CHAPTER IV

RESULTS AND DISCUSSION

By means of repeated chromatographic techniques, two compounds, Sp1 and Sp2 were isolated from crude MeOH extract of *Sapindus rarak* fruits.

Spectroscopic data (IR, MS, and NMR) were interpreted to determine the chemical structures of the two compounds. The structures were confirmed by comparative analysis using previous reports as references.

1. Structure determination of the isolated compounds

1.1 Structure determination of compound Sp1

Compound Sp1 was obtained as a white powder (55.32mg) with a melting point of 238-240 °C (lit. 232-234 °C), $[\alpha]_D^{29}$ + 14.0° (CH_3OH, *c* 0.10) [lit. $[\alpha]_D^{24}$ + 6.0° (CH_3OH, *c* 0.2)]. The FT-IR spectrum (Figure 11) showed the presence hydroxyl and carbonyl groups at 3319 and 1695 cm^{-1}, respectively. Other signals were tentatively assigned as shown in Table 19.

Table 19 The FT-IR absorption band assignments of compound Sp1

Wave number (cm^{-1})	Tentative assignments
3319	O-H stretching
2942	alkane C-H stretching
1695	C = O stretching
1453, 1386	C-H bending of methylene group
1265, 1136, 1054	C-O-C stretching

The ^{1}H-NMR spectrum (Figure 13-16) of compound Sp1 showed methyl groups at δ_H1.16 (3H, s, H-24), δ_H0.95 (3H, s, H-25), δ_H1.03 (3H, s, H-26), δ_H1.24 (3H, s, H-27), δ_H0.95 (3H, s, H-29), δ_H1.00 (3H, s, H-30), and other signals of δ_H4.28 (1H, overlapped, H-3), δ_H 3.30 (1H, dd, *J* = 13.7,3.7, H-18), δ_H1.16 (3H, s, H-24), δ_H5.48 (1H, br s, H-12), δ_H4.28 (2H, overlapped ,H-23), δ_H4.00 (1H, d, *J*=10.7, H-23), δ_H5.05 (1H, d, *J*= 6.6, H-1Ara), δ_H6.40 (1H, br s, H-1Rham), δ_H 5.44 (1H, d, *J*=7.8, H-1Xyl).

The ^{13}C - NMR spectrum (Figure 17) of compound Sp1 showed 30 carbons resonances from the aglycone part of the molecule (Table 20-21) confirming the presence of hederagenin aglycone with a glycosidic chain at C-3. The chemical shifts of C-3 at δ_c81.3 and C-28 at δ_c180.2 suggested that compound Sp1 is a monodesmoside with a glycosyl linkage at C-3. The signal arising from the carboxylic carbons in the

compound Sp1 appeared at δ_c180.2, indicating a free carboxylic acid group. In addition, the spectrum showed 16 carbon resonances due to the sugar part of the molecule (Table 22). The anomeric carbons gave rise to signals at δ_c104.7, δ_c101.4, and δ_c107.2 indicating the presence of three sugar and a signal at δ_c81.3 indicated a C-3 substituted glucose residue.

The EI-MS spectrum (Figure 12) of compound Sp1 suggested a molecular weight 882 Da ($[M-H]^-$ at m/z 881.3). The molecular formula $C_{46}H_{74}O_{16}$ as determined by EI-MS spectrometry with the $[M-H]^-$ peak appeared at m/z 881.3. Fragment ion peaks at m/z 749[(M-H)-132]$^-$, 603[(M-H)-(132+146)]$^-$, and 471 [(M-H)-(132+146+132)]$^-$ correspond to the loss of xylose, rhamnose and arabinose units from the molecular ion, respectively.

The molecular formula of compound Sp1 was assigned as $C_{46}H_{74}O_{16}$ by EI-MS spectrometry, ^{1}H and ^{13}C NMR spectra (Tables 20-24). From the molecular formula $C_{46}H_{74}O_{16}$ compound Sp1 must consist of five rings in addition to one double bond, one hydroxyl group, one carbonyl group and three sugar units. Several 2D-NMR techniques were then used to assist in the interpretation of the structure of this compound. All of the carbon-proton spin systems were traced by using data from a HMQC experiment (Figure 21). Heteronuclear correlation experiments, HMBC (Figures 18-20) allowed unambigouous assignment of all the ^{1}H-NMR and ^{13}C-NMR resonances of compound Sp1.

