CHAPTER IV

RESULTS AND DISCUSSION

1. Extraction yield

The three dried powders of whole plants *Ophiorrhiza trichocarpon* Bl., *Ophiorrhiza rugosa* Wall. and *Ophiorrhiza* aff. *nutans* Cl. *ex* Hk. f. were exhaustively extracted by hot methanol for 12 hr; the combined methanolic extracts were evaporated to dryness under reduced pressure, The percentage yields are shown in Table 4.1.

Table 4.1 Percentage yields of crude extracts from three *Ophiorrhiza* species.

Plant Name	Powdered plant wt. (kg)	Crude methanol extract wt. (g)	% yield*
Ophiorrhiza trichocarpon Bl.	2.15	303.10	14.1
Ophiorrhiza rugosa Wall.	0.12	12.07	10.23
Ophiorrhiza aff. *nutans* Cl. *ex* Hk. f.	1.45	199.60	13.77

* The percentage yield was calculated on dried weight basis

The crude methanolic extract of *O.* aff. *nutans* Cl. *ex* Hk. f. was partitioned by hexane, dichloromethane, ethyl acetate and n-butanol respectively. Each crude extract was evaporated to dryness under reduced pressure. The percentage yields of crude extracts were presented in Table 4.2.

Table 4.2 Percentage yields of crude extracts of three *Ophiorrhiza* species in various solvents.

Crude extract from solvent partition	crude extract wt. (g)	% yield*
***O. trichocarpon* Bl. 80.20 g.**		
Hexane extract	32.45	5.7
Dichloromethane extract	6.20	1.09
Ethyl acetate extract (Crude alkaloids)	1.58	0.27
n-Butanol extract	6.60	1.16
***O. rugosa* Wall. 11.03 g.**		
Hexane extract	4.45	4.06
Dichloromethane extract	0.56	0.51
Ethyl acetate extract (Crude alkaloids)	0.22	0.20
n-Butanol extract	1.48	1.35
***O.* aff. *nutans* Cl. *ex* Hk. f. 158.30 g.**		
Hexane extract	66.00	5.74
Dichloromethane extract	3.80	0.33
Ethyl acetate extract (Crude alkaloids)	1.50	0.13
n-Butanol extract	12.80	1.11

* The percentage yield was calculated on dried weight basis

2. Phytochemical screening studies

The phytochemical in crude methanolic extracts of *O. trichocarpon* Bl., *O. rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk. f. were screened by using chemical test and thin layer chromatography. Results indicated that alkaloid, coumarins, anthaquinone glycosides presented in all crude extracts except flavonoids. Scopoletin and saponins presented in only methanolic extracts of *O. trichocarpon* Bl. and *O.* aff. *nutans* Cl. *ex* Hk. f. Results are summarized in **Table 4.3.**

Table 4.3 Phytochemical screening of crude methanolic extracts of three *Ophiorrhiza* species.

Test	Plant to be tested		
	***O. trichocarpon* Bl.**	***O. rugosa* Wall.**	***O.* aff. *nutans* Cl. *ex* Hk. f.**
Alkaloids			
Kraut's reagent	Turbidity +	Turbidity +	Turbidity +
Dragendroff's reagent	Turbidity ++	Turbidity ++	Turbidity ++
Mayer's reagent	Yellow turbidity + + +	Yellow turbidity + + +	Yellow turbidity + + +
Velser's ragent	Yellow turbidity + + + +	Yellow turbidity + + + +	Yellow turbidity + + + +
Wagner's reagent	Yellow turbidity + + + + +	Yellow turbidity + + + + +	Yellow turbidity + + + + +
Flavonoids	Not detected	Not detected	Not detected
Coumarins	Bright blue green	Bright blue green	Bright blue green
Antraquinoneglycoside	A bit of pink red	Moderate of pink red	Increase of pink red
Scopoletin	Bright at 365 nm	Not detected	A bit bright at 365 nm
Saponin	Beehive bubble	Not detected	Beehive bubble

3. Bioactivity studies

3.1 Antioxidant activity

The radical-scavenging activity on DPPH$^{\bullet}$ was expressed as IC_{50}. This value was the concentration of crude extract of three *Ophiorrhiza* species and crud extracts of *O.* aff. *nutans* Cl. *ex* Hk.f. required to inhibit 50% of the initial DPPH$^{\bullet}$ free radical. The IC_{50} of all crude extracts are shown in **Table 4.4** and **Table 4.5.**

Table 4.4 Antioxidant activities of crude methanolic extracts of *O. trichocarpon* Bl., *O. rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk. f. extract by DPPH$^{\bullet}$ assay.

