

Isolation of a new quinic acid derivative and its antibacterial modulating activity

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ABSTRACT

Background and the purpose of the study: The species *Hymenocrater calycinus*, belongs to the plant family Lamiaceae and grows wildly in the north-east of Iran. Previously, the antimicrobial activity of the plant extracts was reported. In the present study, the bioactivity-guided fractionation of the methanol extract of *H. calycinus* and the combination effects of the isolated compound with cell wall active agents against *S. aureus* and *E. coli* was investigated.

Methods: Column and thin layer chromatographic methods were used for isolation and purification and spectroscopic data (MS, ¹H- and ¹³C-NMR, HMQC, HMBC and ¹H-¹H COSY) were employed for identification of the compound isolated from the extract. A disk diffusion method was used to determine the antibacterial activity of the isolated compound against *S. aureus* and *E. coli* in comparison with 7 different antibiotics.

Results: The isolated compound **1** was identified as 3-(3, 4- dihydroxyphenyl) lactic acid 2-O-quinic acid. Compound **1** (500 µg/disc) enhanced antibacterial effect of ampicillin, ciprofloxacin, vancomycin and cefepime against *S. aureus* and activated the effects of ampicillin and vancomycin against *E. coli*.

Conclusion: Results showed that the compound **1** was not active against both tested strains at any concentration below 1 mg/disk, and as a result the enhancing effect of the compound could be due its association with antibiotics.

Keywords: *Hymenocrater calycinus*, Lamiaceae, 3-(3, 4- dihydroxyphenyl) lactic acid 2-O- quinic acid, Antibacterial effect

INTRODUCTION

Infectious diseases are the main cause of approximately one-half of all death in tropical countries (1). During the past decade life-threatening fungal and bacterial infections have increased dramatically in immunocompromised patients (2). Among the microorganisms, first *Escherichia coli* and second *Staphylococcus aureus* are pathogens responsible for most primary and secondary skin infections. *S. aureus* is also colonized in 30% of burn wounds (3). In recent years, the resistance of these microorganisms to other antimicrobial agents have increased significantly (4).

Plants have provided a good source of novel anti-infective compounds (5, 6). The first generation of the plant drugs were usually simple botanicals which were used in crude form and the second generation were the pure and active isolated molecules (1).

In the search for potent herbal antimicrobial compound, the activity of an extract of *Hymenocrater calycinus* (Lamiaceae), which is growing wildly in the

north east of Iran and some parts of Turkmania as an endemic plant and is called Gol-e-Arvaneh in Persian language, was investigated (7, 8). The synonym of this plant is *Hymenocrater macrophyllum* Bunge (8) and there are several reports on the biological and phytochemical properties of this genus in the literatures (9, 10). Antibacterial and antifungal activity of the methanol extract of *H. sessilifolius*, by agar well diffusion and disk diffusion assays has also been reported previously (10).

It has been found that the efficacy of antimicrobial agents against different pathogens, including *S. aureus*, *P. aeruginosa*, and *E. coli* can be improved by combining with crude plant extracts (eg. *Salvia officinalis*) (11).

This paper reports isolation and identification of an active compound from the methanol extract of *H. calycinus*, which is used traditionally in infectious diseases (10), by the bioactivity-guided fractionation and its antibacterial synergy with cell wall active antibiotics against *S. aureus* and *E. coli*.

Table 1. The NMR data of the compound **1** in DMSO-*d*₆.

Carbon No.	¹³ C- NMR (ppm)	¹ H- NMR (ppm)	HMBC	H-HCOSY
1	132.2		H-5, H-7a, H-7b, H-8	
2	117.8	6.65 (d, J=1.6 Hz, 1H)	H-6, H-7a, H-7b	
3	145.5		H-5	
4	144.0		H-2, H-6	
5	116.0	6.57 (d, J=8.0 Hz, 1H)		H-6
6	120.7	6.44 (dd, J=8.0, 1.6 Hz, 1H)	H-2, H-7a, H-7b	H-5
7	41.4	2.36 (dd, J=13.8, 8.7 Hz, 1H) 2.84 (dd, J=13.8, 2.8 Hz, 1H)	H-2, H-6, H-8	H-7b, H-8 H-7a, H-8
8	73.5	3.64 (dd, J=8.7, 2.9 Hz, 1H)	H-7a	H-7a, H-7b
9	177.7		H-7a, H-7b, H-8	
1'	74.5		H-2'b, H-6'a, H-6'b	
2'	40.7	1.51 (dd, J=12.1, 3.8 Hz, 1H) 1.61 (dd, J=12.1, 11.2 Hz, 1H)		H-2'b, H-3' H-2'a, H-3'
3'	67.2	3.93 (m, 1H)	H-2'b	H-2'a, H-2'b, H-4'
4'	74.2	3.41 (t, 1H)	H-5', H-3'	H-3', H-5'
5'	69.8	3.57 (m, 1H)	H-8, H-4'	H-4', H-6'a, H-6'b
6'	37.5	1.45 (dd, J=13.8, 3.5 Hz, 1H) 1.72 (dd, J=13.8, 4.4 Hz, 1H)	H-2'b	H-5', H-6'b H-5', H-6'a
7'	180.3		H-2'b, H-6'a, H-6'b	