The HMBC (Figures 18-20) spectrum showed correlation peaks of AraH-1 with C-3 and H-3 with AraC-1 suggesting that the arabinose unit was linked to C-3 position of the aglycone unit, whereas the correlations between RhamH-1 and AraC-2, and AraH-2 and RhamC-1 indicated that

the rhamnose unit was linked to C-2 position of the arabinose unit. The correlations between XylH-1 and RhamC-3, and RhaH-3 and XylC-1 revealed that the terminal xylose unit was linked to C-3 position of the rhamnose unit.

By comparison of the spectroscopic data with those reported for sapindoside B, compound Sp1 was determined to be sapindoside B.

The data of compound Sp1 have been assigned as hederagenin 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (Sapindoside B) (Figure 9). Sapindoside B was previously isolated from *Sapindus mukurossi*, *Sapindus emarginatus* and *Sapindus delavayi.* [Kanchanapoom, Kasai and Yamasaki, 2001]

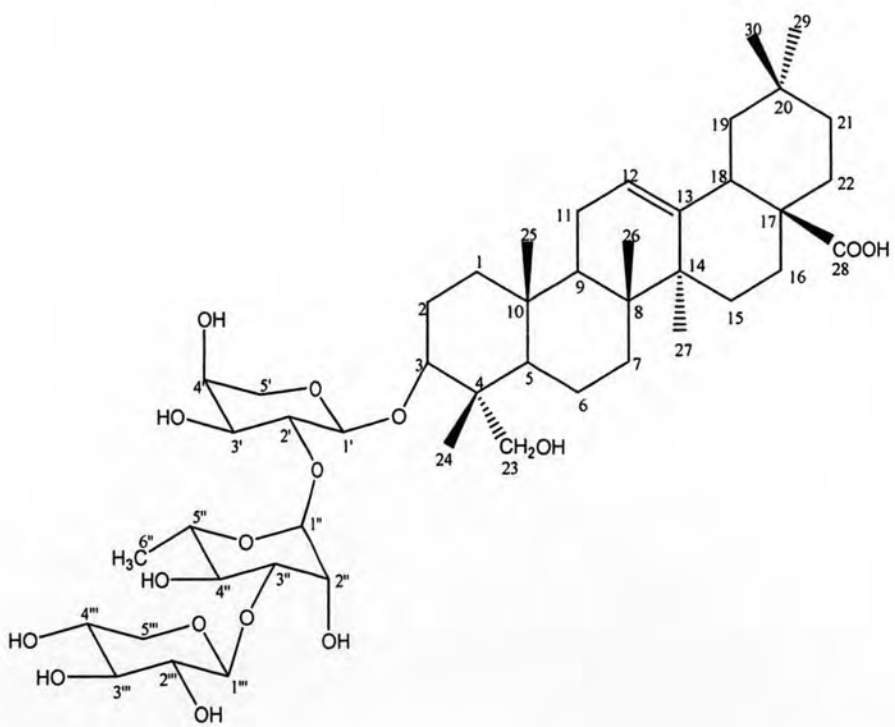

Figure 9 Structure of compound Sp1(Sapindoside B)

Table 20 ^{13}C-NMR Spectral Data of the Aglycone part of Compound Sp1 and Sapindoside B (recorded in pyridine-d_5)

Position	δ_C Compound Sp1(ppm)	δ_C Sapindoside B (ppm)
1	39.0	39.0
2	26.4	26.4
3	81.2	81.2
4	43.6	43.7
5	47.8	47.7
6	18.1	18.2
*7	33.2	33.2
8	39.7	39.8
9	48.2	48.3
10	36.9	36.9
11	23.8	23.9
12	122.6	122.6
13	144.8	144.8
14	42.1	42.2
15	28.3	28.4
16	23.7	23.7
17	46.6	46.7

Table 21 ^{13}C-NMR Spectral Data of the Aglycone part of Compound Sp1 and Sapindoside B (Cont.)