Sample	IC_{50}(mg mL^{-1})
***O. trichocarpon* Bl.**	5.09
***O. rugosa* Wall.**	3.02
***O.* aff. *nutans* Cl. *ex* Hk. f.**	2.76
Standard	
Ascorbic acid	0.05
Trolox	0.08
Quercetin	0.05

The methanolic extract of *O.* aff. *nutans* Cl. *ex* Hk. f. exhibited the highest antioxidant activity with the IC_{50} of 2.76 mg mL^{-1}. But the methanolic extract of *O. trichocarpon* Bl. showed the lowest antioxidant activity with the IC_{50} of 5.09 mg mL^{-1}, using ascorbic acid, Trolox and quercetin as reference standard (**Table 4.4**).

Table1 4.5 Antioxidant activities of crude extracts of *Ophiorrhiza* aff. *nutans* Cl. *ex* Hk. f. by $DPPH^{\bullet}$ assay.

Sample	IC_{50}(mg mL^{-1})
Crude extract of *O.* aff. *nutans* Cl. *ex* Hk. f.	
Hexane extract	5.17
Dichloromethane extract	3.36
Ethyl acetate extract	1.99
n-Butanol extract	2.35
Standard	
Ascorbic acid	0.05
Trolox	0.08
Quercetin	0.05

From **Table 4.5** it is indicated that the ethyl acetate extract showed the highest antioxidant activity (IC_{50} = 1.990 mg mL^{-1}), whereas the hexane extract showed the lowest antioxidant activity (IC_{50} = 5.710 mg mL^{-1}).

3.2 Antimicrobial activity

The antibacterial activities of three *Ophiorrhiza* species were studied. The crude methanolic extracts of *O. trichcarpon* Bl., *O. rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk. f. showed antibacterial activities against gram-positive and gram-negative bacteria, using gentamycin 75 μg mL^{-1} as reference standard. Results are shown in **Table 4.6**. All crude extracts showed inhibition against *Escherichia col*i 0.029, 0.021 and 0.022 μg mL^{-1}, *Staphylococcus aureus* 0.172, 0.176, 0.185 μg mL^{-1} respectively and also showed inhibition against *Pseudomonas aeruginosa* 0.044 and 0.221 μg mL^{-1} respectively.

The antifungal activities of three methanolic extracts of *Ophiorrhiza* species were also investigated, using ketoconazole 250 μg mL^{-1}as reference standard. All crude extracts showed antifungal activities against *Candida albican, Aspergillus flavas* and *Trichrophyton mentagrophyte.* Results were shown in **Table 4.6.**

Table 4.6 Antimicrobial activities crude methanolic extracts of three *Ophiorrhiza* species.

sample	inhibition activity (mg mL^{-1})			inhibition activity (mg mL^{-1})		
	E. coli	***P. aeruginosa***	***S. aureus***	***C. albican***	***A. flavas***	***T. mentagofyte***
O. trichocarpon Bl.	75.00	10.00	10.00	50.00	75.00	30.00
O. rugosa Wall.	100.00	50.00	10.00	50.00	75.00	30.00
O. aff. *nutans* Cl.ex Hk. f.	100.00	10.00	10.00	50.00	75.00	30.00

Reference standard of antibacterial = Gentamicin tandard (75 µg mL^{-1})
Reference standard of antifungal = Ketoconazole standard (250 µg mL^{-1})
Minimum of inhibition zone= 11.00 mm

The antibacterial activities of each crude extract from *O.* aff. *nutans* Cl. *ex* Hk. f. were investigated by means of agar well diffusion method. The inhibition zones were measured. The crude ethyl acetate extract showed inhibition against all bacterias. But the crude hexane, dichloromethane and n-butanol extracts showed inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus* except *Escherichia coli* as shown in **Table 4.7.** The antifungal activities of crude hexane, dichloromethane and ethyl acetate extracts gave inhibition zones against *Candida albicans* and *Trichophyton mentagophyte* except *Aspergillus flavas.* But the crude n-butanol extract showed only inhibition against *Candida albicans* except *Trichophyton mentagophyte* and *Aspergillus flavas.* Results were presented in **Table 4.7.**

Table 4.7 Antimicrobial activities of each crude *Ophiorrhiza* aff. *nutans* Cl. *ex* Hk. f. extract.

