Extraction, solation and structure identification of the antioxidant chemicals of the fruits of Cudrania Tricuspidata

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Abstract

To obtain compounds from the antioxidant component of fruits of *Cudrania tricuspidata* (Carr.) Bur. Several separation equipments were used to separate, extracte and purify the fruits, NMR and MS were used to identify the structure of compounds.

Keywords

***Cudrania tricuspidata*, extraction, DPPH, oxidation resistance.**

1. Introduction

Cudrania tricuspidata (Carr.), a deciduous tree, mainly distributed in Eastern Asia[1] and Several kinds of compounds have been found in C.tricuspidata, such as xanthones[2], flavonoids[3], alkaloids, sugars[4]. Biological effects of whole plant are main concentrated in flavonoids and xanthones. These compounds have a number of pharmacological activities including antioxidant, anti-tumor, anti-inflammatory, liverprotection[6-9] and so on. The fruit of C.tricuspidata has also been used as traditional medicine juices, fermented jams and alcoholic beverages with sugar [10].

2. Experimental

2.1 Plant Material

The fruit of *Cudrania tricuspidata* picked from Shandong province.

2.2 General Experimental Procedures

Column chromatography was performed using octadecyl silane(ODS) column chromatography. Thin layer chromatography was performed using pre-coated silica gel plates GF254 (0.25 mm, Merck). HPLC was conducted by using the Agilent-DAD system: YMC-Pack ODS-A (250×4.6 mm; i.d., 5 μm, Kyoto, Japan). ESI-MS was performed on a Waters Q-TOF micromass spectrometer. NMR spectra were acquired using a Varian 500-MHz NMR spectrometer.

2.3 Extraction and Isolation

Fresh fruits of C. tricuspidata (5kg) was extracted at room temperature with MeOH to yield of total residue. Then we added the total residue to a macroporous resin separation column, successively eluted with 30%, 50%, 75%, 95% ethanol-water mixed solvent and merged of the eluent. After spray drying, we got four parts of crude Ethanol extract: A (30% Ethanol extract), B (50% Ethanol extract), C (75% Ethanol extract), D (90% Ethanol extract)

2.4 Determination of Antioxidant Activity of Crude Extract

We used DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity assay to determine the crude Ethanol extract antioxidant activity. Briefly we added 20 μl of extract (1 mg/ml) to 180 μl of DPPH reagent in a 96-well plate. The absorbance was measured at 490 nm after 45 minutes by using microplate reader. Experiments were performed in triplicates. The results were expressed as percentage inhibition (I%), which was calculated using the following formula:

I%=[(AC-AS)/AC] ×100%

AC：the absorbance of the control DPPH at time 0

AS：the absorbance of the control DPPH at time 90 min

2.5 Purification and Structure Identification

The Ethanol extract was subjected to octadecyl silane(ODS) column chromatography, eluted with gradient methanol-water (3:7-10:0), ultimately we got 170 fractions, combined and collected on TCL analysis leading to 13 major series. Further purification of the series were conducted by HPLC, using the Agilent-DAD system: YMC-Pack ODS-A (250×10mm; i.d. 5μm） column with a 1 mL/min flow rate. Finally, we used NMR, MS to identify the high purity monosome.

3. Result and Discussion

3.1 Antioxidant Activities of the Ethanol Extract

The absorbance values of the four parts of ethanol extract (A, B, C, D) are shown in Table 3-1. The crude extract (A, B, C, D) ability of clear • DPPH were respectively equivalent to 375, 430, 460, 396 nmol /mL of Trolox. It showed 75% ethanol extract had a better ability of clear • DPPH than the other three groups.

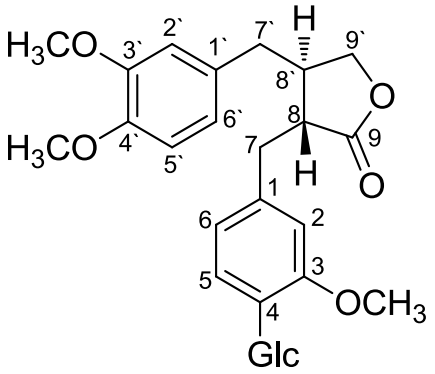
Tab.3-1 The optical density of four crude extract (DPPH) （$\bar{x}$ ±s）(490nm)

Ethanol extract	Number of replication	OD value
A (30%Ethanol extract)	3	0.3394±0.020
B (50%Ethanol extract)	3	0.2742 ±0.23
C (75%Ethanol extract)	3	0.2370 ±0.015
D (90%Ethanol extract)	3	0.3106 ±0.017

3.2 Analysis of Compound Structure

We obtained 2 compounds from 75% Ethanol extract, the structure analyed as follow:

Compound1: Arctiin, colorless crystal, $C_{27}H_{34}O_{11}$, ESI-MS m/z: 557.2016[M+Na]+, Mr=534



Structure of compound 1

^{1}H NMR (500 MHz，CDCL3) spectrum showed compound 1 has 6 Benzene ring proton signals: δH6.87(1H,d,J=8.0), δH6.71(1H,d,J=8.0), δH6.58(1H,s), δH6.55(1H,m), δH6.52(1H,m), δH6.46(1H,d,J=1.5). 3 methoxy unimodal proton signals: δH3.64 (3H,s), 3.74(3H,s), 3.78(3H,s), see Fig 3-1.

^{13}C NMR (400 MHz, CDCL3) spectrum date, see table 3-2.