Position	δ_C Compound Sp1 (ppm)	δ_C Sapindoside B (ppm)
18	42.0	42.0
19	46.4	46.4
20	30.9	31.0
21	34.2	34.3
22	32.9	32.9
23	64.1	64.1
24	14.1	14.2
25	16.0	16.1
26	17.4	17.5
27	26.1	26.2
28	180.2	180.2
29	33.2	33.3
30	23.7	23.8

Recorded in pyridine-d_5.

Table 22 ^{13}C-NMR Spectral Data of the Sugar part of Compound Sp1 and Sapindoside B

Position	δ_C Compound Sp1(ppm)	δ_C Sapindoside B (ppm)
Ara-1'	104.7	104.7
2'	75.7	75.7
3'	74.8	74.8
4'	69.6	69.6
5'	65.6	65.6
Rha-1"	101.4	101.4
2"	71.9	71.9
3"	82.6	82.6
4"	73.0	73.0
5"	69.6	69.6
6"	18.4	18.4
Xyl-1"'	107.2	107.5
2"'	75.2	75.3
3"'	74.8	78.4
4"'	72.8	71.2
5"'	63.4	67.4

Recorded in pyridine-d_5

Table 23 ^{13}C and ^{1}H-NMR spectral data of the Compound Sp1

Position	δ_C Compound Sp1 (ppm)	δ_H Compound Sp1 (ppm) *J* (Hz)
3	81.2	4.28*
12	122.6	5.45 (br t)
18	42.0	3.27 (dd)
23	64.1	3.93 (d, 10.4), 4.28*
24	14.2	1.12 (s)
25	16.1	0.92 (s)
26	17.5	1.01(s)
27	26.2	1.23 (s)
29	33.3	0.91 (s)
30	23.8	0.98 (s)
Ara-1'	104.7	5.10*
2'	75.7	4.59 (dd, *J*=8.3, 7)
3'	74.8	4.02 (dd, *J*=8.3, 3.4)
4'	69.6	4.13*
5a'	} 65.6	3.67 (br d, *J*=10.9)
5b'		4.23*

Recorded in pyridine-d_5, * Overlapping signals.

Table 24 ^{13}C and ^{1}H-NMR spectral data of the Compound Sp1(Cont.)

Position	δ_C Compound Sp1 (ppm)	δ_H Compound Sp1 (ppm) *J* (Hz)
Rha-1"	101.4	6.35 (br s)
2"	72.0	4.92 (br s)
3"	83.0	4.77 (dd, *J*=9.5, 2.8)
4"	73.0	4.48 (t, *J* = 9.5)
5"	69.6	4.73*
6"	18.5	1.55 (d, *J* = 6.1)
Xyl-1'"	107.5	5.35 (d, *J* = 7.5)
2'"	75.3	4.06 (dd, *J*=8.5, 7.5)
3'"	78.4	4.13*
4'"	71.2	4.15*
5a'"	}67.4	3.67*
5b'"		4.22*

Recorded in pyridine-d_5.

* Overlapping signal

1.2 Structure determination of Compound Sp2

Compound Sp2 was obtained as white a powder (44.68mg) with a melting point of 192-195 °C (lit. 229-232 °C), $[\alpha]_D^{29}$ + 23.2 °(CH_3OH, *c* 0.10), [lit. $[\alpha]_D^{24}$ + 40 ° (CH_3OH, *c* 0.2)]. The FT-IR spectrum (Figure 22) showed the presence hydroxyl and carbonyl groups at 3321 and 1727 cm^{-1}, respectively. Other signals were tentatively assigned as shown in Table 25.

