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A novel 29-*nor*-3,4-*seco*-friedelane triterpene and a new guaiane sesquiterpene from the roots of *Phyllanthus oxyphyllus*

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Abstract—An unusual 29-*nor*-3,4-*seco*-friedelan-4(23),20(30)-dien-3-oic acid and a new 5-hydroxy-6,9-epoxyguaiane have been isolated along with other rare terpenes and lignans from *Phyllanthus oxyphyllus* roots. Their structures were elucidated from spectroscopic data. The radical scavenging properties of some of these compounds were evaluated. *seco*-Isolariciresinol showed strong antioxidant activity (IC_{50} 0.017±0.001 mM).

1. Introduction

A large number of *Phyllanthus* species are found throughout most of the tropical and subtropical part of the world and several of which have been widely used in traditional medicines. A considerable number of species have been investigated and some recent studies revealed various bioactive constituents such as alkaloids,[1–3] lignans,[4–6] terpenes,[7–9] flavonoids[10] and tannins.[11,12]

In our ongoing search for bioactive compounds from the Euphorbiaceae we have studied *Phyllanthus oxyphyllus* Miq. (symnonymous name, *P. frondosus* Wall.ex Muell. Arg.), known in Thai as 'yaai chuung laan'. This plant is a shrubby tree which grows to about 1–3 m in height. A hot water decoction of its leaves is used to wash new-born babies as an anti-infective and also as drink to relieve fever, diaphoretic and gonorrhea.[13]

2. Results and discussion

The dichloromethane extract of the roots which caused 100% death to the *Artemia salina* (brine shrimps) nauplii at 100 ppm concentration[14] and exhibited radical scavenging properties towards the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical in a TLC autographic assay was chosen for further purification. Repeated column chromatography of the extract resulted the isolation of a novel 29-*nor*-3,4-*seco*-friedelane triterpene (**1**), a new guaiane sesquiterpene (**2**), from which a mono-acetate derivative (**3**) was prepared, in addition to eight known compounds. The known compounds were identified as lupeol (**4**), β-sitosterol (**5**), 3,12-dihydroxy-cleistantha-8,11,13,15-tetraene, spruceanol (**6**),[15,17] 2,3,12-trihydroxy-cleistantha-8,11,13,15-tetraene, cleistanthol (**7**),[16,17] 2,3-bis-(4′-hydroxy-3′-methoxybenzyl)-butane-1,4-diol, *seco*-isolariciresinol (**8**),[18] 2,6-di-(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane, pinoresinol (**9**),[19] 3-oxofriedelan-29-oic acid, polpunonic acid (**10**)[20] and stigmast-4,5-en-3-one (**11**) by comparison of their physical and spectroscopic data with those of the related compounds previously reported.

Compound **1** was obtained as a colourless sticky liquid. The FT-IR spectrum showed the presence of a carboxyl group at ν_{max} 3065 and 1707 cm^{-1} as well as C=C stretching at ν_{max} 1647 cm^{-1}. The EI-MS spectrum gave the molecular ion peak at *m/z* 426 corresponding to $C_{29}H_{46}O_2$. The ^{13}C NMR and DEPT spectra indicated twenty nine carbons comprising five methyl carbons, thirteen methylene carbons of which two were olefinic, four methine carbons and seven quaternary carbons, including one carboxyl and one olefinic quaternary. The presence of a vinylic double bond was revealed from the ^1H chemical shifts at δ_H 5.60 (dd, *J*=17.4, 10.8 Hz, H-4) and a pair of double doublets at δ_H 4.91 (dd, *J*=10.7, 1.1 Hz), 4.88 (dd, *J*=17.3, 1.1 Hz) in addition to the ^{13}C shifts at δ_C 150.9 (d), 111.0 (t). An exocyclic double bond was also implied from the ^1H chemical shift at δ_H 4.57