Sample	Inhibition activity (mg mL^{-1})			Inhibition activity (mg mL^{-1})		
	E. coli	***P. aeruginosa***	***S. aureus***	***C. albican***	***A. flavas***	***T. mentagophyte***
Hexane extract	0.00	10.00	30.00	30.00	0.00	30.00
Dichloromethane extract	0.00	30.00	30.00	30.00	0.00	30.00
Ethyl acetate extract	30.00	10.00	10.00	30.00	0.00	30.00
n-Butanol extract	0.00	10.00	30.00	30.00	0.00	0.00

Reference standard of antibacterial = Gentamicin tandard (75 µg mL^{-1})
Reference standard of antifungal = Ketoconazole standard (250 µg mL^{-1})
Minimum of inhibition zone= 11.00 mm.

3.3 Cytotoxicity and Anticancer activity

The cytotoxicity of crude *O. trichocarpon* Bl. *O. rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk. f. extracts were studied using SRB assay (as shown in **Table 4.8**). All of the crude extracts showed inhibition against Vero cell (African green monkey kidney) with the IC_{50} of 27.51 µg mL^{-1}, 15.07 µg mL^{-1} and 12.17 µg mL^{-1} respectively.

Table 4.8 Anticancer activity of crude ethyl acetate extract of *O. trichocarpon* Bl., *O.rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk.f.

Sample	[a]Cytotoxicity against Vero cells IC_{50} ($\mu g\ ml^{-1}$)	Anti cancer cell line IC_{50} ($\mu g\ ml^{-1}$)		
		[b]KB-Oral Cavity cancer	[c]NCI-H187-Small cell lung cancer	[d]MCF7-Breast cancer
***O. trichocarpon* Bl.**	27.51	10.64	13.33	40.79
***O. rugosa* Wall.**	15.07	6.10	0.89	-
***O.* aff. *nutans* Cl. *ex* Hk. f.**	12.17	11.17	19.41	45.23

[a]Reference standard of cytotoxicity= Ellipticine 0.617 $\mu g\ ml^{-1}$

[b]Reference standard of anticancer=Ellipticine 0.475 $\mu g\ ml^{-1}$ and Doxorubicine= 0.133 $\mu g\ ml^{-1}$

[c]Reference standard of anticancer=Ellipticine 0.617 $\mu g\ ml^{-1}$ and Doxorubicine= 0.042 $\mu g\ ml^{-1}$

