CONTRIBUTION TO THE STUDY ON CHEMICAL CONSTITUENTS OF *HYDROCOTYLE VULGARIS* (L.), APIACEAE

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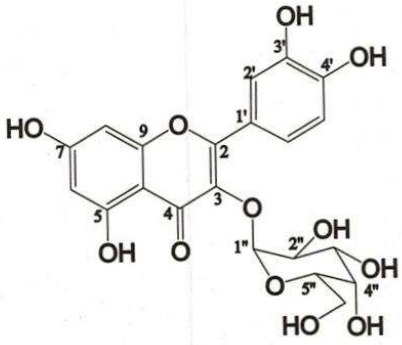
ABSTRACT: *The essential oil of Hydrocotyle vulgaris was analyzed by GC-MS then tested citotoxicity on the cancer cells. The result showed that the essential oil of Hydrocotyle vulgaris had weaker bioactities than the one of Hydrocotyle bonariensis, and in two species had the same main compounds. In addition, from the ethyl acetate extract, quercetin 3-O-galactopyranoside was isolated and identified by the spectrum data 1D, 2D-NMR and MS.*

Key works: *Hydrocotyle vulgaris, quercetin 3-O-galactopyranoside, bioactivities on RD, Hep-G2 and LU cancer cells.*



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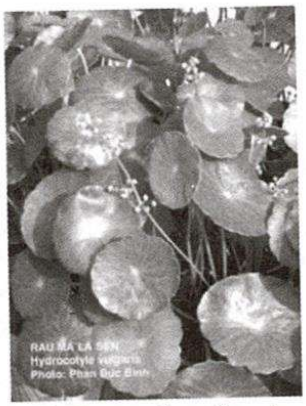

Hydrocotyle vulgaris

Hydrocotyle bonariensis

1. INTRODUCTION

Hydrocotyle vulgaris (marsh pennywort, water pennywort, Rau ma la sen) is a new plant growing in the Mekong–delta and used with *H. asiatica, H. sibthorpioides* as a jumble vegetable. *Hydrocotyle* and *Centella* species (Apiaceae) produce characteristic essential oils throughout the whole plant. It is known that *H. sibthorpioides* Lam and *H. maritima* Honda have hemostatic and antitumor activities [1]. Besides, *H. vulgaris* has the botanical aspect similar to the one of the species *H. bonariensis.*

In the continuation of the study on *Hydrocotyle* genus, now we report the chemical constituents of *H. vulgaris* and their bioactivities *in vitro* on RD and Hep-G2, LU cancer cells. These compounds of essential oil and quercetin 3-O-galactopyranoside were identified by the spectrum data NMR, MS or GC-MS.

2. RESULT AND DISCUSSION

Compound (**1**) was isolated from the ethyl acetate extract and presented as yellow crystals. Its TLC, showed indigo-blue spot when the layer was sprayed by solution $FeCl_3$ 5% in

methanol, so perhaps (**1**) was a flavonoid compound. The EI-MS gave a peak with *m/z* = 464.6 $[M]^+$. IR, KBr, ν cm^{-1}: 3399 (O-H), 2923 (C-H), 1688 (C=O flavone), 1606 (C=C). ^{1}H-NMR (500 MHz, DMSO) δ ppm: 3.2 – 5.0 (protons of sugar), 6.2 (1H, d, J = 1.5 Hz, =CH); 6.9 (1H, d, J = 8.5 Hz, -CH=), 7.7 (1H, =CH). ^{13}C-NMR spectral data showed 21 carbons. The chemical shiffs δ ppm of the carbons were: 177.2 (-C=O), 164.1 (=C-OH), 161.2 (=C-OH), 148.4, 144.8, 133.4, 121.9, 115.1, 98.6, 93.4. These signals manifested that (**1**) was a flavonol. In additon, the present of sugar was showed by the chemical shiffs of the (H-1" – H-4") protons at (3.4 – 5.2) ppm and of the (C-1"–C-6") in turn carbons at δ ppm: 101.8 (carbon anomer), 71.2 , 73.1, 75.8 and 67.9 (CH-OH), 60.1 (CH_2-OH). The sugar was suggested as galactopyranose. The spectral data were showed on the table 1. The HMBC spectrum showed long-range coupling between H-1" with C-3 so the sugar linked to the flavonol at its C-3. Base on the 1D and 2D-NMR, as well as comparison with the authentic sample, in one previously reported of *Hydrocotyle sibthorpioides* [4], compound (**1**) was identified as quercetin 3-*O*-galactopyranoside.