Keywords: *Phyllanthus oxyphyllus*; Euphorbiaceae; friedelane; guaiane; cleistanthane; lignans; antioxidant.
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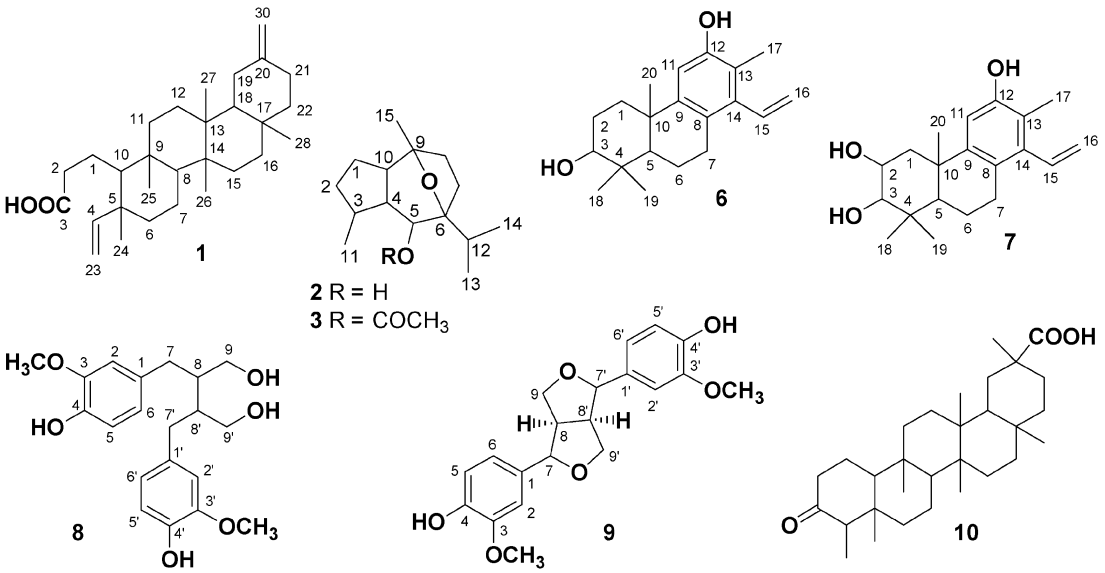

(m, $w_{1/2}$=8.5 Hz) and ^{13}C chemical shifts at δ_C 149.3 (s) and 107.3 (t).

The presence of the key ^3J ^{1}H–^{13}C correlations between H-8/C-10, C-11, C-25 and C-26; as well as H-10/C-1, C-2, C-8, C-24 and C-25 demonstrated the connectivities that would be encountered only in the moiety shown in boldface of the friedelane skeleton (Fig. 1). The presence of a carboxyl group at C-3 which also implied the rupture of a bond joining C-3 and C-4 was deduced from the ^3J ^{1}H–^{13}C correlations between H-1 and H-2/C-3 as well as H-10/C-1, C-2 and C-24. Correlations particularly between H_3-24/C-4, C-6 and C-10 together with correlations between H-4/C-5, C-6, C-10 and C-24 indicated a vinylic double bond at C-4(23). An exocyclic double bond at C-20(30) was implied from the correlations between H-18 (δ_H 1.51)/C-20 and C-28 as well as the correlations between H-30 (δ_H 4.57)/C-19, C-20 and C-21. Full assignment of the ^{1}H and ^{13}C chemical shifts (Table 1) was based on the HMQC and HMBC spectra. The relative stereochemistry of **1** (Fig. 2) was deduced from the NOESY spectrum. Compound **1** was concluded to be 29-*nor*-3,4-*seco*-friedelan-4(23),20(30)-dien-3-oic acid.

Compound **2** was obtained as a colourless liquid. The FT-IR spectrum revealed the presence of a hydroxyl group (ν_{max} 3430 cm^{-1}) and a cyclic ether (ν_{max} 1261, 939 and 888 cm^{-1}). The HREIMS showed the molecular ion at *m/z* 238.1929 corresponding to $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum showed fifteen carbon signals comprising four methyl, four methylene, five methine and two quaternary carbons. The presence of one oxymethine group and two oxygenated quaternary carbons were deduced from the ^{1}H and ^{13}C chemical shifts at δ_H 3.69 and δ_C 71.8 (d), 83.0 (s) and 86.4 (s). The guaiane sesquiterpene skeleton and location of oxygenated carbons at C-5, C-6 and C-9 were revealed from the existence of an isopropyl group (δ_H 1.01

