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Phytochemical Study of *Hedychium malayanum* (Zingiberaceae)

(Kajian Fitokimia *Hedychium malayanum* (Zingiberaceae))

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ABSTRACT

A phytochemical study was conducted on the stems and leaves of Hedychium malayanum *(Zingiberaceae). Three steroids namely stigmasterol* ***(1)****, sitostenone* ***(2)*** *and stigmast-4-ene-3,6-dione* ***(3)*** *as well as one triterpene, lupenone* ***(4)*** *and one oxygenated sesquiterpene, caryophyllene oxide* ***(5)*** *were successfully isolated from the respective stems and leaves, utilizing several chromatographic techniques. Their structures were elucidated by spectroscopic means (IR, MS, NMR), and by comparison with the literature data.*

Keywords: Hedychium malayanum*; NMR; steroids; terpenoids; Zingiberaceae*

ABSTRAK

Kajian fitokimia dijalankan terhadap batang dan daun Hedychium malayanum *(Zingiberaceae). Tiga steroid stigmasterol* ***(1)****, sitostenon* ***(2)*** *dan stigmast-4-ena-3,6-dion* ***(3)*** *berserta triterpena, lupenon* ***(4)*** *dan seskuiterpena beroksigen, kariofilena oksida* ***(5)*** *berjaya dipisahkan masing-masing daripada batang dan daun, menggunakan beberapa teknik kromatografi. Struktur mereka dikenal pasti dengan cara spektroskopi (IM, SJ, RMN) dan secara perbandingan dengan data kepustakaan.*

Kata kunci: Hedychium malayanum*; RMN*; *steroid; terpenoid; Zingiberaceae*

INTRODUCTION

Most of *Hedychium* species are well known for their terpenes. To date, eight *Hedychium* have been subjected to phytochemical investigations namely *H. coronarium* (Chen et al. 2013), *H. gardnerianum* (Kumrit et al. 2010), *H. spicatum* (Reddy et al. 2009), *H. chrysoleucum* (Luo et al. 2009), *H. forrestii* (Liu et al. 2004), *H. villosum* (Xiao et al. 2001), *H. yunnanense* (Li et al. 2016) and *H. longipetalum* (Zhao et al. 2015) and all of them were found to contain labdane diterpenes. Many sesquiterpenoids identified from the essential oils such as cryptomeridiol, β-eudesmol, oplopanone, elemol, oplodiol, caryophyllene oxide, *trans*-nerolidol and α-eudesmol were also chromatographically isolated from the *H. spicatum* (Suresh et al. 2013), *H. coronarium* (Kiem et al. 2011), *H. chrysoleucum* (Luo et al. 2009), *H. gardnerianum* (Carvalho et al. 2003) and *H. yunnanense* (Zhao et al. 1995). Lupeol as a tritepenoid was isolated once from *Hedychium* i.e. *H. spicatum* (Sravani et al. 2012).

The availability of steroids in *Hedychium* species comes in second after terpenoids. They are distributed in five plants namely *H. spicatum* (Sravani et al. 2014, 2012), *H. coronarium* (Kiem et al. 2011), *H. chrysoleucum* (Luo et al. 2009), *H. forrestii* (Liu et al. 2004) and *H. villosum* (Xiao et al. 2001). Xanthones and flavonoids which have similar structures can be found in *Hedychium gardnerianum* (Carvalho et al. 2003; De Medeiros et al. 2008). There are several reports on the compounds isolated from some *Hedychium* species, but diarylheptanoids in particular were absent except in *H. coronarium* (Lin et al. 2015). To the best of our knowledge, no phytochemical study has been carried out on the *H. malayanum* extracts. Therefore, in this paper, we report the isolation and identification of stigmasterol, sitostenone, stigmast-4-ene-3,6-dione, lupenone and caryophyllene oxide from the stems and leaves of *H. malayanum*.

MATERIALS AND METHODS

The stems and leaves of *Hedychium malayanum* were collected in December 2013 from Fraser Hills, Pahang, Malaysia. A voucher specimen of WAY 538 was deposited at the Universiti Kebangsaan Malaysia Herbarium (UKMB).