Table 25 The FT-IR absorption band assignments of compound Sp2

Wave number (cm^{-1})	Tentative assignments
3321	O-H stretching
2932	alkane C-H stretching
1727	C=O stretching
1455, 1375	C-H bending of methylene group
1250, 1136, 1050	C-O-C stretching

The ^{1}H-NMR spectrum (Figure 24-28) of compound Sp2 showed methyl groups at δ_H1.16 (3H, s, H-24), δ_H0.95 (3H, s, H-25), δ_H1.03 (3H, s, H-26), δ_H1.24 (3H, s, H-27), δ_H 0.95 (3H, s, H-29), δ_H1.00 (3H, s, H-3), δ_H 1.90 (3H, s, H-Ac). and δ_H 4.28 (1H, overlapped, H-3), δ_H3.30(1H, dd, *J*=13.7, 3.7, H-18), δ_H1.16 (3H, s, H-24), δ_H5.48 (1H, br s, H-12), δ_H4.28 (2H, overlapped, H-23), δ_H4.00 (1H, d, *J*=10.7, H-23), δ_H5.05 (1H, d, *J*=

6.6, H-1 Ara), δ_H6.40 (1H, br s ,H-1Rha), δ_H5.44(1H, d, *J*=7.8, H-1Xyl).

The presence of an acetyl group was demonstrated by the proton signal at δ_H1.90 (3H, s).

The ^{13}C-NMR spectrum (Figure 29) of compound Sp2 showed 30 carbons resonances from the aglycone part of the molecule (Tables 21-22) confirming the presence of hederagenin aglycone with a glycosidic chain at C-3. The chemical shifts of C-3 at δ_c81.3 and C-28 at δ_c180.2 suggested that compound Sp2 is a monodesmoside with a glycosyl linkage at C-3. The signal arising from the carboxylic carbons in the compound Sp2 appeared at δ_c180.2, indicating a free carboxylic acid group.

The ^{13}C-NMR spectrum (Figure 29) of compound Sp2 showed 18 carbon resonances from the sugar part of the molecule (Table 28). The anomeric carbons appeared at δ_c104.7, δ_c101.4, and δ_c107.2 indicating the presence of three sugar units. The carbon signals at δ_c170.5 and δ_c20.8 indicated the presence of an acetyl group. A signal at δ_c81.3 indicated a C-3 substituted glucose residue.

The presence of an acetyl group was demonstrated by the proton signal at δ_H1.90(3H, s) and the carbon signals at δ_c20.8, 170.5. Comparison of the ^{13}C-NMR data of compound Sp2 with those of compound Sp1 revealed the downfield shift of C-4 of terminal xylose unit (+1.7 ppm),and upfield shift of C-3 and C-5(-3.6, -4.0ppm), respectively, while the rest of the carbon signals remained almost unchanged.

The EI-MS spectrum (Figure 22) of compound Sp2 gave a molecular weight 924 Da ([M-H]$^-$ at m/z 923.4). The molecular formula $C_{48}H_{76}O_{17}$ as determined by EI-MS spectrometry with the [M-H]$^-$ peak appeared at m/z 923.4. Fragment ion peaks at m/z 881 [(M-H)-42]$^-$, 749 [(M-H)-132]$^-$, 603 [(M-H)-(132+146)]$^-$,and 471 [(M-H)-(132+146 +132)]$^-$ correspond to the loss of acetyl group, xylose unit, rhamnose unit and arabinose units from the molecular ion, respectively.

The molecular formula of compound Sp2 was assigned as $C_{48}H_{76}O_{17}$ by EI-MS spectrometry, ^{1}H and ^{13}C-NMR spectrum (Tables 25-30). From the ^{13}C-NMR spectrum and the molecular formula $C_{48}H_{76}O_{17}$ compound Sp2 must consist of five rings in addition to one double bond, one hydroxyl group, one carbonyl group and three sugar units. Several 2D-NMR techniques were then used to assist in the interpretation of the structure of this compound. All of the proton-proton spin systems were traced by using data from a COSY experiment (Figure 35). Heteronuclear correlation experiments, HMQC (Figure 34) and HMBC (Figures 30-33) allowed unambigouous assignments of all the ^{1}H-NMR and ^{13}C-NMR resonances in compound Sp2.