[d]Reference standard of anticancer=Doxorubicine= 0.649 $\mu g\ ml^{-1}$

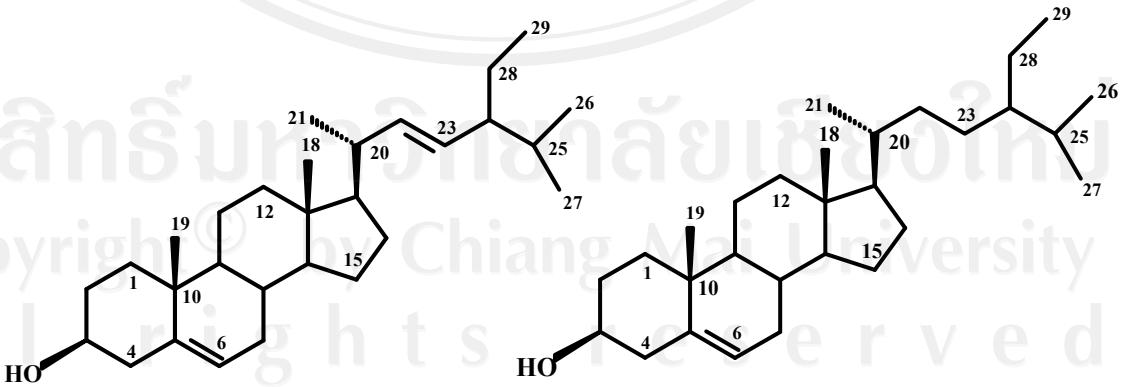
The anticancer activity of the crude *O. trichocarpon* Bl., *O. rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk.f. were investigated using NIC-H187-Small cell lung cancer, MCF7-Breast cancer and KB-Oral Cavity cancer. The crude *O. trichocarpon* Bl. and *O. rugosa* Wall. and *O.* aff. *nutans* Cl. *ex* Hk.f. extracts showed activities against KB-Oral cancer and Lung cancer. The IC_{50} for KB-Oral cavity cancer and NIC- H187 small cell lung cancer were 10.64 $\mu g\ mL^{-1}$, 6.10 $\mu g\ mL^{-1}$, 11.17 $\mu g\ mL^{-1}$ and 13.33 $\mu g\ mL^{-1}$, 0.89 $\mu g\ mL^{-1}$, 19.41 $\mu g\ mL^{-1}$ respectively. Only *O. trichocarpon* Bl. and *O.* aff. *nutans* Cl. extracts showed inhibition against MCF7- Breast cancer with the IC_{50} of 40.79 $\mu g\ mL^{-1}$ and 45.23 $\mu g\ mL^{-1}$ respectively except *O. rugosa* Wall. extract. Results were presented in **Table 4.8.**

4. Structure elucidation of the isolated compounds

4.1 Compound from crude dichloromethane extract

4.1.1 Compound G6P

The ^{1}H NMR ($CDCl_3$ 600 MHz) spectrum of a mixture of compound G6P indicated resonances for olefinic proton at δ 5.15 (1H, *dd*, *J*= 8.74, 8.80 Hz), 5.01 (1H, *dd*, *J*= 8.80, 0.80 Hz) and 5.35 (2H, *t*, *J*= 3.30, 1.93 Hz). The protons at δ 5.15, 5.01 and one of the protons at 5.35 were assigned to stigmasterol. The other hydrogen at δ 5.35 was attributed to β- sitosterol. The multiplet at 3.53 (1H) was assigned to the carbinyl protons of both compounds. Methyl groups of stigmasterol were deduced from the resonances at δ 0.70 (*s*), 0.68 (*s*), 1.08 (*d, J*= 4.40 Hz), 0.77 (*d*, *J*= 1.93 Hz), 0.83 (*d*, *J*=1.92 Hz), and 1.05 (*t, J*=6.05, 6.32 Hz). Due to the similarity in the structure of compound G6P, the methyl and methine resonances for β-sitosterol were overlapping with those of stigmasterol. The stigmasterol and β-sitosterol was deduced from the integrals of the resonances for the δ 0.70 methyl of stigmasterol and the 0.93 methyl of β-sitosterol. The ^{13}C NMR spectrum of stigmasterol and β-sitosterol were compare with data that published shown in the **Table 4.9**. The signal corresponding to the molecule formula of $C_{29}H_{48}O$ (stigmasterol) and $C_{29}H_{50}O$ (β-sitosterol), which was indentified by comparison with data that published (125, 131). Compound is β-sitosterol has also been found in *O. liukiuensis* (19).



Stigmasterol **β- sitosterol**

Figure 4.1 Structure of Stigmasterol and β- sitosterol (G6P)

Table 4.9 ^{13}C NMR analysis and relative ^{13}C-enrichment of b-sitosterol and stigmasterol of compound G6P from crude dichloromethane extract of *O.* aff. *nutans* Cl. *ex* Hk.f. compare with reference.

	Compound G6P		**Reference**	
	Chemical shift (ppm)		Chemical shift (ppm)	
Position	β-sitosterol	stigmasterol	β-sitosterol[a]	stigmasterol[a]
1"	37.22		37.21	
2"	31.63		31.62	
3"	71.79		71.80	
4"	42.18	42.27	42.18	42.29"
5"	140.7		140.72	
6"	121.7		121.71	
7"	31.86		31.87	
8"	31.86		31.87	
9"	50.10		50.08	
10"	36.48		36.48	
11"	21.07		21.07	
12	39.73	39.64	39.73	39.64
13"	42.27		42.29	
14	56.73	56.83	56.73	56.83
15	24.28	24.34	24.28	24.34
16	28.24	28.92	28.23	28.92
17	56.00	55.89	56.00	55.90
18	11.84	12.03	11.84	12.03
19"	19.39		19.38	
20	36.13	40.51	36.12	40.50
21	18.75	21.04	18.75	21.05
22	33.89	138.32	33.90	138.32
23	25.98	129.22	25.99	129.22
24	45.78	51.22	45.78	51.21
25	29.08	31.86	29.08	31.87"
26	19.82	21.20	19.81	21.20
27	19.00	18.96	19.00	18.95
28	23.02	25.40	23.02	25.40
29	11.96	12.26	11.96	12.25"