Air-dried ground stems of *Hedychium malayanum* (420 g) were soaked in *n*-hexane at room temperature for six days to give 1.6 g (0.38%) of a green gummy extract after solvent removal using a rotary evaporator. The extract was subjected to column chromatography (CC) using silica gel (Merck 9385) by eluting with *n*-hexane containing increasing percentages of chloroform. The collected fractions were combined according to their silica gel thin layer chromatography (TLC) (Merck 5554) profiles to yield five fractions (A-E). Fractions B (80 mg) and D (91 mg) were separately rechromatographed by preparative thin layer chromatography (PTLC) over silica gel (Merck 7749) to produce compound **1** (6.4 mg) and

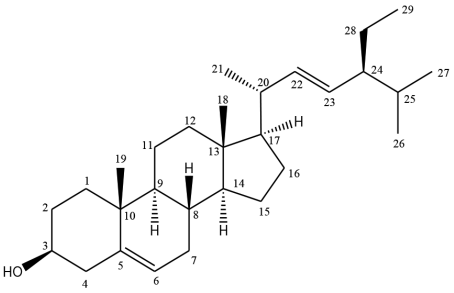
3 (4.6 mg), respectively. Fraction C (132 g) underwent silica gel radial chromatography (RC) (Merck 7749) using increasing polarity of *n*-hexane:ethyl acetate by 10% to afford compound **2** (4.2 mg).

A 480 g of air-dried leaves of *Hedychium malayanum* were macerated with *n*-hexane for six days at room temperature. After filtration and evaporation, the greenish black residue (5.4 g, 1.1%) was fractionated utilizing vacuum liquid chromatography (VLC) (Merck 7747), analysed by TLC and combined into three fractions (A, B and C) for further separation. The VLC was run on silica gel by using increasing polarity of *n*-hexane-ethyl acetate. Fraction C (66 mg) was subjected to PTLC to yield compound **4** (12.3 mg).

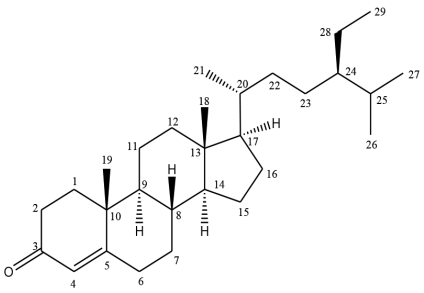
Air-dried ground leaves of *Hedychium malayanum* (160 g) were consecutively extracted using solvents of increasing polarity from *n*-hexane to chloroform to ethyl acetate to acetone and lastly methanol (3 days for each). The methanol filtrate was concentrated using a rotary evaporator to yield 3.3 g (2.1%) of green extract. A 2.5 g portion of the extract was subjected to CC using 10% polarity increment from 90:10 chloroform-methanol to 100% methanol. The collected fractions that showed similar TLC profile were combined to yield three fractions I-III. Fraction II (189 mg) was subjected to RC by eluting with 90:10 of *n*-hexane-ethyl acetate in 10% polarity increment to produce other three sub-fractions. The sub-fraction 3 was then fractionated over silica gel on PTLC to give compound **5** (11.1 mg).

Results and Discussion

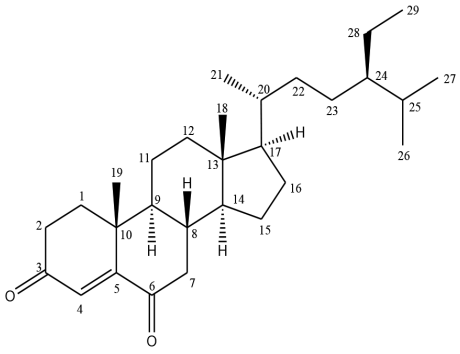
The NMR spectral data (^{1}H and ^{13}C APT) of stigmasterol (Table 1), sitostenone (Table 2) and stigmast-4-ene-3,6-