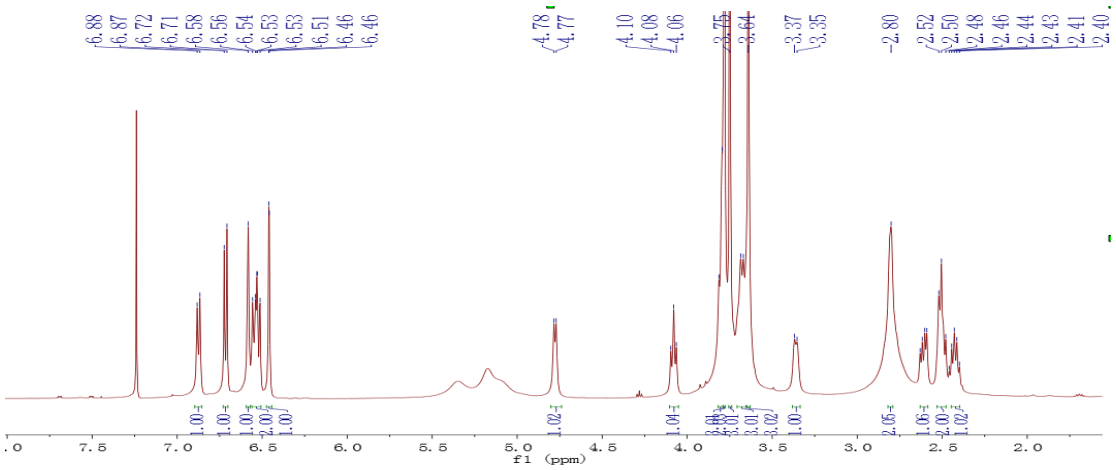


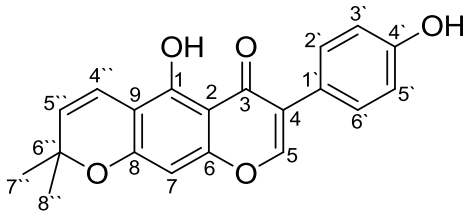
Fig.3-1 1H NMR spectrum of compound 1

Table 3-2 ^{13}C NMR data of compound 1(δ, in CDCL3)

position	^{13}C
1	130.5
2	112.1
3	148
4	145.2
5	111.6
6	122.1
7	34.6
8	46.6
9	178.9
1`	133.2
2`	113.4
3`	149.4
4`	149.2
5`	117.1
6`	120.9
7`	38.2
8`	41.3
9`	71.4
Glc-1	102
Glc-2	76.3
Glc-3	73.4
Glc-4	69.5
Glc-5	76.1
Glc-6	61.6
3-OMe	56.1
3`-OMe	56.1
4`-OMe	56.1

Compound 2: erythrivarone A, yellow powder, $C_{20}H_{16}O_5$, ESI-MS m/z: 337.1075[M+H]+, Mr=336. ^1H NMR (500 MHz，CD3OD) spectrum showed the compound has 6 Benzene ring proton signals: δH7.37(2H,d,J=8.5), δH6.84(2H,d,J=8.5), δH6.68(1H,d，J=10.0)、δH6.33(1H,s), δH5.70 (1H,d, J=10.0), δH8.04(1H,s). 2 methoxy unimodal proton signals: δH1.46(6H, s), see Fig 3-2.

^{13}C. NMR (500 MHz，CD_3OD) spectrum date, see table 3-3



Structure of compound 2

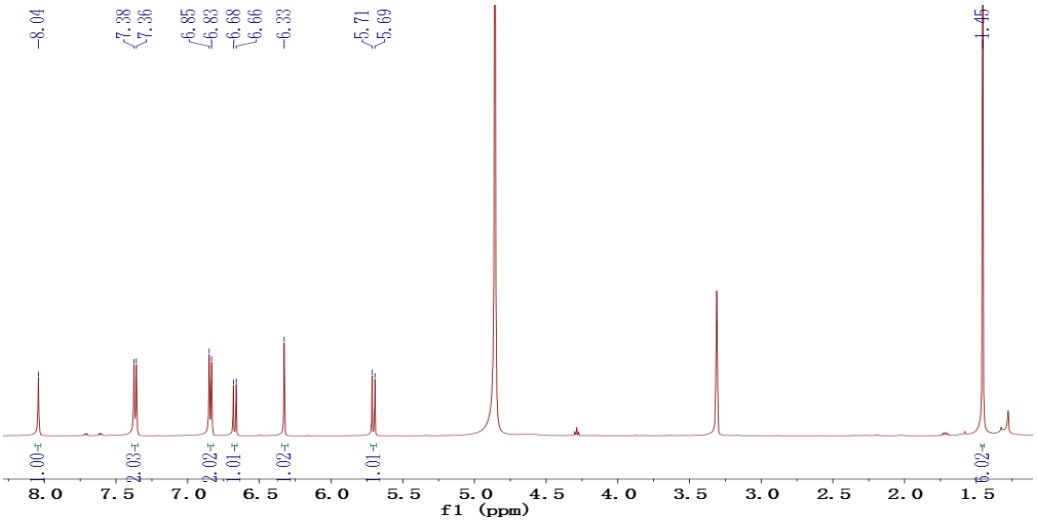


Fig.3-2 1H NMR spectrum of compound 3

Table 3-3 ^{13}C NMR data of compound 3 (δ, in CD_3OD)

position	13C
1	155
2	106.7
3	182.6
4	123.3
5	157.9
6	159
7	96
8	158.9
9	107.1
1`	125
2`	131.5
3`	116.4
4`	161
5`	116.4
6`	131.5
4``	116.2
5``	129.8
6``	79.4
7``	28.7
8``	28.7

4. Conclusion

In this study we found that the 75% ethanol extract had a better antioxidant activities. Futher we extracted, purified, identified 2 compounds from the 75% ethanol extract: Arctiin and erythrivarone A.

Acknowledgements

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