Study on Extraction, Isolation and Structure Identification of the Chemical Constituents of Hippeastrum vittatum

JI Yu-bin¹², Xue qin-bing¹², XIN Guo-song¹²*, Li Xin¹²

(¹The life science and environmental science research center of Harbin University of Commerce; Heilongjiang Harbin, 150076; ²national ministry of education anti-tumor natural drug engineering research center; Heilongjiang Harbin, 150076;)

*Corresponding author’s E-mail: 13766801150@163.com

Abstract: The MTT assay results show that the four parts extraction of amaryllis leaves significantly and dose-dependently inhibit HepG2 cellular proliferation. After separate and purify the compounds in n-butanol section by multiplex chromatography, two compounds were acquired. According to the data of 1H-NMR, 13C-NMR, MS and other spectroscopic data along with the physical and chemical properties, two compounds structure are identified, namely (2S)-7,3’-dihydroxy-4’-methoxy-flavan and (2S)-7-hydroxy-3”,4’-dimethoxy-flavan. This two compounds is isolated from Hippeastrum.

1. Introduction

Hippeastrum vittatum is original from central and South America, Brazil and Peru, Native to the Andes of Peru Amaryllis in 1789 in Europe, now the world widely cultivated Amaryllis which is original from South Africa in Cape of Good Hope in 1633 and introduced to Europe[1]. The middle of the nineteenth Century to twentieth Century, more than 50 Hippeastrum wild species were introduced to Europe[2]. Amaryllis China introduced in early twentieth Century, there are about 75 species, many of the existing interspecific hybrid species, mainly cultivated in the Yangtze River in the south area can be cultivated, many other potted ornamental[3]. In recent years, domestic and foreign scholars have conducted extensive research on Amaryllis bulbs, roots and flowers, extracts of this plant have been isolated from various chemical constituents[4].

Hippeastrum vittatum, Hippeastrum also has antitumor, antiviral, anti-inflammatory and other pharmacological activities. Malignant tumor has become one of the major threats to human health and death.[5-9]. Many alkaloids in nature have antitumor activity, and the anti-tumor activity of the alkaloids is very striking. A wide range of physiological activities of Lycoris plants have been studied. Studies have shown that the chemical constituents in plants of Lycoris have certain anti-tumor and anticancer activity. Red spider lily alkali as an important component of its red extraction, which have strong inhibitory activity of on tumor cells.

2. Effect of detection method of MTT red Amaryllis extract effect on human hepatocarcinoma

HepG-2 cell proliferation From 37 degrees, 5% CO2 incubator removed HepG-2 cells in the exponential growth phase, in the sterile operation table in the drained cell culture medium in the original bottle, using 3 mL PBS buffer washed cells, crisscross washing three times, action should be gentle, after washing out trypsin digestion cells with 1mL 0.25%, burnt on the inverted microscope cell digestion, when cells fall off each other, never rule lepidic smaller circle that digest completely, quickly transferred to the clean bench, pour the pancreatin, adding 3mL containing 12% FBS
RPMI-1640 digestion was terminated, glue dropper sterilization repeated lightly mixed cultured adherent cells in the bottle until the bottle blew on the wall of cells, the action should be gentle, and should be uniform. The counting board will blow uniform cell suspension was adjusted to 5 * 10^4/mL cell suspension, each hole by adding 100 L cell suspension in 96 well plates after sterilization were cultured, the edge of hole by adding of 100 L PBS, 5% CO2, 37 C in the culture box.Cultured HepG-2 cells after 24h, remove the 96 hole plate, with complete medium with different concentrations of tested Amaryllis extracts (petroleum ether, dichloromethane, ethyl acetate part and n-butanol), the drug concentration was 160 g/ml, 80 g/ml, 40 g/ml, 20 g/ml, 10 g/ml, per hole 100 L were added to 96 well plates. Each dose is provided with 6 sets of parallel holes to ensure the accuracy of the experimental data. The blank control group did not take the medicine, but added 100 mu L RPMI1640 culture medium. After that, the 96 hole plate is cultured in a 5% CO2 incubator, 37 DEG C.

3. Experimental results and analysis

Extraction by heating reflux method hippeastrum vittatum dry leaves on 3kg, 84.9g, petroleum ether dichloromethane, ethyl acetate, 17g 23.8g n-butanol 140.4g respectively. The n-butanol layer was selected and purified by silica gel column chromatography and ODS column chromatography.