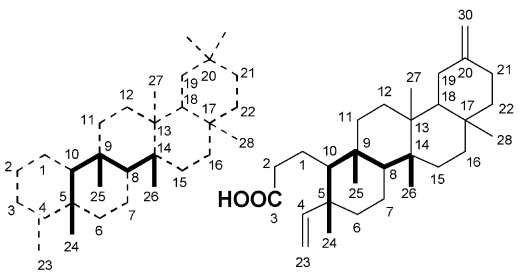

Figure 1.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** (in $CDCl_3$)

Position	δ_H	δ_C	HMBC
1	1.46	21.2 (t)	C-2, 3, 10
2	2.33	37.1 (t)	C-1, 3, 10
3		179.2 (s)	
4	5.60 (dd, 17.4, 10.8)	150.9 (d)	C-5, 6, 10, 24
5		42.0 (s)	
6	1.44 (α-H), 1.32	41.4 (t)	C-5, 7, 8, 24
7	1.39, 1.30	17.7 (t)	C-5, 6
8	1.29	49.5 (d)	C-7, 10, 11, 15, 25, 26
9		38.7 (s)	
10	0.91	58.4 (d)	C-1, 2, 4, 8, 24, 25
11	1.43, 1.34 α-H	34.9 (t)	C-8, 10, 12, 13, 25
12	1.48, 1.33	28.8 (t)	C-9, 11, 13, 27
13		40.6 (s)	
14		39.5 (s)	
15	1.35, 1.24 α-H	28.3 (t)	C-13, 16, 22, 26
16	1.74, 1.26 α-H	36.1 (t)	C-15, 17, 18, 22, 28
17		31.3 (s)	
18	1.51	45.4 (d)	C-12, 13, 14, 17, 19, 20, 22, 27, 28
19	2.37, 2.27	29.8 (t)	C-13, 17, 18, 20, 21, 30
20		149.3 (s)	
21	2.31, 2.16 (dd, 13.1, 5.0)	30.8 (t)	C-17, 19, 20, 30
22	2.02 (α-H, dt, 13.6, 5.1), 1.10	38.2 (t)	C-16, 17, 20, 21, 28
23	4.91, (dd, 10.7, 1.1), 4.88 (dd, 17.3, 1.1)	111.0 (t)	C-4, 5, 10
24	0.95 (s)	18.1 (q)	C-4, 5, 6, 10, 23
25	0.89 (s)	19.3 (q)	C-8, 9, 10, 11
26	0.83 (s)	15.1 (q)	C-8, 13, 14, 15
27	1.02 (s)	18.1 (q)	C-12, 13, 14, 18
28	1.09 (s)	31.4 (q)	C-16, 17, 18, 22
30	4.57 (m, $w_{1/2}$=8.5)	107.3 (t)	C-19, 20, 21

Assignments were based on COSY, HMQC and HMBC experiments. Coupling constants are listed in parentheses in Hertz.

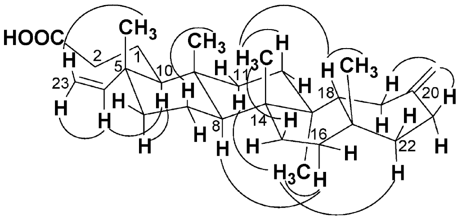

Figure 2. The NOE interactions of **1**.

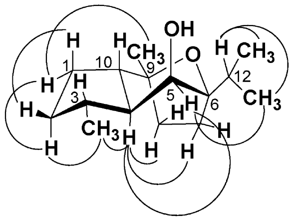

Figure 3. The NOE interactions of **2**.

