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CHEMICAL EXAMINATION OF *HYDROCOTYLE BONARIENSIS* L. (APIACEAE)

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TON NU LIEN HUONG[1], NGUYEN KIM PHI PHUNG[2], NGUYEN NGOC SUONG[2]

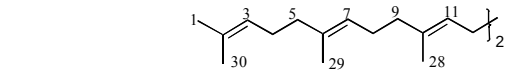
[1]*Department of Sciences, University of Can Tho*

[2]*University of Natural Sciences, National University Ho Chi Minh City*

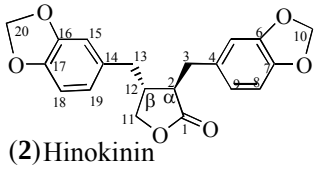
ABSTRACT

*Hydrocotyle bonariensis, growing in the Mekong-delta, is used as vegetable and has not yet been chemically studied. From the aerial parts of H. bonariensis, three compounds had been isolated: a triterpene squalene (**1**) and a mixture of two lignans: hinokinin (**2**) and α-[3,4-methylendioxy phenylmethylidene]-β-[3,4-methylendioxyphenylmethyl]-γ-butyrolactone (**3**). The fresh plant was divided into stem-leaf and flower and each part was distilled with steam to afford essential oils. The major components of stem-leaf are: (Z)-3-hexen-1-ol, trans-caryophyllene, α-farnesene, copaene while the major components of flower are: α-pinene, 2-β-pinene, β-myrcene, limonene, α-caryophyllene, epibicyclosesquiphellandrene. The essential oil of the stem-leaf showed cytotoxic activities in vitro on RD and Hep-G2 cancer cells with the IC_{50} values of 16.1 and 19.9àg/ml, respectively. The study on the plant is being continued.*

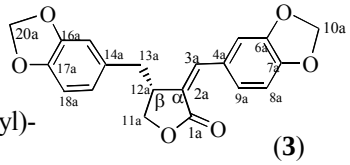
Hydrocotyle bonariensis



(**1**) Squalene



(**2**) Hinokinin
[α,β- bis(3,4-Methylendioxyphenylmethyl)-
-γ-butyrolactone]



(**3**)
α-(3,4-Methylendioxyphenylmethyliden)-
-β-(3,4-methylendioxyphenylmethyl)-
-γ-butyrolactone

1 - INTRODUCTION

Hydrocotyle bonariensis, growing in the Mekong —delta, like *H. asiatica* and *H. sibthorpioides,* is used as vegetable. *H. sibthorpioides* Lam and *H. maritima* Honda showed hemostatic and antitumor activity [1]. Whole plant of *Hydrocotyle* and *Centella* species (Apiaceae) produce characteristic essential oils. The major component of *H. sibthorpioides* and *H. maritima* is *trans*-β-farnesene. *H. maritima* also elaborates α-terpinene and thymol methyl ether in respectable amount. The essential oil of *H. asiatica,* contained β-caryophyllene, *trans*-β-

farnesene and D-germacrene [2].

In the present paper, we report constituents of essential oil of *Hydrocotyle bonariensis*. In addition, we also report the isolation and characterization of a triterpene squalene (**1**) and a mixture of two lignans: hinokinin (**2**) and *α*-[3,4-methylendioxy phenylmethylidene]-*β*-[3,4-methylendioxyphenylmethyl]-*γ*-butyrolactone (**3**). The essential oil showed antimicrobial and cytotoxic activities *in vitro* on RD and Hep-G2 cancer cells.

II - EXPERIMENTAL

1. Plant material

Hydrocotyle bonariensis was collected in Can Tho province in May 2007. The identification of the scientific name of the plant was performed by Mr. Phan Duc Binh, University of Medicine—Ho Chi Minh City.

2. Extraction and isolation

✳Dried and powdered whole plant of *Hydrocotyle bonariensis* (1810 g) was macerated in ethanol 95% at room temperature to give ethanol extract (100 g). This crude extract was separated into petroleum ether, chloroform, ethyl acetate and methanol extracts, respectively by technique of silica gel solid phase extraction. The petroleum ether extract (4.80 g) was subjected to column chromatography, eluted with different solvents, yielded 4 fractions. From the fraction 1, the squalene was isolated (**1**, 36 mg). The chloroform extract (18.70 g) was applied on column chromatography, eluted with different solvents, yielded 5 fractions. Fraction 2 (0.76 g) was rechromatographed to afford a mixture of Hinokinin (**2**) and its analogue (**3**) with the ratio of (3 : 2).

✳Whole raw plant of *Hydrocotyle bonariensis* was divided into stem-leaf and flower. Each part was distilled with steam then extracted with diethyl ether. The yield of the essential oil of stem-leaf and flower is 0.249 and 0.141%, respectively. The essential oils were studied by GC-MS. GC-MS spectra were obtained under the following conditions, GC column: 30 m × 250 μm, HEWLETT PACKARD 6890 series; HP5MS, MSD; temp. 60 - 280°C at 10°/min. after the first 3 minutes and 30°/min. after 100° C, injector temp. 250°C, He 0.9 ml/min., p = 6.9 psi.

