Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Content determination of benzyl glucosinolate and anti-cancer activity of its hydrolysis product in *Carica papaya* L.

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ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 15 December 2011
Accepted 15 January 2012
Available online 20 March 2012

Keywords:
Benzyl glucosinolate
Anti-cancer activity
Carica papaya L.

ABSTRACT

Objective: To determine the content of benzyl glucosinolate (BG) in the pulp and the seed and investigate the anti-cancer activity of its hydrolysis product in *Carica papaya* L. **Methods:** Determination of BG was performed on an Hypersil BDS C_{18} column at the wavelength of 214 nm with 0.1% trifluoroacetic acid (TFA) aqueous solution (A) and 0.1%TFA acetonitrile (B) as the mobile phase. *In vitro* activity test was adopted with cultured human lung cancer H69 cell *in vitro* to investigate the inhibition rate of cell proliferation of benzyl isothiocyanate (BITC) against H69 cell. **Results:** The pulp has more BG before the maturation of papaya and it nearly disappeared after papaya matured, while the seed contains BG at every stage. Activity test demonstrated that the a higher concentration of BITC would have better inhibition rate of cell proliferation on H69 cell, and the IC_{50} was 6.5 μmol/L. **Conclusions:** BG also can be produced in the pulp of papaya and it will be stored in the seed after the fruit has been matured. The hydrolysis product of BG has certain cancer-prevention anti-cancer activities for human.

1. Introduction

A number of studies support the fact that a thioglycoside constituent, benzyl glucosinolate (BG) exists in all tissues except the mature pulp of *Carica papaya*[1]. In the catalysis of myrosinase, BG can be hydrolyzed into benzyl isothiocyanate (BITC), a compound which has cancer-preventive and anti-cancer activities[2,3].

Carica papaya L. is a perennial evergreen herbaceous plant belongs to Family Caricaceae and Genus Carica, which has a short growth period and its fruit is of great nutritive and health-care values[4]. Further research and exploitation of high added-value products from papaya will benefit the development of papaya industry. In this study, Cultivar "Sunrise Solo" papaya was used as the plant material. The contents of BG in the papaya pulps and seeds collected at different mature stages were determined and the inhibition effect of the hydrolysis product of BG against tumor cell H69 was investigated as well for the further research and development to provide the theory basis.

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Foundation project: Supported by National Key Technologies R & D Program of China (2009BADA2B02-04) and Natural Science Fund of Hainan Province (No. 309042), Natural Science Fund of China (No. 31171822).

2. Materials and methods

2.1. Assay of BG[2]

2.1.1. Chromatographic conditions

Chromatographic separation was achieved on an Hypersil BDS C_{18} chromatography column (200 mm×4.6 mm, 5 μm). The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) aqueous solution (A) and 0.1% TFA acetonitrile (B). Gradient elution began with 100% A gradually changed to 90% and B from 0% to 10% in the first 20 min, then A was gradually changed from 90% to 0% and B from 10% to 100% during 20-30 min, and then kept for 5 min. For the next 10 min, A gradually changed from 0% to 100% and B from 100% to 0%, and then kept for another 5 min. The analyses were detected at 214 nm and at a rate of 1.0 mL/min with the column temperature kept at 30 ℃. The injection volume was 10 μL.

2.1.2. Preparation of standard solution and sample solutions

An accurately weighed BG reference standard was dissolved in water and diluted quantitatively as standard solution.

About 5 g sample was put into a ceramic mortar with 20 mL of distilled water, covered it with a piece of plastic wrap and heated for 3 min in a microwave oven. Then the sample was homogenized with a ceramic pestle after taken out from the microwave oven. The sample solution was then centrifuged for 3 min at 10 000 g. The supernatant was transferred into a volumetric flask, and was diluted with water to 25 mL. Finally the solution was filtered through 0.45 μm membrane filter.

2.2. In vitro inhibition of BITC against human lung cancer H69 cell

2.2.1. Cell strains

Human lung cancer H69 cell (Peking Union Medical College Institute of Medicinal Plant Development) was maintained in RPMI1640. The media was supplemented with 10% heat-inactivated fetal calf serum, 100 μg/mL penicillin, 100 μg/mL streptomycin , and 0.2% $NaHCO_3$ and grown in an atmosphere of 95% air and 5% CO_2 at 37℃.