The HMBC spectrum showed correlation peaks of AraH-1 with C-3 and H-3 with AraC-1 suggesting that the arabinose unit was linked to C-3 position of the aglycone unit, whereas the correlations between RhamH-1 and AraC-2, and AraH-2 and RhamC-1 indicated that the rhamnose unit was linked to C-2 position of the arabinose unit. The correlations between XylH-1 and RhamC-3, and RhamH-3 and XylC-1 revealed that the terminal xylose unit was linked to C-3 position of the rhamnose unit.

In addition, the HMBC spectrum showed the correlation peaks of XylH-4 (proton at C-4 position of xylose unit) with C-AcO (acetyl carbonyl carbon) and the other correlation peaks were similar to those of compound Sp1. On the basis of this evidence, the acetyl group was located at C-4 of terminal xylose unit. Comparison of the spectroscopic data with reported values, compound Sp2 was determined to be mukurozi-saponin E_1.

The data of compound Sp2 have been assigned as hederagenin 3-*O*-(4-*O*-acetyl-*β*-D-xylopyranosyl)-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside (Mukurozi-saponin E_1). Compound Sp2 was previously isolated from *Sapindus mukurossi*, *Sapindus emarginatus* and *Sapindus delavayi.* [Kanchanapoom, Kasai and Yamasaki, 2001]

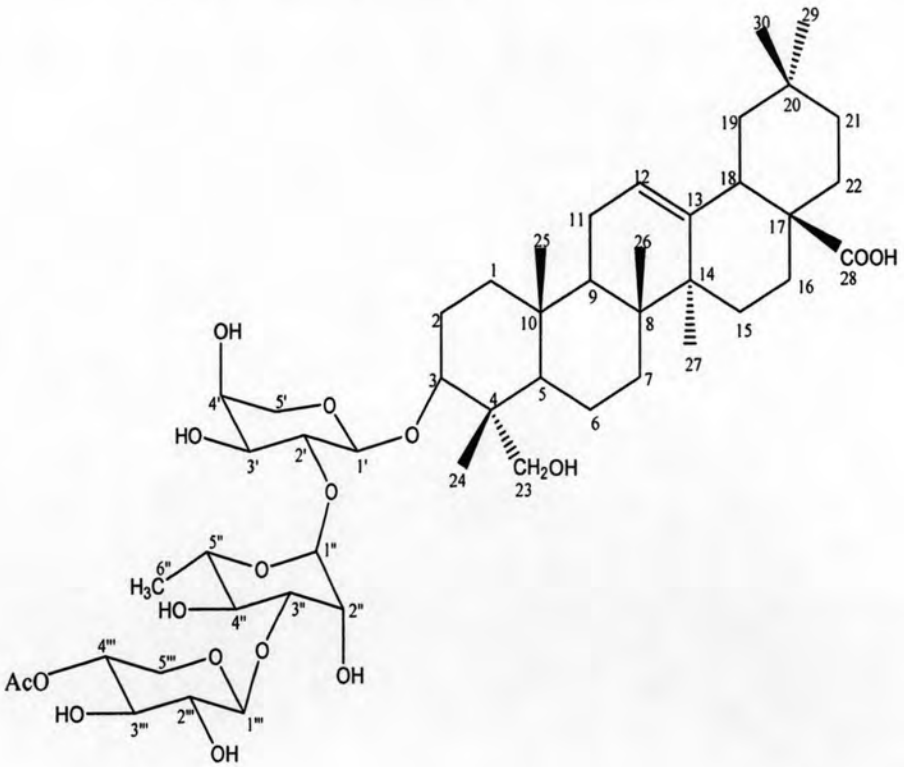

Figure 10 Structure of compound Sp2 (Mukurozi-saponin E_1)

Table 26 ^{13}C-NMR Spectral Data of the Aglycone part of Compound Sp2 and Mukurozi-saponin E_1 (recorded in pyridine-d_5)

Position	δ_C Compound Sp2 (ppm)	δ_C Mukurozi-saponin E_1 (ppm)
1	39.0	39.0
2	26.2	26.3
3	81.3	81.3
4	43.6	43.6
5	47.8	47.8
6	18.1	18.1
7	33.2	33.2
8	39.7	39.7
9	48.2	48.1
10	36.9	36.9
11	23.8	23.8
12	122.6	122.6
13	144.8	144.8
14	42.1	42.1
15	28.3	28.3
16	23.7	23.7
17	46.6	46.6

Table 27 ^{13}C-NMR Spectral Data of the Aglycone part of Compound Sp2 and Mukurozi-saponin E_1 (Cont.)