"The carbons with signals overlapping of the stigmasterol and β-sitosterol (carbon Nos. 1-3, 5-11, 13 and 19) or of different carbons (carbon Nos. [4 and 25 of stigmasterol] with carbon Nos. 13 and 7-8 of both phytosterols respectively).
a = reference 131

4.1.2 Compound P6bP

Compound P6bP was isolated as a colorless amorphous powder (1.06 mg). The ^{1}H-NMR (DMSO-d_6, 600 MHz) spectrum was showed δ 5.11 (1H, *(brs)*, H-12), 4.29 (1H, *d*, *J*= 5.28 Hz, -OH), 2.99 (1H, *m*, H-3), 2.09 (1H, *(brd)*, *J*= 11.22 Hz, H-18), 1.03 (3H, *s*, Me-27), 0.90 (3H, *s*, Me-30), 0.88 (3H, *s*, Me-24), 0.85 (3H, *s*, Me-25), 0.80 (3H, *d*, *J*= 6.27 Hz, Me-29), 0.74 (3H, *s*, Me-26) and 0.66 (3H, *s*, Me-23). ^{13}C-NMR (DMSO-d_6, 150 MHz) spectrum was showed δ 178.31 (COOH), 138.21 (C-13), 124.58 (C-12), 76.85 (C-3), 54.79 (C-5), 52.39 (C-18), 46.93 (C-17), 41.66 (C-14), 38.39 (C-4), 40.04 (C-8), 38.24 (C-1), 36.33 (C-22), 36.54 (C-10), 32.71 (C-7), 38.58 (C-19), 38.44 (C-20), 27.55 (C-15), 31.19(C-21), 23. 28 (C-27), 23.86 (C-11), 26.99 (C-2), 21.09 (C-30), 28.27(C-23), 23.82 (C-16), 17.03 (C-29), 15.23 (C-24), 16.00 (C-25) and 16.94 (C-26) respectively. The ^{1}H-NMR spectrum of P6bP showed seven tertiary methyl groups at δ 0.66 (*s*), 0.88 (*s*), 0.85 (*s*), 0.73 (*s*), 1.03 (*s*), 0.80 (*d*, *J*= 6.27 Hz) and 0.90 (*s*). Furthermore, the broad singlet at δ 5.11 showed the urs-12-en type in structure. The multiplete of proton with oxygented carbon at δ 2.99 showed 3β-hydroxyl functionality. The ^{13}C-NMR spectrum was showed two olefinic signals at δ 124.58 and δ 138.21, one acid signal at 178.31 and oxygened carbon of C-3 at 76.85. The molecule formula was determined to be $C_{30}H_{48}O_3$. Accordingly, the structure of P6bP was elucidated as ursolic acid (3-hydroxy-urs-12-en-28-oic acid), which was indentified by comparison of data with the published value shown in **Table 4.10** (126, 132). This compound has also been found in *O.liukiuensis* (19).

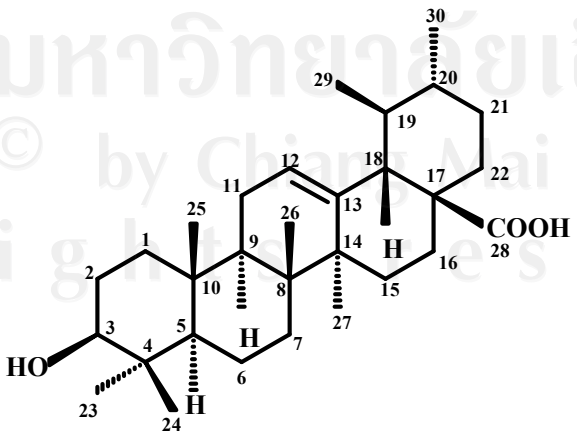


Figure 4.1 Structure of Ursolic acid (P6bP)

Table 4.10 ^{1}H and ^{13}C NMR analysis of compound P6bP from crude dichloromethane extract of *O.* aff. *nutans* Cl. *ex* Hk.f. compare with reference.