Table 1. ^{13}C NMR δppm values in DMSO-d_6 solutions

Assigned position	Compounds (**1**)	Quercetin 3-galactoside
C-2	156.2	156.3
C-3	133.4	133.4
C-4	177.4	177.4
C-5	161.2	161.1
C-6	98.6	98.6
C-7	164.1	164.0
C-8	93.4	93.5
C-9	156.2	156.3
C-10	103.9	103.9
C-1'	121.9	121.9
C-2'	115.1	115.1
C-3'	144.8	144.7
C-4'	148.4	148.4
C-5'	115.9	115.9
C-6'	121.0	121.0
C-1"	101.8	101.9
C-2"	71.2	71.2
C-3"	73.1	73.1
C-4"	67.9	67.9
C-5"	75.8	75.7
C-6"	60.1	60.1

Hydrcotyle vulgaris was divided into stem, leave and flower and each part was subjected to the steam distillation. Each essential oil was analyzed by GC-MS and the results were presented in table 2. The major components of essential oil of the stem and the flower are the same with the presence of some sesquiterpenes such as: santalene, β-farnesene, β-sesquiphellandrene, β-bisabolene. On the contrary, the essential oil of leave contained alcohol and aldehyde such as 3-hexen-1-ol and 2-hexenal with the great amount, like the one of *H. bonariensis*.

The essential oil was evaluated the antimicrobial activity on *E. coli, P. aeruginosa, S. aureus, F. oxysporum* (table 3) and was also tested the cytotoxicity on the RD, Hep-G2, LU cancer cells by the Likhiwitayawuid assay (table 4). The essential oil of *H. vulgaris* had weaker bioactivities than the one of *H. bonariensis*.

Table 2. The essential oil of *H. vulgaris*

Peak No	*Compounds*	R_t	Leaves (%)	Stem (%)	Flower (%)
1	***Hexenal***	3.38	6.59	-	-
2	(2*E*)-Hexenal	4.23	18.53	-	-
3	3-Hexen-1-ol	4.36	36.01	-	-
4	Santalene	10.61	10.07	29.81	29.51
5	*β*-Farnesene	10.78	6.84	21.71	21.42
6	*β*-Cubebene	10.91	-	-	8.25
7	*γ*-Muurolene	10.96		7.96	-
8	*β*-Bisabolene	11.05	7.90	14.68	14.46
9	*β*-Sesquiphellandrene	11.13	7.32	20.68	19.56
10	Nerolidol	11.28	-	-	1.18
11	Caryophyllene oxid	11.54	1.19	-	0.77
12	Ledol	11.69		-	3.71
13	Z-α-Bisabolene epoxide	11.71	5.55	-	-
14	Globulol	11.73		5.16	-
15	*epi*-Globulol	11.82		-	0.74
16	5,5-Dimethyl-4-(3-methyl-1,3-butadienyl)-1-oxaspiro[2.5]octane	11.89		-	0.41

Table 3. Result of the antimicrobial assay of the essential oil.

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

Table 4. Result of the cytotoxicity test on 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 of *H. bon.*	50.0 ± 0.50	84.59 ± 1.00	6.87 ± 0.60	19.93	16.10
4	Essential oil of *H. vul.*	100 ± 0.00	100 ± 0.00	88.9 ± 0.55		> 20

3. EXPERIMETAL

3.1. General

^{1}H- and ^{13}C-NMR were recorded on Bruker Avance 500 MHz and 125 MHz, respectively, in MEOD. LC-MS spectra were carried out on Agilent-MSD-Trap-SL with the column of

Adserbphere UHF C18. All spectrums were recorded in the Institute of Chemistry, Vietnamese Academy of Science and Technology, Cau Giay Dist., Ha Noi.

GC-MS spectra were obtained under the following conditions, GC column: 30 m x 250 µm, Agilent 6890N; MD Agilent 5973 inert; 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.