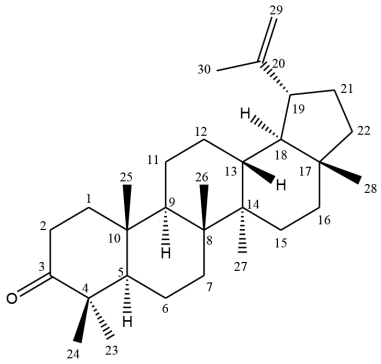
Stigmasterol (**1**)



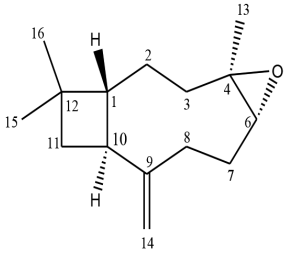
Sitostenone (**2**)



Stigmast-4-ene-3,6-dione **(3)**



Lupenone (**4**)



Caryophyllene oxide (**5**)

TABLE 1. ^{1}H and ^{13}C APT NMR data of compound **1** and *literature ($CDCl_3$, 600 MHz)

Position	δ_H ppm (number of protons, *multiplicity*, *J* Hz)	*δ_H ppm (number of protons, *multiplicity*, *J* Hz)	δ_C ppm	*δ_C ppm
1			37.3	37.2
2			31.6	31.6
3	3.53 (1H, *m*)	3.51 (1H, *m*)	71.8	71.8
4			42.3	42.3
5			140.8	140.7
6	5.35 (1H, *bd*, *J* = 3.0)	5.35 (1H, *m*)	121.8	121.7
7			32.0	31.9
8			31.9	31.9
9			50.1	50.1
10			36.5	36.5
11			21.0	21.1
12			39.7	39.7
13			42.2	42.3
14			56.9	56.9
15			24.4	24.3
16			29.0	28.9
17			55.9	55.9
18	0.70 (3H, *s*)	0.67 (3H, *s*)	12.1	12.0
19	1.01 (3H, *s*)	1.00 (3H, *s*)	19.4	19.4
20			40.5	40.5
21	0.92 (3H, *d*, *J* = 6.6)	0.92 (3H, *d*, *J* = 6.0)	21.1	21.1
22	5.15 (1H, *dd*, *J* = 8.4, 15.0)	5.14 (1H, *dd*, *J* = 6.5, 15.0)	138.4	138.3
23	5.02 (1H, *dd*, *J* = 9.0, 15.0)	5.04 (1H, *dd*, *J* = 9.0, 15.0)	129.3	129.3
24			51.3	51.2
25			31.9	31.9
26	0.85 (3H, *d*, *J* = 6.0)	0.84 (3H, *d*, *J* = 6.0)	21.2	21.2
27	0.80 (3H, *d*, *J* = 6.0)	0.80 (3H, *d*, *J* = 6.0)	19.0	19.0
28			25.4	25.4
29	0.82 (3H, *t*, *J* = 7.5)	0.82 (3H, *t*, *J* = 6.5)	12.3	12.2

TABLE 2. ^{1}H and ^{13}C APT NMR data of compound **2** and *literature ($CDCl_3$, 600 MHz)

Position	δ_H ppm (number of protons, *multiplicity*, *J* Hz)	*δ_H ppm (number of protons, *multiplicity*, *J* Hz)	δ_C ppm	*δ_C ppm
1			35.7	35.7
2			34.0	34.0
3			199.8	199.7
4	5.73 (1H, *s*)	5.72 (1H, *s*)	123.7	123.7
5			171.9	171.8
6			33.0	32.9
7			32.1	32.0
8			35.6	35.6
9			53.8	53.8
10			38.6	38.6
11			21.0	21.0
12			39.6	39.6
13			42.4	42.4
14			55.9	55.8
15			28.2	28.2
16			24.2	24.2
17			56.0	55.9
18	0.72 (3H, *s*)	0.71 (3H, *s*)	11.9	11.9
19	1.19 (3H, *s*)	1.18 (3H, *s*)	17.4	17.4
20			36.1	36.1
21	0.92 (3H, *d*, *J* = 6.6)	0.92 (3H, *d*, *J* = 6.5)	18.7	18.7
22			33.9	33.8
23			26.0	26.0
24			29.1	29.1
25			45.8	45.8
26	0.83 (3H, *d*, *J* = 6.6)	0.83 (3H, *d*, *J* = 6.5)	19.9	19.8
27	0.82 (3H, *d*, *J* = 7.2)	0.81 (3H, *d*, *J* = 6.8)	19.0	19.0
28			23.1	23.0
29	0.85 (3H, *t*, *J* = 7.2)	0.85 (3H, *t*, *J* = 6.7)	12.0	11.9