and 1.02, each d, H-13 and H-14 and δ_H 1.94, H-12) together with the $^1H-^1H$ COSY correlations between H-11/H-3; H-3/H-4; H-4/H-10 and H-4/H-5 as well as the long range $^1H-^{13}C$ correlations between H-4/C-5, C-6, C-9, C-10, C-11; H-5/C-3, C-6, C-12; H-10/C-3, C-4, C-9, C-15; H-13/C-6, C-12, C-14 and H-14/C-6, C-12, C-13. Upon acetylation (using acetic anhydride, DMAP and pyridine), the H-5 signal of the acetylated product (**3**) was observed at the less shielded position (δ_H 5.11) indicating the presence of a free hydroxyl group in **2** at C-5. The C–O–C bridge between C-6 and C-9 could thus be proposed. The ^1H and ^{13}C NMR chemical shifts were fully assigned (Table 2) by the use of HMQC and HMBC spectral data. The relative stereochemistry of **2** (Fig. 3) was obtained from the NOESY spectrum. Compound **2** was established as 5-hydroxy-6,9-epoxyguaiane.

Due to the scarcity of the pure compounds, the brine shrimp toxicity test of the pure compounds was thus not carried out. The observed toxicity in the crude dichloromethane extract is believed to be due partly to the presence of the cytotoxic constituents, such as compounds **6**[15] and **10**.[20] Only compounds **6–8** were evaluated for their antioxidant properties using the DPPH stable radical.[21] Compound **8** showed an IC_{50} 0.017±0.001 mM which is about two times stronger than the standard antioxidant, 2,6-di-(*tert*-butyl)-4-methylphenol (BHT), IC_{50} 0.031±0.001 mM. Compounds **6** and **7** also exhibited rather significant antioxidant properties (IC_{50} 0.124±0.022 and 0.285±0.038 mM, respectively).

3. Experimental

3.1. General

The specific rotations were measured by a Jasco DIP 1020 polarimeter. The IR spectra were obtained on a Perkin–Elmer 1760x FT-IR spectrophotometer. EI-MS and HR-EIMS spectra were recorded on a Finnigan MAT 90 instrument. ^1H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

3.2. Plant material

The roots of *Phyllanthus oxyphyllus* Miq. (Euphorbiaceae) were collected from Hin Phoeng Village, Ampur Klongtom, in Krabi Province in July 1997. The botanical identification was achieved through comparison with voucher specimens No. SN 237290-237299 in the herbarium collections of the Sirindhorn Museum, Botanical Section, Department of

Table 2. ^1H and ^{13}C NMR spectral data of compounds **2** and **3** (in $CDCl_3$)

Position	Compound **2**			Compound **3**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	1.59 (β-H, m), 0.99 (α-H, m)	25.9 (t)	C-2, 4, 9	1.58 (m); 1.07 (ddd, 11.6, 7.5, 4.1)	24.9 (t)	C-3, 9, 10
2	1.97 (β-H, ddd, 13.0, 11.1, 2.3), 1.16 (α-H, dddd 11.2, 8.4, 5.1, 2.7)	31.6 (t)	C-1, 3, 11	1.91 (m), 1.20 (m)	31.2 (t)	C-1, 3, 4, 11
3	2.28 (β-H, obs. dddq 15.3, 13.0, 7.2, 2.6)	30.5 (d)	C-1, 10, 11	2.04 (br q, 6.9)	31.2 (d)	C-1, 10, 11
4	1.38 (α-H, m)[a]	48.7 (d)	C-1, 2, 3, 5, 6, 9, 10, 11	1.50 (m)	47.5 (d)	C-3, 5, 10, 11
5	3.69 (β-H, d, 10.4)	71.8 (d)	C-3, 4, 6, 7, 12	5.11 (d, 10.3)	72.7 (d)	5-O*CO*CH3, C-4, 6, 7
6		86.4 (s)		–	85.8 (s)	
7	1.78 (ddd, 11.6, 7.2, 2.4), 1.66 (m)	29.2 (t)	C-5, 6, 8, 12	1.74 (m)	30.3 (t)	C-5, 6, 8, 9, 12
8	1.71 (m), 1.38 (m)[a]	31.7 (t)	C-6, 7, 9, 10	1.72 (m)[a];1.41 (ddd, 12.4, 6.9, 3.5)	31.5 (t)	C-6, 7, 9, 10, 15
9	–	83.0 (s)	–	–	83.1 (s)	–
10	1.61 (β-H, m)	49.4 (d)	C-1, 2, 3, 4, 8, 9, 15	1.71 (m)[a]	48.9 (d)	C-1, 5, 8, 9, 15
11	0.88 (d, 7.1)	17.0 (q)	C-2, 3, 4	0.87 (d, 7.1)	17.1 (q)	C-3, 4
12	1.94 (qq, 6.9, 6.7)	32.5 (d)	C-5, 6, 7, 13, 14	1.76 (m)	33.3 (d)	C-6, 7, 13, 14
13	1.02 (d, 7.1)[b]	17.5 (q)[b]	C-6, 12, 14	0.96 (d, 6.9)[b]	17.5 (q)[b]	C-6, 12, 14
14	1.01 (d, 6.8)[b]	18.4 (q)[b]	C-6, 12, 13	0.91 (d, 7.1)[b]	18.2 (q)[b]	C-6, 12, 13
15	1.22 (β, s)	24.3 (q)	C-8, 9, 10	1.23 (s)	24.4 (q)	C-8, 10
*CH3*CO				2.00 (s)	21.4 (q)	5-O*CO*CH3
CH3*CO*					169.9 (s)	