MATERIAL AND METHODS

General experimental procedures

¹H- and ¹³C-NMR spectra were measured on a Bruker Avance 500 DRX (500 MHz for ¹H and 125 MHz for ¹³C) spectrometer with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). EI-MS data were recorded on Agilent Technology (HP TM) instrument with 5973 Network Mass Selective Detector (MS model). The FT-IR spectra were recorded on a Nicolet 550 instrument. Silica gel 60F₂₅₄ pre-coated plates (Merck) were used for TLC. The components were detected by spraying with anisaldehyde-H₂SO₄ reagent followed by heating.

Plant collection

Aerial parts of *Hymenocrater calycinus* (Boiss.) Benth., were collected from Firuzkuh near to Delichae village, during flowering stage in July 2005. Two Herbarium specimens were identified by Dr. Ali Reza Naghinejad from the Faculty of Sciences, University of Tehran. One specimen was deposited at Central Herbarium of Tehran

University and another one housed at Herbarium of Medicinal Plants Research Center, Tehran University of Medical Sciences.

Extraction and isolation procedure

The flowered aerial parts of *H. calycinus* (400 g) was cut into small pieces and percolated with methanol at room temperature for 72 hrs. Percolation was repeated two more times. The MeOH extract (7.4 g) was successively subjected to reverse phase silica gel column chromatography with MeOH: H₂O (4:6, 7:3 and 1:0) to give five fractions (A-E). Fraction A (3.3 g) was further fractionated by a silica gel CC with AcOEt: MeOH (6:4) to yield five fractions (A₁- A₅). Compound **1** (35 mg) was obtained from A₃ (0.5 g) using sephadex LH₂₀ CC with MeOH.

Evaluation of the combination effects of compound **1** with different antibiotics

A disk diffusion method, based on National Committee for Clinical Laboratory Standards (12), was used to determine the activity of different antibiotics and compound **1** against tested strains (*S. aureus*, ATCC 29737 and *E. coli*, ATCC 8739)

Table 2. Zone of inhibition (mm) of different antibiotics against *S. aureus* (in the absence and presence of compound **1** at concentration of 500 (µg/disk).

Antibiotics (µg/disk)	Inhibition zone against <i>S. aureus</i>		Increase in the inhibition zone (%)
	Antibiotic (A)	Antibiotic + compound 1 (B)	
Ampicillin (30)	23	28	48
Cefixime (5)	16	16	0
Ciprofloxacin (5)	19	21	22
Vancomycin (30)	12	14	36
Penicillin G (10UT)	24	24	0
Cefepime (30)	17	20	38
Cephalexine (30)	21	21	0

Table 3. Zone of inhibition (mm) of different antibiotics against *E. coli* (in the absence and presence of compound **1** at concentration of 500 (µg/disk).

Antibiotics (µg/disk)	Inhibition zone against <i>E. coli</i>		Increase in the inhibition zone (%)
	Antibiotic (A)	Antibiotic + compound 1 (B)	
Ampicillin (30)	16	18	26
Cefixime (5)	0	0	0
Ciprofloxacin (5)	0	0	0
Vancomycin (30)	18	21	36
Penicillin G (10UT)	0	0	0
Cefepime (30)	31	31	0
Cephalexine (30)	22	22	0

on Müeller-Hinton agar (Merck, Germany) plates. The standard antibiotics disks were supplied from Mast Co., UK. To determine the combination effect, each standard disc paper (ampicillin, ciprofloxacin, vancomycin, cephalexine, penicillin G, cefixime and cefepime) was impregnated with the tested compound (500 µg). A single colony of the test strains were grown overnight in Müeller -Hinton broth medium on a rotary shaker (200 rpm) at 35°C. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard and prepared disks containing of tested compound (500µg/disc). Similar experiments were carried out with isolated compound alone. After incubation at 37°C for 24 hrs, the zones of inhibition were measured. Mean surface area of the inhibition zone (mm²) was calculated for each tested antibiotic from the mean diameter. The percentage of increase in the inhibition zone for different antibiotics were calculated as $(b^2 - a^2) / a^2 \times 100$ where *a* and *b* are the inhibition zones A (antibiotic only) and B (antibiotic

plus compound **1**), respectively. All experiments were carried out in triplicates and standard deviations were negligible.