(1) compound 1: faint yellow block crystal (CH3OH). Methanol dissolved, capillary dipped in a small amount of points, on silica gel GF-254 plate after activation, after drying, the expansion cylinder in saturated ammonia, solvent ratio of dichloromethane: methanol =20:1, were detected in 254nm under UV light, the spots shown as blue purple dark spots. The three-phase system for further inspection of the pure solvent respectively, petroleum ether: acetone (1:2), petroleum ether: ethyl acetate (1:1), dichloromethane: methanol (25:1), and found it was a single spot, prove that this compound is a monomeric compound indeed.

(2) compound 2: faint yellow block crystal (CH3OH). Methanol dissolved, capillary dipped in a small amount of points, on silica gel GF-254 plate after activation, after drying, the expansion cylinder in saturated ammonia, solvent ratio of dichloromethane: methanol =9:2, were detected in 254nm under UV light, the spots shown as blue purple dark spots. The three-phase system for further inspection of the pure solvent respectively, petroleum ether: acetone (1:2), petroleum ether: ethyl acetate (1:1), dichloromethane: methanol (25:1), and found it was a single spot, prove that this compound is a monomeric compound indeed.

4. Structure identification

The planar structures of two compounds were identified on the basis of physicochemical properties, spectroscopic analysis and literature comparison. The results are as follows.

Compound 1: Canary yellow crystal (CH3OH), [α]D = -45.5(MeOH, 0.30). Cation EI-MS m/z display: 272[M+H]+. Speculative formula C16H16O4. In the 1H-NMR (CD3OD,600 MHz)spectrum, δH: 6.89 (1H, d, J = 1.8 Hz), 6.86 (1H, d, J = 8.4 Hz), 6.81 (1H, dd, J = 8. 4, 1.8 Hz) and 6.82 (1H, d, J = 8.4 Hz),6.32 (1H, dd, J = 8.4, 2.4 Hz),6.27 (1H, d, J = 2.4 Hz),Contains 2 ABX coupled systems,3.83 (6H, s) it's a methoxy signal, δH 2.8 -2. 61 and 2.10-1. 92 is characteristic signals of protons in the alkane C ring.

In the13C-APT (CD3OD, 150 MHz)spectrum, δC : 157.6 (C-9), 157.1 (C-7), 148.6 (C-4'), 147.5 (C-3')It’s a O carbon signal on the benzene ring,136.4 (C-1'), 131.0 (C-5)It's a quaternary carbon signal on the benzene ring,118.6 (C-6'), 114.4 (C-10), 114.3 (C-5'), 112.7 (C-2'), 109.1 (C-6), 104.1 (C-8)The benzene ring C signal is the 2 ABX system,78.7 (C-2)Connect the C ring with the O carbon signal,56.9 (4'-OCH3)It’s Methoxy signal,31.6 (C-4), 25.6 (C-3)Secondary carbon signals for C rings.The above data are consistent with those reported in the literature,The compound was identified as (2S) -7,3'-Two hydroxyl radical-4'-Methoxy urethane ( 2S) -7,3'-dihydroxy-4'-methoxy-flavan ,Formula for C16H16O4,Molecular weight of272.30.,1H-NMR is 4-1
Compound 2: Canary yellow crystal (CH3OH), Rotatory power is $[\alpha]_D^{20} = +8.33$(MeOH, 0.30), Cation EI-MS m/z display: 286[M+H] +, Speculative formula C17H18O4. In 1H-NMR (CD3OD, 600 MHz) Spectrum, $\delta$H: 7.03 (1H, d, J = 1.8 Hz), 6.95 (1H, d, J = 8.4 Hz), 6.96 (1H, dd, J = 8.4, 1.8 Hz) and 6.85 (1H, d, J = 8.4 Hz), 6.32 (1H, dd, J = 8.4, 2.4 Hz), 6.27 (1H, d, J = 2.4 Hz). Contains 2 ABX coupled systems, 3.83 (6H, s) and 3.84 (6H, s). It's the two methoxy signal, $\delta$H 2.8 - 2.61 and 2.10 - 1.92 is a characteristic signal of proton in the alkane C ring.