dione (Table 3) were in agreement with those of De-Eknamkul and Potduang (2003)/Hussain et al. (2008), Pardo et al. (2000) and Seca et al. (2000), respectively. Stigmasterol was reported previously from other two *Hedychium* species of *H. forresti*i (Liu et al. 2004) and *H. coronarium* (Kiem et al. 2011) and also found in many other plant families including Zingiberaceae such as *Renealmia* (Lognay et al. 1989), *Alpinia* (Le et al. 2007; Phan & Phan 2004; Phan et al. 2012, 2007, 2005), *Zingiber* (Peng et al. 2007), *Etlingera* (Mahdavi 2014; Yahya et al. 2011) and *Curcuma* (Shamim et al. 2011). To the best of our knowledge, this is the first time sitostenone and stigmast-4-ene-3,6-dione have been isolated from genus *Hedychium*, which contributes to the database of steroids from *Hedychium*. Additionally, these two steroids have never been reported previously from any other genus of Zingiberaceae except in *Etlingera* (Mohamad et al. 2005).

Comparison of the ^{1}H and ^{13}C APT NMR spectral data of lupenone (Table 4) with the previously published data of Prachayasittikul et al. (2010) confirmed that both are of the same compound. Based on literature review, triterpenoid lupenone was isolated for the first time from Zingiberaceae. In fact, the presence of other triterpenoids in Zingiberaceae species of *Curcuma longa* (Mohamed et al. 2003), *Globba racemosa* (Qiao et al. 1999), *Renealmia alpinia* (Lognay et al. 1989) and *Hedychium gardnerianum* (Saleh et al. 1981) supports its existence in *Hedychium malayanum*.

The similarity of the ^{1}H and ^{13}C APT NMR spectral data of caryophyllene oxide (Table 5) with those of the published data (Orihara et al. 1994) confirmed that both are of the same structure. Besides frequent identification of the sesquiterpene caryophyllene oxide as a major compound in essential oils from many *Hedychium* species, it was also purified from *H. spicatum* (Suresh et al. 2013) and *Alpinia conchigera* (Aziz et al. 2013).

Conclusion

The isolation and characterization of stigmasterol (**1**), sitostenone (**2**), stigmast-4-ene-3,6-dione (**3**), lupenone (**4**) and caryophyllene oxide (**5**) from the stems and leaves of *Hedychium malayanum* were the first ever to be reported from this plant. This is the first report of sitostenone, stigmast-4-ene-3,6-dione, lupenone from *Hedychium*

TABLE 3. ^{1}H and ^{13}C APT NMR data of compound **3** and *literature ($CDCl_3$, 600 MHz)

Position	δ_H ppm (number of protons, *multiplicity*, *J* Hz)	*δ_H ppm (number of protons, *multiplicity*, *J* Hz)	δ_C ppm	*δ_C ppm
1			35.5	35.5
2			34.0	34.0
3			199.6	199.5
4	6.18 (1H, *s*)	6.17 (1H, *s*)	125.5	125.4
5			161.1	161.1
6			202.4	202.4
7	2.69 (1H, *dd*, *J* = 4.2, 16.2)	2.68 (1H, *dd*, *J* = 3.7, 15.6)	46.9	46.8
8			34.2	34.2
9			51.0	51.0
10			39.8	39.8
11			20.9	20.9
12			39.1	39.1
13			42.5	42.5
14			56.7	56.5
15			24.0	24.0
16			28.0	28.0
17			55.8	55.8
18	0.73 (3H, *s*)	0.72 (3H, *s*)	11.9	11.9
19	1.17 (3H, *s*)	1.17 (3H, *s*)	17.5	17.5
20			36.1	36.0
21	0.94 (3H, *d*, *J* = 6.6)	0.93 (3H, *d*, *J* = 6.4)	18.7	18.7
22			33.8	33.8
23			26.0	26.0
24			29.1	29.1
25			45.8	45.8
26	0.82 (3H, *d*, *J* = 6.6)	0.81 (3H, *d*, *J* = 6.5)	19.0	19.0
27	0.83 (3H, *d*, *J* = 7.2)	0.84 (3H, *d*, *J* = 6.5)	19.9	19.8
28			23.1	23.0
29	0.85 (3H, *t*, *J* = 7.2)	0.85 (3H, *t*, *J* = 6.7)	12.0	12.0