The essential oil was evaluated the antimicrobial activities on *E. coli, P. aeruginosa, S. aureus, F. oxysporum* and cytotoxic activities on the RD, Hep-G2, LU by the Likhiwitayawuid assay.

3.2. Plant material

The leaves of *Hydrocotyle vulgaris* L. were collected in Tien Giang province in December 2007. A voucher specimen was determined by Mr. Phan Đuc Binh, Assistant editor of Journal Medicine and Health–Ho Chi Minh City and was deposited in Department of Sciences, Can Tho University.

3.3. Extraction and isolation

Dried and powdered whole plant of *Hydrocotyle vulgaris* (1800 g) was macerated in ethanol 95% at room temperature to give methanol extract (150 g). This crude extract was separated into petroleum ether, chloroform, ethyl acetate and methanol extracts, respectively, by the technique of silica gel solid phase extraction. The ethyl acetate extract (1.20 g) was subjected to column chromatography, eluted with different solvents, yielded 16 fractions (E1.1 – E1.16). From the fraction E1.12, the quercetin 3-*O*-galactoside was isolated (**1**, 140 mg).

3.4. Quercetin 3-*O*-galactoside

Yellow crystals from MeOH; mp.:230 – 232°C; EI-MS, *m/z* = 464.6 $[M]^+$ ($C_{21}H_{20}O_{12}$, M= 464); IR, ν_{max}^{KBr} cm^{-1}: 3399 (O-H), 2923 (C-H), 1688 (C=O flavone), 1606 (C=C); ^{1}H-NMR (500 MHz, DMSO) δ ppm: 7.7 (1H, *d*, J = 2.7 Hz, H2′), 7.6 (1H, *dd*, J = 2.7 and 8.1 Hz, H6′), 6.9 (1H, *d*, J = 8.5 Hz, H5′), 6.4 (1H, *d*, J = 1.5 Hz, H8), 6.2 (1H, *d*, J = 1.5 Hz, H6), 3.2 – 5.0 (protons of sugar); ^{13}C-NMR (125 MHz, DMSO) δ ppm: 177.2 (C-4), 164.1 (C-7), 161.2 (C-5), 156.2 (C-2), 156.2 (C-9), 144.7 (C-3′),148.4 (C-4′), 121.9 (C-1′), 121.0 (C-6′), 115.1(C-5′), 103.9 (C-10), 101.8 (C-1"), 98.6 (C-6), 93.4 (C-8), 75.8 (C-5"), 73.1(C-3"), 71.2 (C-2"), 67.9 (C-4") and 60.2 (C-6").

3.5. Biotest

Whole raw plant of *H. vulgaris* was divided into stem, leave and flower and each part was subjected to the steam distillation. The essential oils are faint aroma light yellow oil, with small content (0,057 %), (d_{30}): 0,737, (n_D^{30}): 1,571. Each essential oil was studied by GC-MS. Because of the lack of sample, the essential oil of different parts of the plant were mixed together and then was evaluated the antimicrobial activities and cytotoxic activities on the RD, Hep-G2, LU cancer cells.

GÓP PHẦN TÌM HIỂU THÀNH PHẦN HÓA HỌC CÂY RAU MÁ LÁ SEN *HYDROCOTYLE VULGARIS* (L.), HỌ NGÒ (APIACEAE)

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TÓM TẮT: *Cây Rau má lá sen, Hydrocotyle vulgaris, là loài mới phát hiện ở vùng đồng bằng sông Cửu Long, được sử dụng xen lẫn với các loài rau má khác làm rau ăn. Các cây Rau má lá sen có hình dáng tương tự nhau rất dễ nhầm lẫn khi thu hái, vì các cây Hydrocotyle vulgaris và Hydrocotyle bonariensis có hình dạng rất giống nhau chi khác ở hoa. Hydrocotyle bonariensis chưa được khảo sát trên thế giới, còn Hydrocotyle vulgaris chưa được khảo sát ở Việt Nam.*

Nhằm tiếp tục các nghiên cứu trên chi Hydrocotyle, tiếp theo phần báo cáo về Hydrocotyle bonariensis, trong bài báo này chúng tôi trình bày về thành phần hóa học và hoạt tính kháng các vi sinh vật, độc tính kháng tế bào ung thư RD, Hep-G2, LU in vitro của cây Hydrocotyle vulgaris. Các chất đã được định danh từ các dữ liệu phổ NMR, MS hoặc GC-MS.

