Fig. 27) showed signals. The chemical at 2.58-2.74 ppm (AB quartet) was methylene proton which was attached to the aromatic ring and at 3.44-3.76 ppm indicate the methylene proton (AB'quartet) which was attached to a carbon bearing a hydroxy group. The chemical shift at 4.14 ppm was a hydroxy group at 5.88 ppm was a methylene proton of a methylenedioxy group and the signal at 6.57-6.69 ppm indicated the presence olefinic proton of an aromatic rings.

The <sup>13</sup>C, DEPT 135 and DEPT 90 NMR spectra (Fig. 28-29) revealed this compound had 3 methylene carbons, 4 methine carbons and 3 quaternary carbons.

The mass spectrum (Fig. 30) showed molecular ion peaks (*m/e* 358), the dominant fragmentation were found at *m/e* 340, 204, 149, 135, 105 and 77

The elemental analysis found C=5.57% , H=6.30% and O=1.67%

From the elemental analysis and spectroscopic data, this compound was 2,3 Bis (1,3-benzodioxol-5-ylmethy) -1,4- butanediol (II)

## CONCLUSION

In this research, the stems of *Piper ribesoides* Wall. which was the plant in the Piperaceae, were investigated for their chemical constituents because of their utilization as tribal medicine. From the stem of *Piper ribesoides* Wall., one chemical constituent was obtained from chloroform extracts which was separated by using flash chromatography. It was :

VI, a bright brown solid was 2,3-Bis (1,3-benzodioxol-5-ylmethyl)-1,4-butanediol (Dihydrocubebin),  $C_{20}H_{22}O_6$ , m.p 112-113 °C



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