	Compound P6bP			**Reference**		
	Chemical shift			Chemical shift		
Position	^{1}H	*J*(Hz)	^{13}C	$^{1}H^{a}$	J^{a}(Hz)	$^{13}C^{a}$
1	-	-	38.24	-	-	39.2
2	-	-	26.99	-	-	28.2
3	2.99	*m*	76.85	3.00	*m*	78.2
4	-	-	38.39	-	-	39.6
5	-	-	54.79	-	-	55.9
6	-	-	18.00	-	-	18.8
7	-	-	32.71	-	-	33.7
8	-	-	40.04	-	-	40.1
9	-	-	47.02	-	-	48.1
10	-	-	36.54	-	-	37.5
11	-	-	22.86	-	-	23.7
12	5.11	*br s*	124.58	5.13	*m*	125.7
13	-	-	138.21	-	-	139.3
14	-	-	41.66	-	-	42.2
15	-	-	27.55	-	-	28.8
16	-	-	23.82	-	-	25.0
17	-	-	46.85	-	-	48.1
18	2.09	*br d*	52.39	2.10	*d*, 11.25	53.6
19	-	-	38.52	-	-	39.5
20	-	-	38.44	-	-	39.4
21	-	-	30.19	-	-	31.1
22	-	-	36.33	-	-	37.4
23	0.66	*s*	28.27	0.68	*s*	28.8
24	0.88	*s*	15.23	0.89	*s*	16.5
25	0.85	*s*	16.00	0.87	*s*	15.7
26	0.74	*s*	16.94	0.75	*s*	17.5
27	1.03	*s*	23.28	1.04	*s*	24.0
28	-	-	178.31	-	-	179.7
29	0.80	*d*, 6.27	17.03	0.81	*d*, 6.25	17.5
30	0.90	*d, 5.52*	21.09	0.92	*d*, 6.50	21.4
-OH	4.29	*br s*	-	4.31	*br s*	-

a = reference 132

4.1.3 Compound P6fP

The ^{1}H NMR spectrum (600 MHz, $CDCl_3$) displayed a clear doublet centered at δ 6.27 (1H, *d*, 9.35 Hz) and 7.61 (1H, *d*, 9.62 Hz), which were typical for H-3 and H-4. The spectrum also showed a singlet at 6.85(H-5), 6.92(H-8) and broad singlet at 6.18, each of one proton intensity. These could assign aromatic protons at C-5, C-8 and a hydroxyl group proton at C-7 respectively. A three proton singlet in the spectrum at 3.96 revealed the presence of a methyl group. Comparison of chemical shifts of the methoxyl and hydroxyl groups allows place this substituent at C-6 and C-7 respectively. On this basic compound P6fP was characterized as 7-hydroxy-6-methoxycoumarin or Scopoletin ($C_{10}H_8O_4$) which was indentified by comparison with data that published in **Table 4.11** (133, 134), which has also been found in *O. liukiuensis* (19).

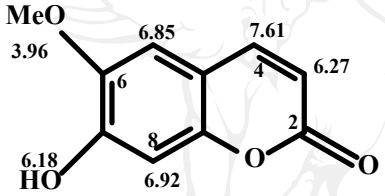


Figure 4.2 Structure of Scopoletin (P6fP)

Table 4.11 ^{1}H and ^{13}C NMR analysis of compound P6fP from crude dichloromethane extract of *O.* aff. *nutans* Cl. *ex* Hk.f. compare with reference.