In 13C-APT (CD3OD, 150 MHz) Spectrum, $\delta$C: 157.8 (C-9), 157.3 (C-7), 150.6 (C-4'), 150.2 (C-3') It's a O carbon signal on the benzene ring, 136.5 (C-1'), 131.2 (C-5') It's a quaternary carbon signal on the benzene ring, 119.9 (C-6'), 114.5 (C-10), 113.0 (C-5'), 111.3 (C-2'), 109.4 (C-6), 104.2 (C-8) The benzene ring C signal is the 2 ABX system, 79.0 (C-2) Connect the C ring with the O carbon signal, 56.9 and 56.6 for two methoxy signals, 31.6 (C-4), 25.6 (C-3) Secondary carbon signals for C rings. The above data are consistent with those reported in the literature. The compound was identified as (2S)-7-hydroxyl-3',4'-Two methoxy urethane (2S)-7-hydroxy-3',4'-dimethoxy-flavan). Formula for C17H18O4, Molecular weight of 286.12, 1H-NMR is 4-2.
5. Conclusion

This paper extracted by reflux method hippeastrumvittatum dry leaves of 3kg, petroleum ether and dichloromethane 84.9g part 17g, part 23.8g, ethyl acetate n-butanol fraction 140.4g. Through the experimental study of MTT red Amaryllis part extract on HepG-2 cell activity study found that inhibition of the fourth part of its extract on human hepatoma HepG-2 were. Study on the separation of high purity two compounds, the use of spectroscopic analysis (MS, 1H-NMR, 13C-NMR) to analyze the structures of the compounds, determine which two compounds were respectively two (2S) -7,3'-hydroxy -4'-methoxy flavanone ((2S) -7,3'-dihydroxy-4'-methoxy-flavan), (2S) -7- hydroxy -3'', 4'-methoxy flavanone ((2S) -7-hydroxy-3", 4'-dimethoxy-flavan), these two compounds were first isolated from the red sea. Through the MTT method to study the two compounds isolated from the Red Sea on human hepatocellular carcinoma HepG-2 cells cytotoxicity. These two compounds were found to inhibit the proliferation of human hepatoma HepG-2 cells, and their inhibitory effects increased with increasing doses.

Acknowledgment

Fund Project : Fund Project : Harbin municipal science and technology bureau project(2016RQQXJ124,2016RAXXJ064); Innovation talent project of education department of heilongjiang province(UNPYSCT-2016181);

Reference

[1] Ma Hui, Wang Qi, Yuan Yanbo. Germplasm Resources of Hippeastrums spp.and Their Application to Landscaping [J]. World Forestry Research, 2012, 04: 28-33.
[2] Ma Shasha, Wu Shasha, Jiao Xuehui, Agricultural Engineering Technology (Greenhouse
[3] Ma Hui, Wang Qi, Yuan Yanbo. Germplasm Resources of Hippeastrums spp. and Their Application to Landscaping [J]. World Forestry Research, 2012, 04: 28-33.

[4] Wang Yongxiang, Xin Guosong, Li Yinglin. Study on the Chemical Composition and Pharmacological Effects of Hippeastrum Vittatum [J]. Heilongjiang Medical Journal, 2015, 02: 231-235.

[5] KIM Y H, PARK E J, PARK M H, et al. Crinamine from Crinum asiaticum var. japonicum Inhibits Hypoxia Inducible Factor-I Activity But Not Activity of Hypoxia Inducible Factor-2 [J]. Biological & Pharmaceutical Bulletin, 2006, 29 (10): 2140-2142.

[6] Chen Wanqing, Zheng Rongshou, Zeng Hongmei,. Report of Cancer Incidence and Mortality in China, 2012 [J]. China Cancer, 2015, 01: 1-10.

[7] Wang Yisu, Lin Haiyu, Lin Xiaoji. Risk factors oral infections in hematological malignancies patients after chemotherapy [J]. Chinese Journal of Nosocomiology, 2015, 01: 154-156.

[8] Liu Caiping, Hu Hao, Zhang Yanping. Analysis of malignant tumor death in Yinchuan city in 2012 [J]. Journal of Medical Pest Control, 2015, 01: 35-38.

[9] Chen Wanqing, Zhang Siwei, Zeng Hongmei. Incidence and mortality of malignant tumors in China in 2010 [J]. China Cancer, 2014, 01: 1-10.