Position	δ_C Compound Sp2 (ppm)	δ_C Mukurozi- saponin E_1 (ppm)
18	42.0	42.0
19	46.4	46.4
20	30.9	30.9
21	34.2	34.2
22	32.9	32.9
23	64.1	64.1
24	14.1	14.0
25	16.0	16.0
26	17.4	17.4
27	26.1	26.1
28	180.2	180.2
29	33.2	33.2
30	23.7	23.7

Recorded in pyridine-d_5.

Table 28 ^{13}C-NMR Spectral Data of the Sugar part of Compound Sp2 and Mukurozi-saponin E_1

Position	δ_C Compound Sp2 (ppm)	δ_C Mukurozi-saponin E_1 (ppm)
Ara-1'	104.7	105.1
2'	75.7	75.7
3'	74.8	74.8
4'	69.6	69.2
5'	65.6	65.5
Rha-1"	101.4	101.5
2"	71.9	71.8
3"	82.6	82.6
4"	73.0	72.9
5"	69.6	69.6
6"	18.4	18.4
Xyl-1"'	107.2	107.0
2"'	75.2	75.5
3"'	74.8	74.4
4"'	72.8	72.8
5"'	63.4	63.4
$CH_3\underline{C}O$	170.5	170.5
$\underline{C}H_3CO$	20.8	20.8

Table 29 ^{13}C-NMR Spectral Data of the Aglycone part of Compound Sp1 and Compound Sp2

Position	δ_C Compound Sp1 (ppm)	δ_C Compound Sp2 (ppm)
1	39.0	39.0
2	26.4	26.2
3	81.2	81.3
4	43.7	43.6
5	47.7	47.8
6	18.2	18.1
7	33.2	33.2
8	39.8	39.7
9	48.3	48.2
10	36.9	36.9
11	23.9	23.8
12	122.6	122.6
13	144.8	144.8
14	42.2	42.1
15	28.4	28.3
16	23.7	23.7
17	46.7	46.6

Recorded in pyridine-d_5

Table 30 ^{13}C-NMR Spectral Data of the Aglycone part of Compound Sp1 and Compound Sp2 (continued)

Position	δ_C Compound Sp1 (ppm)	δ_C Compound Sp2 (ppm)
18	42.0	42.0
19	46.4	46.4
20	31.0	30.9
21	34.3	34.2
22	32.9	32.9
23	64.1	64.1
24	14.2	14.1
25	16.1	16.0
26	17.5	17.4
27	26.2	26.1
28	180.2	180.2
29	33.2	33.2
30	23.8	23.7

Recorded in pyridine-d_5.

Table 31 ^{13}C-NMR Spectral Data of the Sugar part of Compound Sp1 and Compound Sp2

Position	δ_C Compound Sp1 (ppm)	δ_C Compound Sp2 (ppm)
Ara-1'	104.7	104.7
2'	75.7	75.7
3'	74.8	74.8
4'	69.6	69.6
5'	65.6	65.6
Rha-1"	101.4	101.4
2"	72.0	71.9
3"	83.0	82.6
4"	73.0	73.0
5"	69.6	69.6
6"	18.5	18.4
Xyl-1"'	107.5	107.2
2"'	75.3	75.2
* 3"'	78.4	74.8
4"'	71.2	72.8
5"'	67.4	63.4
$CH_3\underline{C}O$	-	170.5
$\underline{C}H_3CO$	-	20.8