[a] Overlapped signals.
[b] Chemical shifts in the same column may be interchangeable.

Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10903. A voucher specimen (SSPO/1997) is kept at the Chemistry Department, Faculty of Science, Ramkhamhaeng University.

3.3. Extraction and isolation

The dried roots of *Phyllanthus oxyphyllus* were milled to obtain 4.2 kg of powder. The pulverized roots were extracted successively with *n*-hexane, dichloromethane and methanol using a Soxhlet extraction apparatus. Extracts were filtered and concentrated to remove solvent under reduced pressure on a rotary evaporator to yield the pale yellow sludge of *n*-hexane (25.8 g, 0.6% dry wt), reddish brown sludge of dichloromethane (21.2 g, 0.5% dry wt) and reddish brown sludge of methanol (80.8 g, 1.9% dry wt) extracts.

The dichloromethane extract of the roots (21.2 g) was subjected to a silica gel column chromatography with gradient of *n*-hexane/$CHCl_3$ 20:80 to $CHCl_3$/MeOH 50:50 to obtain five fractions. Fraction 3 was subjected to column chromatography (silica gel, *n*-hexane/CH_2Cl_2 80:20 to CH_2Cl_2/EtOAc 10:90) to give 11 subfractions (subfrs. 3.1–3.11). Subfraction 3.5 was purified by recrystallization to give compound **4** (5.3 mg, 1.26×10^{-4}% based on dry wt). Fraction 4 was column chromatographed (silica gel, $CHCl_3$ to $CHCl_3$/MeOH 50:50) to yield six subfractions (subfrs. 4.1–4.6). Subfraction 4.5 was further purified by column chromatography (silica gel, *n*-hexane/CH_2Cl_2 80:20 to CH_2Cl_2/MeOH 50:50) to give 7 subfractions (subfrs. 4.5.1–4.5.7). Subfraction 4.5.5 was subjected to additional column chromatography (2×, silica gel, *n*-hexane/$CHCl_3$ 75:25 to $CHCl_3$/EtOAc 50:50 then $CHCl_3$) to yield subfraction 4.5.5.5 which contained compound **5** (5.5 mg, 1.31×10^{-4}%). Subfraction 4.5.7 was further purified using column chromatography (silica gel, CH_2Cl_2/EtOAc 98:2 to EtOAc/MeOH 50:50) to obtain 18 subfractions (subfrs. 4.5.7.1–4.5.7.18). Additional column chromatography of subfraction 4.5.7.10 (2×, silica gel, *n*-hexane/CH_2Cl_2 to CH_2Cl_2/MeOH 50:50 then *n*-hexane/CH_2Cl_2 10:90 to CH_2Cl_2/MeOH 50:50) yielded compound **6** (13.6 mg, 3.24×10^{-4}%). Column chromatography of subfraction 4.5.7.15 (silica gel, *n*-hexane/EtOAc 80:20 to EtOAc/MeOH 90:10) yielded compounds **7** (11.1 mg, 2.64×10^{-4}%) and **8** (3.5 mg, 8.33×10^{-5}%). Subfraction 4.5.7.11 was further column chromatographed (silica gel, *n*-hexane/EtOAc 85:15 to EtOAc/MeOH 50:50) to obtain 11 fractions. The most polar fraction was purified using HPLC (RP-C-18, MeOH/H_2O 25:75, 0.5 ml/min) to yield compound **9** (R_t=4.0 min, 0.9 mg, 2.14×10^{-5}%). The moderately polar fraction was purified by recrystallization to yield compound **10** (24.6 mg, 5.86×10^{-4}%). Subfraction 4.5.5.10 was purified by column chromatography (silica gel, *n*-hexane/CH_2Cl_2 50:50) to yield further nine subfractions. The moderately polar subfraction was column chromatographed (2×, silica gel, *n*-hexane/EtOAc 95:5 then reversed phase RP C-18, MeOH) to yield compound **2** (24.3 mg, 5.79×10^{-4}%) and compound **11** (13.4 mg, 3.19×10^{-4}%). Subfraction 4.5.7 was column chromatographed (EtOAc/CH_2Cl_2 2:98 to EtOAc/MeOH 50:50) to yield 18 subfractions. Subfraction 4.5.7.5 contained compound **1** (31.2 mg, 7.43×10^{-4}%).