3. Biological test

The oil of whole plant was evaluated the antimicrobial and cytotoxic activities on Rhabdosarcoma (RD) cells and Heptocellular carcinoma (Hep-G2), by the Likhiwitayawuid assay, at the Lab of experimental Biology, Institute of Chemistry of Natural Products, Hanoi.

Table 1: Constituents of essential oil of different parts of *Hydrocotyle bonariensis*

No	Stem and leaf	(%)	No	Flower	(%)
1	(*Z*)-3-Hexen-1-ol	47.4	1	(*Z*)-3-Hexen-1-ol	3.03
2	1-Hexanol	4.17	2	*α*-Pinene	12.80
3	(*Z*)-3-Hexenyl acetate	0.75	3	2-*β*-Pinene	8.03
4	Benzeneacetic acid	3.40	4	*β*-Myrcene	11.07
5	4-Methylphenol	2.70	5	Limonene	14.87
6	*α*-Humulene	3.50	6	*γ*-Terpinene	0.73
7	Camphene	1.45	7	3-Methylphenol	3.42
8	2-Methyl-2-cyclopenten-1-one	1.04	8	*α*-Terpinolene	1.53
9	3-Methyl-6-(1-methylethenyl) cyclohexene	3.13	9	1-Methyl-4-(1-methylethyl)-1, 4-cyclohexadiene	0.46
10	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	3.38	10	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	4.82
11	*α*- Bisabolol	0.49	11	(*Z*)-3-Dodecene	0.47
12	Copaene	1.39	12	Copaene	1.17

No	Stem and leaf	(%)	No	Flower	(%)
13	*trans*-Caryophyllene	8.16	13	*trans*-Caryophyllene	3.25
14	α-Farnesene	2.86	14	α-Caryophyllene	2.10
15	Vulgarol B	0.90	15	*epi*Bicyclosesquiphellandrene	3.60
16	2,4-Nonadien-6-yn-1-ol	0.72	16	Caryophyllene oxide	2.36
17	2,6-*Bis*(1,1-dimethylethyl)-4-methylphenol	0.91	17	1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)cyclohexene	1.24
18	3,7-Dimethyl-6-octenyl acetate	0.29	18	Hexadecane	0.44
19	Hexadecane	0.71	19	Octadecane	1.50
20	Pentacosane	0.80	20	Docosane	1.24
21	Octacosane	1.28	21	Pentacosane	1.48
22	Diisooctyl phthalate	1.00	22	Di-*n*-octyl phthalate	2.13
23	Triacontane	2.76	23	Dotriacontane	1.78
24	Hexatriacontane	1.89	24	Pentatriacontane	1.46
			25	*N*-Methyl-*N*-[4-[4-methoxy-1-hexahydropyridyl]-2-butynyl]acetamide	2.69
			26	Hexatriacontane	6.32

Table 2: Result of the antimicrobial assay of the essential oil of *H. bonariensis*

Sample	MIC (μg/ml)							
	Bacteria Gram (-)		Bacteria Gr (+)		Fungus		Yeast	
	E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*Asp. niger*	*F. oxy-sporum*	*S.cere-visiae*	*C. albicans*
Ess. oil	100	200	(-)	100	(-)	100	(-)	(-)

Table 3: Result of the cytotoxicity test on the cancer cells of the essential oil

N°	Sample	Cell survival (%)			IC_{50} (μg/ml)	
		Hep-G2	LU	RD	Hep-G2	RD
1	DMSO	100.0 □0.00	100.0 □0.00	100.0 □ 0.00		
2	Ellipithine	3.00 □ 0.01	1.50 □ 0.00	1.70 □ 0.00	0.19	0.10
3	Essential oil	50.0 □ 0.50	84.59 □ 1.00	6.87 □ 0.60	19.93	16.1

III - RESULT AND DISCUSSION

Compound (**1**) was isolated from the petroleum ether extract. Its TLC showed red spot when the plate was sprayed by concentrated sulfuric acid, so perhaps (**1**) was a triterpenoid compound. Although the EI-MS did not give the ion peak $[M]^+$ but the library of MS instrument suggested that (**1**) was squalene ($C_{30}H_{48}$) with the compatibility of 91%. The chemical structure of (**1**) was well determined by ^{1}H- and ^{13}C-NMR spectrum (table 4) as well as comparison with published data [3].

Mixture of (**2**) and (**3**) was transparent oil. The EI-MS (70eV) gave an ion peak with m/z = 354 $[M]^+$. The library of the MS instrument suggested that was Hinokinin ($C_{20}H_{18}O_6$) with the compatibility of 87%. The mass spectrum also showed another peak with m/z = 352. The IR spectrum showed absorption at ν_{max} cm^{-1} = 3449 (O-H), 1764 (C=O lactone), 1744 (C=C) and 1034 (C-O). ^{13}C-NMR spectral data showed 40 carbons, belong them, 20 carbons had chemical shifts that well suited to the ones of Hinokinin. The 20 carbons left had chemical

shifts agreed with an analogue of Hinokinin that was then identified as α-[3,4-methylenedioxyphenylmethylidene]-β-[3,4-methylendioxyphenylmethyl]-γ-butyrolactone. The comparisons of ^{1}H- and ^{13}C-NMR of this mixture with published data were presented in table 5. This oil was suggested as a mixture of two compounds Hinokinin (**2**) and its analogue (**3**) with the ratio of (3 : 2).