TABLE 4. ^{1}H and ^{13}C APT NMR data of compound **4** and *literature ($CDCl_3$, 600 MHz)

Position	δ_H ppm (number of protons, *multiplicity*, *J* Hz)	*δ_H ppm (number of protons, *multiplicity*, *J* Hz)	δ_C ppm	*δ_C ppm
1			39.6	39.6
2			34.2	34.1
3			218.4	218.2
4			47.4	47.3
5			54.9	54.9
6			19.7	19.2
7			33.6	33.5
8			40.8	40.7
9			49.8	49.7
10			36.9	36.8
11			21.5	21.4
12			25.1	25.1
13			38.2	38.1
14			42.9	42.8
15			27.4	27.4
16			35.5	35.5
17			43.0	42.9
18			48.2	48.2
19			48.0	47.9
20			150.9	150.8
21			29.7	29.6
22			40.0	39.9
23	1.08 (3H, *s*)	1.04 (3H, *s*)	26.7	26.6
24	1.03 (3H, *s*)	1.00 (3H, *s*)	21.1	21.0
25	0.94 (3H, *s*)	0.90 (3H, *s*)	16.0	15.9
26	1.26 (3H, *s*)	1.22 (3H, *s*)	15.8	15.7
27	0.96 (3H, *s*)	0.93 (3H, *s*)	14.5	14.4
28	0.80 (3H, *s*)	0.77 (3H, *s*)	18.0	17.9
29	4.58 (1H, *bs*, H-29a)	4.55 (1H, *bs*, H-29a)	109.4	109.3
30	4.70 (1H, *bs*, H-29b)	4.66 (1H, *bs*, H-29b)	20.3	19.6
	1.69 (3H, *s*)	1.66 (3H, *s*)		

TABLE 5. ^{1}H and ^{13}C APT NMR data of compound **5** and *literature ($CDCl_3$, 600 MHz)

Position	δ_H ppm (number of protons, *multiplicity*, *J* Hz)	*δ_H ppm (number of protons, *multiplicity*, *J* Hz)	δ_C ppm	*δ_C ppm
1	1.76 (1H, *t*, *J* = 9.9)	1.76 (1H, *dd*, *J* = 10.1, 4.2)	50.7	50.8
2			27.2	27.4
3			39.1	39.3
4			59.9	60.0
6	2.88 (1H, *dd*, *J* = 10.8, 4.2)	2.86 (1H, *dd*, *J* = 10.6, 4.2)	63.8	63.9
7			30.2	30.4
8			29.8	29.9
9			151.8	152.0
10	2.62 (1H, *q*, *J* = 9.6)	2.62 (1H, *dd*, *J* = 9.9, 8.5)	48.7	48.9
11			39.8	39.9
12			34.0	34.2
13	1.20 (3H, *s*)	1.20 (3H, *s*)	17.0	17.2
14	4.86 (1H, *bd*, *J* = 1.2, H-14a)	4.86 (1H, *d*, *J* = 1.2, H-14a)	112.8	113.0
	4.97 (1H, *bd*, *J* = 0.6, H-14b)	4.97 (1H, *d*, *J* = 1.2, H-14b)		
15	1.00 (3H, *s*)	1.00 (3H, *s*)	29.9	30.1
16	0.98 (3H, *s*)	0.98 (3H, *s*)	21.6	21.8

genus and lupenone from Zingiberaceae family. The work was done using several chromatographic techniques and spectroscopic analyses.

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