RESULTS AND DISCUSSION

In this research, dried aerial parts of *H. calycinus*, was percolated with methanol to give 7.4 g of the extract (18.5% based on the dried weight of the plant sample). The extract has shown antibacterial and antifungal activity (13) and in this study one of its active components was isolated by the reverse phase silica gel and sephadex column chromatography. The isolated compound showed a pink spot with anisaldehyde-H₂SO₄ reagent on thin layer chromatogram On the basis of physicochemical and spectral data its structure was established as 3-(3, 4-dihydroxyphenyl) lactic acid 2-O-quinic acid: Pale yellow amorphous crystal; m. p. 160-161 °C; $[\alpha]_D^{25}$ -9.0° (*c*=0.003, MeOH); IR (CHCl₃) ν_{max} 3332, 2925, 2229, 1731, 1605, 1445, 1228, 1073, 749 cm⁻¹; EI-MS (70eV) *m/z* (%): 123 (100), 110 (42), 198 (18), 152 (11); ¹H and ¹³C-NMR spectra are shown in table 1.

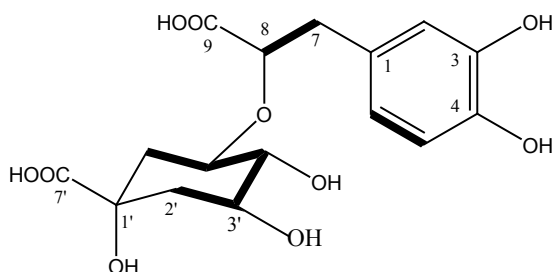


Fig 1. Chemical structure of the isolated compound **1** from *H. calycinus* and H-H COSY correlations (bold bands).

The $^1\text{H-NMR}$ spectrum of compound **1** exhibited aromatic ABX -type signals at 6.44 (*dd*, $J=8.0$, 1.6 Hz, 1H), 6.57 (*d*, $J=8.0$ Hz, 1H) and 6.65 (*d*, $J=1.6$ Hz, 1H), assignable to H-6, 5 and 2, respectively. Aliphatic ABX-type signals at 2.36 (*dd*, $J=13.8$, 8.7 Hz, 1H), 2.84 (*dd*, $J=13.8$, 2.8 Hz, 1H) and 3.64 (*dd*, $J=8.7$, 2.9 Hz, 1H) are assignable to H-7, H-7' and H-8 of 3, 4- dihydroxyphenyl- lactic acid part of the compound **1** respectively. The $^{13}\text{C NMR}$ data revealed the presence of a quinic acid moiety characterized with two methylenes (δ 37.5 and 40.7), three oxymethines (δ 67.2, 69.8, and 74.2), one quaternary carbon (δ 74.5), and one carboxyl group (δ 180.3), as well as a 3, 4- dihydroxyphenyl- lactic acid moiety characterized by 6 aromatic carbon (δ 116.0 - 145.5, C-1 to C-6), one methylene at δ 41.4(C-7), one oxygenated CH at δ 73.0 (C-8), and one carboxylic acid at δ 177.7 (C-9).

The OH group of the position of 5' of quinic acid unit is connected (via etheric linkage) to the C-8 of the 3, 4- dihydroxyphenyl- lactic acid based on the HMBC correlations (see Table 1). The molecular formula,

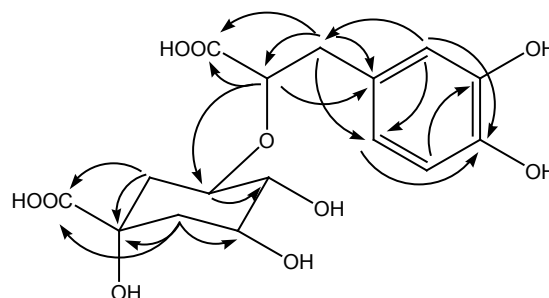


Fig 2. HMBC correlations of compound **1** (H \rightarrow C).