พลสมุดกลาง ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

Table 32 ^{13}C and ^{1}H-NMR spectral data of the Compound Sp2

Position	δ_C Compound Sp2 (ppm)	δ_H Compound Sp2 (ppm) *J* (Hz)
3	81.3	4.30*
12	122.6	5.45 (br t)
18	42.0	3.27 (dd)
23	64.1	3.93 (d, *J*=10.4), 4.28*
24	14.1	1.13 (s)
25	16.0	0.93 (s)
26	17.4	1.01(s)
27	26.1	1.23 (s)
29	33.2	0.90 (s)
30	23.7	0.98 (s)
Ara-1'	104.7	5.05*
2'	75.7	4.59 (dd, *J*=8.4, 7)
3'	74.8	4.00(dd, *J*=8.4, 3.5)
4'	69.6	4.12 (s)*
5a'	}65.6	3.65 (br d, *J*=11.1)
5b'		4.25*

Recorded in pyridine-d_5.

* Overlapping signals.

Table 33 ^{13}C and ^{1}H-NMR spectral data of the Compound Sp2 (Cont.)

Position	δ_C Compound Sp2 (ppm)	δ_H Compound Sp2 (ppm) *J* (Hz)
Rha-1"	101.4	6.35 (br s)
2"	71.9	4.88 (br s)
3"	82.6	4.77 (dd, *J*=9.5, 2.8)
4"	73.0	4.48 (t, *J*= 9.5)
5"	69.6	4.73*
6"	18.4	1.55 (d, *J*= 6.1)
Xyl-1"'	107.2	5.40 (d, *J* = 7.7)
2"'	75.8	4.05 (dd, *J*=8.5, 7.7)
3"'	74.8	4.20*
4"'	72.9	5.32 (t d, *J*=9.8, 5.4)
5a"'	}63.4	3.52 (dd, *J*=11.1, 9.8)
5b"'		4.18 (dd, *J*=11.1, 9.4)
$\underline{C}H_3CO$	20.8	1.91(s)

Recorded in pyridine-d_5.

* Overlapping signals.

Table 34 ^{1}H-NMR spectral data of the Compound Sp1 and Compound Sp2

Position	δ_H Compound Sp1 (ppm) *J* (Hz)	δ_H Compound Sp2 (ppm) *J* (Hz)
3	4.28*	4.30*
12	5.45 (br t)	5.45 (br t)
18	3.27 (dd)	3.27 (dd)
23	3.93 (d, 10.4), 4.28*	3.93 (d, 10.4), 4.28*
24	1.12 (s)	1.13 (s)
25	0.92 (s)	0.93 (s)
26	1.01(s)	1.01(s)
27	1.23 (s)	1.23 (s)
29	0.91 (s)	0.90 (s)
30	0.98 (s)	0.98 (s)
Ara-1'	5.10*	5.05*
2'	4.59 (dd, *J*=8.3, 7)	4.59 (dd, *J*=8.4, 7)
3'	4.02 (dd, *J*=8.3, 3.4)	4.00(dd, *J*=8.4, 3.5)
4'	4.13*	4.12 (s)*
5a'	3.67 (br d, *J*=10.9)	3.65 (br d, *J*=11.1)
5b'	4.23*	4.25*

Recorded in pyridine-d_5 * Overlapping signals

Table 35 ^{1}H- NMR spectral data of the Compound Sp1 and Compound Sp2 (continued)

Position	δ_H Compound Sp1 (ppm) J (Hz)	δ_H Compound Sp2 (ppm) J (Hz)
Rha-1"	6.35 (br s)	6.35 (br s)
2"	4.92 (br s)	4.88 (br s)
3"	4.77(dd, J=9.5, 2.8)	4.77(dd, J=9.5, 2.8)
4"	4.48(t, J = 9.5)	4.48(t, J = 9.5)
5"	4.73*	4.73*
6"	1.55(d, J = 6.1)	1.55(d, J = 6.1)
Xyl-1"'	5.35(d, J = 7.5)	5.40(d, J = 7.7)
2"'	4.06 (dd, J=8.5, 7.5)	4.05 (dd, J=8.5, 7.7)
3"'	4.13*	4.20*
4"'	4.15*	5.32 (t d, J=9.8, 5.4)
5a"'	3.67*	3.52 (dd, J=11.1, 9.8)
5b"'	4.22*	4.18 (dd, J=11.1, 9.4)
$\underline{C}H_3CO$	-	1.91(s)

Recorded in pyridine-d_5.