Table 4: The comparison of ^{1}H-NMR and ^{13}C-NMR of (**1**) with squalene [3]

Number of carbon	^{1}H-NMR ($CDCl_3$, δ ppm)		^{13}C-NMR ($CDCl_3$, δ ppm)	
	(**1**)	Squalene ($CDCl_3$) [3]	(**1**)	Squalene ($CDCl_3$) [3]
1, 24	1.68 (3H, s)	1.69 (3H, s)	25.69	25.63
2, 23			131.25	131.01
3, 22	5.10 (1H, m)	5.11 (1H, m)	124.44	125.45
4, 21	2.07 (2H, m)	2.08 (2H, m)	26.80	26.79
5, 20	2.00 (2H, m)	1.99 (2H, m)	39.75	39.74
6, 19			134.91	134.74
7, 18	5.01 (1H, m)	5.14 (1H, m)	124.30	124.30
8, 17	2.06 (2H, m)	2.09 (2H, m)	26.60	26.66
9, 16	1.99 (2H, m)	2.01 (2H, m)	39.77	39.76
10, 15			135.15	134.94
11, 14	5.15 (1H, m)	5.17 (1H, m)	124.33	124.34
12, 13	2.01 (2H, m)	2.03 (2H, m)	28.29	28.28
25, 30	1.60 (3H, s)	1.61 (3H, s)	17.68	17.60
26, 29	1.60 (3H, s)	1.61 (3H, s)	16.01	15.93
27, 28	1.60 (3H, s)	1.62 (3H, s)	16.05	15.98

The fresh plant of *Hydrocotyle bonariensis* was divided into stem-leaf and flower. Each part was distilled with steam, then the obtaining distilled water was extracted with diethyl ether to afford essential oil. Each essential oil was analyzed by GC-MS and the results were presented in table 1. The major components of flower essential oil were: limonene, α-pinene, β-myrcene, β-pinene and the parafines (16 - 36 carbons). We realize that, the essential oil of *H. bonariensis* is similar with the one of *Hydrocotyle* species, or rather similar with the oil of *H. asiatica*.

The essential oil was evaluated the antimicrobial activity and showed good effect on *E. coli, P. aeruginosa, S. aureus, F. oxysporum* (table 2). It was also tested the cytotoxicity on the RD, Hep-G2 by the Likhiwitayawuid assay (table 3) and showed that it inhibited the growth of Heptocellular carcinoma (Hep-G2) and Rhabdosarcoma (RD) cells.

REFERENCES

1. S. Miyata. *Anticancer crude drugs and their prescription*, Kagakusshoin, Tokyo, 185 - 186 (1981).
2. Yoshinori Asakawa, Reiko Matsuda and Tsunematsu Takemoto. Phytochemistry, **21**(10), 2590 - 2592 (1982).
3. Lionello Pogliani, Maurizio Ceruti, Gabriele Ricchiardi, Davide Vterbo. Chemistry and Physic of Lipids, **70**, 21 - 34 (1994).

Table 5: The ^{13}C-NMR spectrum data of (**2**) and (**3**)

N° of carbon	(**2**) (δ ppm)	Hinokinin (δ ppm)	Type of carbon	No	(**3**) (δ ppm)	Type of carbon
1	178.05	178.05	O-C=O	1a	172.53	O-C=O
2	46.52	46.25	>CH-	2a	131.65	>C=
3	34.88	34.60	$-CH_2-$	3a	137.65	-CH=
4	135.53	131.30	>C=	4a	128.24	>C=
5	108.83	108.30	-CH=	5a	108.71	-CH=
6	147.93	147.55	O-C=	6a	149.22	O-C=
7	146.51	146.05	O-C=	7a	147.99	O-C=
8	108.30	108.25	-CH=	8a	109.19	-CH=
9	121.57	121.25	-CH=	9a	125.91	-CH=
10	101.03	100.90	$O-CH_2-O$	10a	101.78	$O-CH_2-O$
11	71.14	70.90	$O-CH_2-$	11a	69.54	$O-CH_2-$
12	41.32	41.10	>CH-	12a	39.91	>CH-
13	38.41	38.10	$-CH_2-$	13a	37.59	$-CH_2-$
14	131.39	131.10	>C=	14a	126.08	>C=
15	109.47	109.12	-CH=	15a	108.52	-CH=
16	147.91	147.55	O-C=	16a	148.39	O-C=
17	146.60	146.10	O-C=	17a	146.39	O-C=
18	108.37	108.25	-CH=	18a	108.85	-CH=
19	122.25	121.95	-CH=	19’	122.10	-CH=
20	101.07	100.90	$O-CH_2-O$	20’	101.03	$O-CH_2-O$

Corresponding author: **Nguyen Kim Phi Phung**
University of Science.
National University–Ho Chi Minh City. Vietnam
E-mail: kimphiphung@yahoo.fr