3.3.1. 29-*nor*-3,4-*seco*-Friedelan-4(23),20(30)-dien-3-oic acid (1). Colourless sticky liquid; R_f=0.22 [silica gel, *n*-hexane/EtOAc 9:1]; $[\alpha]_D^{26}=-58.44$ (*c* 0.180, $CHCl_3$); IR (film) ν_{max}: 3065 (br), 2994, 2930, 2868, 2676, 1707, 1647, 1541, 1458, 1412, 1388, 1287, 1219, 1140, 1052, 1004, 911, 882, 759, 674, 610, 471 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 1; EI-MS (70 eV) *m/z* (rel. int.): 426 (M^+, 17), 411 (11), 301 (8), 284 (11), 269 (15), 257 (21), 227 (11), 215 (17), 201 (23), 189 (84), 173 (35), 159 (36), 145 (44), 133 (49), 119 (61), 105 (76), 99 (100), 77 (77); HR-EIMS *m/z* 426.3534 $[M]^+$ (calcd for $C_{29}H_{46}O_2$, 426.3498).

3.3.2. 5-Hydroxy-6,9-epoxyguaiane (2). Colourless liquid; R_f=0.25 [silica gel, *n*-hexane/CH_2Cl_2 2:8]; $[\alpha]_D^{26}=-40.18$ (*c* 0.225, $CHCl_3$); IR (film) ν_{max}: 3430, 2955, 1708, 1470, 1379, 1308, 1261, 1105, 1064, 1006, 939, 888, 787, 592 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 2; EI-MS (70 eV) *m/z* (rel. int.): 239 (M^++1, 100), 238 (42), 222 (16), 221 (95), 203 (25), 149 (18), 127 (10), 109 (9); HR-EIMS *m/z*238.1929 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.3. 5-*O*-Acetyl-6,9-epoxyguaiane (3). Colourless sticky liquid, R_f=0.45 [silica gel, *n*-hexane/CH_2Cl_2 2:8]; $[\alpha]_D^{26}=-30.53$ (*c* 0.095, $CHCl_3$); ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 2; EI-MS (70 eV) *m/z* (rel. int.): 281 (M^++1, 60), 280 (30), 222 (15), 221 (100), 203 (23), 177 (21), 149 (29),134 (39), 121 (16), 119 (49), 109 (28), 107 (28), 105 (16), 93 (22); HR-EIMS *m/z* 280.20345 $[M]^+$ (calcd for $C_{17}H_{28}O_3$, 280.20385).

3.4. Bioassay

Compounds **6**–**8** were tested for radical scavenging properties using DPPH.[21] 50 μL of a solution containing the compound to be tested was added to 5 ml of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30 min at 37°C, and the percent of activity was calculated. IC_{50} is the mean±standard deviation of three assays.

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