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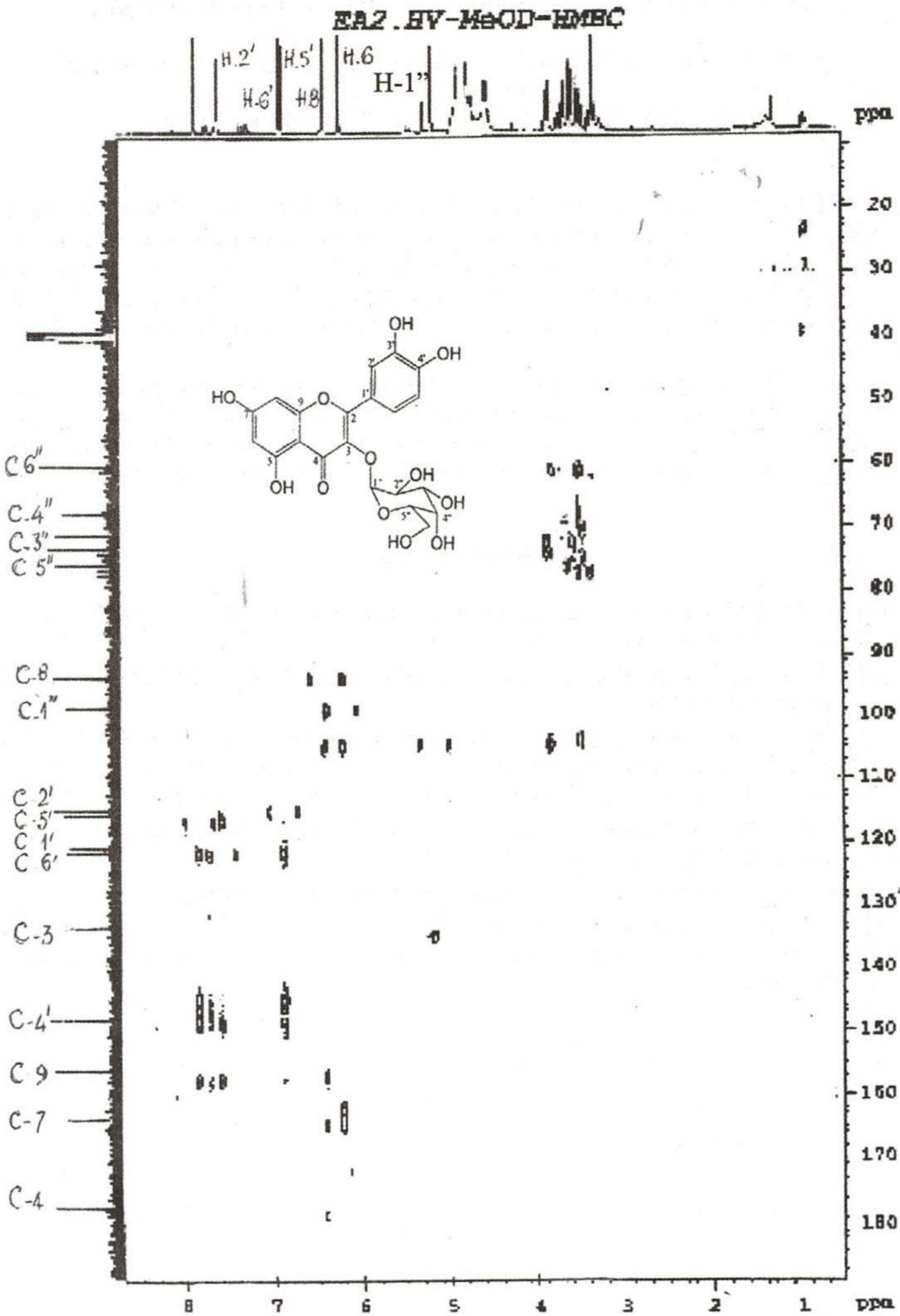
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