$\text{C}_{16}\text{H}_{20}\text{O}_{10}$, revealed the structure of compound **1** as 3-(3, 4- dihydroxyphenyl) lactic acid 2-O-quinic acid. While, many esters of quinic acid have been identified as bioactive natural compounds (14), the compound of this study is considered to be a new phenylpropanoid derivative of quinic acid which has not been reported previously. Antibacterial assays indicated that the compound **1** did not show any activity at the tested concentrations (125, 250 and 500 $\mu\text{g}/\text{disc}$), however, this compound (500 $\mu\text{g}/\text{disc}$) enhanced the activity of several antibiotics against *S. aureus* and *E. coli* (Tables 2, 3). Ampicillin, ciprofloxacin, vancomycin and cefepime caused 48%, 22%, 36% and 38% increase in the inhibition zone of surface area of the compound **1** against *S. aureus* respectively. Also, ampicillin and vancomycin represented 26% and 36% fold increase in the surface area of inhibition zone of compound **1** against *E. coli*.

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REFERENCES

- Iwu MM, Cuncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J, ed. Perspectives on new crops and new uses. Alexandria, ASHS Press. 1999.
- Trevejo RT, Barr MC, Robinson RA. Important emerging bacterial zoonotic infections affecting the immunocompromised. *Vet Res* 2005; 36: 493-506.
- Mahboubi M, Mohammadi-Yeganeh S, Bokae S, Dehdashti H, Feizabadi M. Antimicrobial activity of essential oil from *Oleiveria decumbens* and its synergy with vancomycin against *Staphylococcus aureus*. *Herba Polon* 2007; 53: 69-76.
- Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. Methicillin resistant *S. aureus* in Europe. *Eur J Clin Microb Infect Dis* 2004; 13: 50-55.
- Khan A, Rahman M, Islam MS. Antibacterial, antifungal and cytotoxic activities of 3,5-diacetyltambulin isolated from *Amorphophallus ampanulatus* Blume ex. Decne. *Daru*, 2008; 16: 239-244.
- Amin GR, Dehmoobed Sharifabadi A, Salehi Surmaghi MH, Yasa N, Aynechi Y, Emami M, Shidfar MR, Amin M, Moghadami M, Kordbacheh P, Zeini F. Screening of Iranian plants for antifungal activity: Part 1. *Daru*, 2002; 10: 38-48.
- Rechinger KH. *Hymenocrater* Fisch & CA Mey. In: Rechinger KH, ed. Flora Iranica, Labiatae, No 150. Graz (Austria), Akademische Druck-u Verlagsanstalt. 1982; 239-250.
- Mozaffarian V. A Dictionary of Iranian Plant Names. Farhang Moaser Publication, Tehran. 1996; 282-283.
- Firouznia A, Rustaiyan A, Nadimi M, Masoudi S, Bigdeli M. Composition of the Essential Oil of *Hymenocrater calycinus* (Boiss.) Benth. from Iran. *J Essent Oil Res* 2005; 7: 527-529.
- Zaidi MA, Crow SAJR. Biologically active traditional medicinal herbs from Balochistan, Pakistan. *J Ethnopharm* 2005; 96: 331-334.
- Horiuchi K, Shiota S, Kuroda T, Hatano T, Yoshida T, Tsuchiya T. Potentiation of antimicrobial activity of

- aminoglycosides by carnosol from *Salvia officinalis*. Biol Pharm Bull 2007; 30: 287-290.
12. Momen-Roknabadi N, Gohari AR, Monsef-Esfehani HR, Attar F, Hajiaghaee R, Saeidnia S, Jamalifar H, Kamalinia G, Shahverdi AR. Antifungal and antibacterial activities of *Pentanema divaricatum* and its active constituent. Z Naturforsch 2008; 63c: 649-52.
 13. Gohari AR, Saeidnia S, Shahverdi AR, Yassa N, Malmir M, Mollazade K, Naghinejad AR. Phytochemistry and antimicrobial compounds of *Hymenocrater calycinus*. EurAsia J BioSci 2009; 3: 64-68.
 14. Zidorn C, Peterson BO, Udovicic V, Larsen TO, Duus JO, Rollinger JM, Omgania KH Elmerer EP, Stuppner H. Podospemic acid, 1,3,5-tri-O- (7,8-dihydrocaffeoyl) quinic acid from *Podospermum laciniatum* (Asteraceae). Tetrahed lett 2005; 46: 1291-1294.