* Overlapping signals.

2. Results of Molluscicidal Activity

The results of molluscicidal testing of methanol extract, compound Sp1, compound Sp2 and chemical controls.

Table 36 The results of molluscicidal activity testing against *Pumacea canaliculata* at 24 hours intervals of methanol extract, compound Sp1, compound Sp2 and chemical controls.

Compounds	LC_{50} (ppm) (95 % confidence Limits of LC_{50})	LC_{90} (ppm) (95 % confidence Limits of LC_{90})
Niclosamide	0.27	1.12
Metaldehyde	34.98	89.57
CU1	60.32	99.32
methanol extract	11.19	19.82
Sp1	4.31	7.08
Sp2	4.28	6.83

LC_{50} and LC_{90} value in ml/l

According to the preliminary investigation, the methanol extract (crude saponin extracts) of *Sapindus rarak* DC. fruits exhibited high molluscicidal activity against *Pumacea canaliculata.*

The W.H.O. quantitates toxicity by means of LC_{90} values but LC_{50} values and 100 % snail kill values, all in ppm are also currently used [Marston and Hostettmann, 1985].

The LC_{50} value of the crude saponin extracts showed high molluscicidal activity. Niclosamide showed the highest activity level with LC_{50} value of 0.27 ppm but this pesticides is banned in many countries. [Calumpang *et al.*, 1995] reported that the LC_{50} value of niclosamide was 0.40 ppm. The rapid mortality, probably due to an acute toxic effect, is desirable, as it reduces the possibility of escaping behavior by the mollusc.

CU1 showed moderate activity with LC_{50} value of 60.32 ppm. The result was comparable to that reported by Nantiya [นันทิยา, 2543], in which the LC_{50} value of tea seed cake extract against golden apple snails with 3.5-5.0 cm. shell length was 48.79 ppm.

From molluscicidal activity test, the crude saponins extract showed the highest activity level with LC_{50} value of 11.19 ppm. Compound Sp1 (Sapindoside B) and compound Sp2 (mukurozi-saponin E_1) showed the highest activity level with LC_{50} value of 4.34 and 4.28 ppm, respectively.

3. Results of Cytotoxicity

The in vitro activity of some compounds (10μg / ml) from *Sapindus rarak* DC. against 5 cell lines, for example, KATO-III (gastric cancer), SW620 (colon cancer), BT 474 (breast cancer), HEP-G_2 (hepatoma) and CHAGO (lung cancer) are reported in Table 37.

Table 37 Cytotoxicity data of the saponins from *Sapindus rarak*

Compounds	IC_{50} (μg/ml)				
	KATO-3	SW 620	BT 474	HEP-G_2	CHAGO
Crude saponins extract	4.74	7.27	>10	8.31	>10
Sp1	5.55	8.12	>10	8.49	>10
Sp2	6.17	8.35	>10	>10	>10
Doxorubicine	>10	0.10	0.70	0.61	0.55

Tumor cell lines:

SW 620 = human colon adenocarcinoma

HEP-G_2 = human liver hepatoblastoma

KATO-3 = human gastric carcinoma

BT474 = human breast ductal carcinoma

CHAGO = human undifferentiated lung carcinoma

By MTT colorimetric assay for cytotoxic activity, the crude saponin extracts showed IC_{50} Values against human colon adenocarcinoma (SW 620), human liver hepatoblastoma (HEP-G_2) and human gastric carcinoma (KATO-3) at 7.27, 8.31 and 4.74 μg/ml, respectively.

Compound Sp1(Sapindoside B) showed IC_{50} Values against human colon adenocar cinoma (SW 620), human liver hepatoblastoma (HEP-G_2) and human gastric carcinoma (KATO-3) at 8.12, 8.49 and 5.55 μg/ml, respectively.

Compound Sp2 (Mukurozi-saponin E_1) showed IC_{50} Values against human colon adenocarcinoma (SW620) and human gastric carcinoma (KATO-3) at 8.35 and 6.17 μg/ml, respectively.