	Compound P6fP			**Reference**				
	Chemical shift			Chemical shift				
Position	^{1}H	*J*(Hz)	^{13}C	$^{1}H^{a}$	J^{a}(Hz)	$^{1}H^{b}$	J^{b}(Hz)	$^{13}C^{b}$
2	-	-	161.46	-	-	-	-	160.50
3	6.27	*d*, 9.62	113.38	6.25	*d*, 9.40	6.26	*d*, 9.40	112.50
4	7.61	*d*, 9.40	143.31	7.58	*d*, 9.40	7.58	*d*, 9.40	142.00
5	6.85	*s*	107.42	6.83	*s*	6.89	*s*	107.50
6	-	-	143.97	-	-	-	-	143.00
7	6.18	*br s*	149.65	6.10	*br s*	-	*br s*	149.50
8	6.92	*s*	103.16	6.91	*br s*	6.82	*br s*	102.00
9	-	-	111.47	-	-	-	-	110.50
10	-	-	150.20	-	-	-	-	150.00
6'(MeO)	3.96	*s*	56.38	3.25	*br s*	3.93	*br s*	52.20

a = reference 133, b = reference 134

4.2 Compound from crude ethyl acetate extract

4.2.1 Compound H4d1P

Compound H4d1P was isolated as an amorphous powder. The ^{1}H and ^{13}C NMR spectrum of signal correspond to the molecular formula, $C_{13}H_{20}O_3$. The presence of a –CO-CH=C-Me group was indicated, at δ 1.89 (3H, *d*, J= 1.37 Hz) coupled to a signal at 5.91 (1H, *s*). The presence of the group representing, -CH=CH-CH(OH)Me, was established as follows: 2H signal in the NMR spectrum at δ 5.86 (*dd*, *J*= 15.67, 5.50 Hz) and δ 5.79 (*d*, *J*= 15.67 Hz). Coupling constant suggested the presence of double bond. The signal at δ 1.30 (3H, *d*, *J*=6.32 Hz) was found to be coupled to a multiplet signal at δ 4.42 (1H, *m*, *J*= 6.32 Hz) and coupled to one of the ethylenic protons at δ 5.86 (1H, *dd*, *J*= 6.32 Hz). The ^{1}H NMR spectrum also had signals of tertiary methyl group at 1.01 and 1.08, a pair of isolated methylene protons centered at δ 2.46 and 2.24 (2H, *dd*, *J*= 17.05 Hz). On the basic of above result was determined to be Blumenol A ($C_{13}H_{20}O_{3)}$. The structure was confirmed by measuring the ^{1}H-detected multiple-bond multiple-quantum coherence (HMBC) spectrum and comparison data with the published value shown in **Table 4.12** (128).

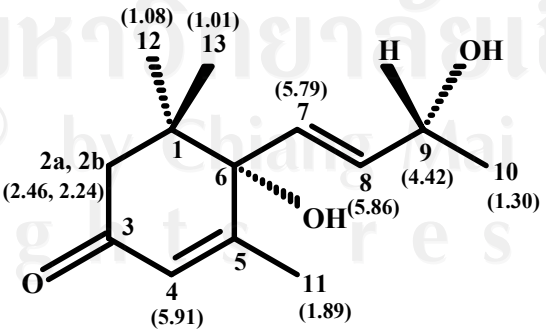


Figure 4.3 Structure of Blumenol A (H4d1P)

Table 4.12 ^{1}H and ^{13}C NMR analysis of compound H4d1P from crude ethyl acetate extract of *O.* aff. *nutans* Cl. *ex* Hk.f. compare with reference.

	Compound H4d1P			**Reference**		
	Chemical shift			Chemical shift		
Position	^{1}H	*J*(Hz)	^{13}C	$^{1}H^{a}$	J^{a} (Hz)	$^{13}C^{a}$
1	-	-	41.22	-	-	41.14
2a	2.46	*d*, 15.05	49.89	2.45	*d*, 17.0	49.67
2b	2.24	*d*, 15.05	49.8	2.25	*d*, 17.0	49.67
3	-	-	197.91	-	-	198.06
4	5.91	*s*	127.03	5.91	*br s*	126.89
5	-	-	162.58	-	-	162.51
6	-	-	79.14	-	-	79.02
7	5.79	*d*, 15.67	135.81	5.79	*d*, 15.5	135.74
8	5.86	*dd*, 15.67, 5.5	129.07	5.86	*dd*, 15.5, 6.0	129.04
9	4.42	*m*	68.14	4.42	*m*	68.01
10	1.30	*d*, 6.32	23.86	1.30	*d*, 1.5	29.71
11	1.01	*s*	22.98	1.02	*s*	22.87
12	1.08	*s*	24.13	1.09	*s*	24.06
13	1.89	*d,* 1.37	18.94	1.90	*br s*	18.87

a = reference 128

4.2.2 Compound I8eP

Ethyl acetate crude extract (0.6691 g) was purified by silica gel chromatography and Medium Pressure Liquid Chromatography (MPLC) to afford 1.4 mg of pure Harman; ^{1}H NMR ($CDCl_3$), δ 8.34 (1H, *d*, *J*= 5.22 Hz), 8.12 (1H, *d*, *J*=7.70 Hz), 7.83 (1H, *d*, *J*= 5.22 Hz), 7.55 (1H, *s*), 7.30 (1H, *t*, *J*= 7.70, 7.97 Hz), 7.26 (1H,*s*), 2.84 (3H, *s*); ^{13}C NMR ($CDCl_3$), δ 141.51 (C-1), 140.16 (C-8a), 138.17 (C-3), 134.48 (C-9a), 128.47 (C-7), 128.38 (C-4a), 121.91 (C-4b), 121.86 (C-5), 120.21 (C-6), 112.98 (C-4), 111.61 (C-8) and 20.03 (C-Me). The compound I8eP has the molecular formula $C_{12}H_{10}N_2$. ^{1}H and ^{13}C NMR spectra of I8eP revealed the presence of β- carboline alkaloids, which was identified as Harman by comparison of ^{1}H and ^{13}C NMR data with published values, shown in **Table 4.13** (129, 130) and this compound has also been found in *O. japonica O. liukiuensis* (17, 19, 25).

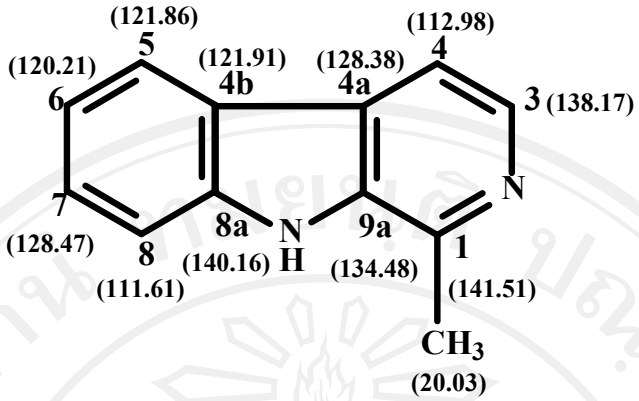


Figure 4.4 Structure of Haman (I8eP)

Table 4.13 ^{1}H and ^{13}C NMR analysis of compound I8eP from crude ethyl acetate extract of *O.* aff. *nutans* Cl. *ex* Hk.f. compare with reference.

	Compound I8eP			**Reference**					
	Chemical shift			Chemical shift					
Position	^{1}H	*J*(Hz)	^{13}C	$^{1}H^{a}$	J^{a}(Hz)	$^{13}C^{a}$	$^{1}H^{b}$	J^{b}(Hz)	$^{13}C^{b}$
1	-	-	141.51	-	-	142.9	-	-	142.1
3	8.34	*d*, 5.22	138.17	8.31	*d*, 5.30	139.0	8.26	*d*, 5.50	137.5
4	7.83	*d*, 5.22	112.98	7.82	*d*, 5.30	113.0	7.94	*d*, 5.50	112.6
4a	-	-	128.38	-	-	128.3	-	-	126.9
4b	-	-	121.91	-	-	122.8	-	-	121.1
5	8.12	*d*, 7.70	121.86	8.12	*d*, 7.90	122.0	8.12	*d*, 7.70	121.7
6	7.30	*t, 7.70*	120.21	7.28	*m*	120.4	7.25	*t, 7.70*	129.2
7	7.30	*t*, 7.70	128.47	7.56	*m*	128.5	7.56	*t*, 7.70	127.8
8	7.55	*s*	111.61	7.55	*m*	111.9	7.65	*d*, 7.70	111.9
8a	-	-	140.16	-	-	140.7	-	-	140.4
9a	-	-	134.48	-	-	135.7	-	-	134.5
1'(Me)	2.84	*s*	20.03	2.80	*s*	20.0	2.83	*s*	20.4

a = reference129, b = reference 130