2.2.2. Preparation of sample solution

BITC reference standard was purchased from Sigma with a purity of 98%. According to the method described in the literature[5], BITC was dissolved in 95% ethanol (no obvious effect on the cell growth when the terminal concentration of ethanol was lower than 0.5%). Stock solution was accurately prepared at a concentration of 100 μmol/L with 50% dimethyl sulfoxide (DMSO) as solvent, and dilute it to 1.25, 2.5, 5, 10, 20 μmol/L in 96-well plates with phosphate buffered saline before use.

2.2.3. MTT assay[6,7]

Human lung cancer H69 cell was inoculated in 96-well plates at a concentration of 4×10^4 units/mL with a volume of 180 μL in each well and then cultured for 12 h in a CO_2 incubator. 20 μL of solution at different concentrations was added after cell adhesion. 50 μL of MTT solution at a concentration of 1 mg/mL was added to each well after the cell which was mixed with sample solution was cultured for 48 h. Then they were incubated for 4 h in the incubator. The supernatant was discarded after taken out and 150 μL of DMSO was added to each well. Then the solution was shaken for 10 min and the absorption value of sample in each well was measured on ELSA meter at the wavelength of 570 nm. The culture medium with no cell was set up as blank group and the control group was set up with culture medium in place of sample. The inhibition of cell proliferation was calculated from the following formula:

Calibration curve was obtained by plotting the inhibition rate(Y) versus logarithmic value of the concentration (X). IC_{50} was calculated through the regression curve.

2.2.4. Statistic analysis

Each sample was parallelly measured 5 wells. Data were statistically analyzed with statistical package SPSS 13.0. Absorption values represent as mean± SD ($\bar{x}\pm s$) and the mean differences between groups were compared with variance analysis.

3. Results

3.1. Contents of BG

The contents of BG in papaya pulps and papaya seeds at different developing stages were also determined, and the results were listed in Table 1 and Figure 1. It showed that BG exists in the pulp before papaya fruit matures and it can not be detected when the pulp has matured, while BG exists in the seed at all stages.

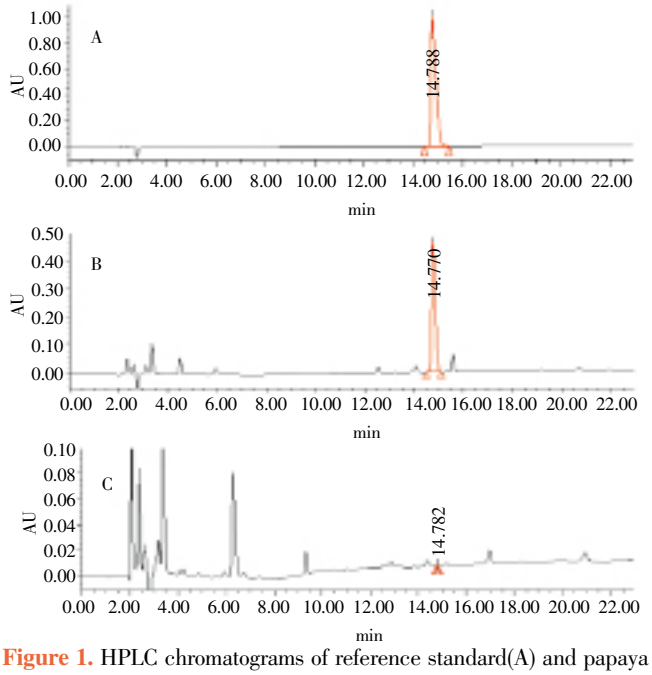

Figure 1. HPLC chromatograms of reference standard(A) and papaya seed (B) and papaya pulp (C)in mature papaya.

Table 1

Contents of BG in pulp and seed in papaya at different developing stages (μmol/g)(Fresh weight).

Sample	No. 1	No. 2	No. 3	Mean±SD
Pulp 1	0.39	0.36	0.39	0.38±0.02
Pulp 2	0.68	0.65	0.59	0.64±0.04
Pulp 3	0.38	0.34	0.30	0.34±0.04
Pulp 4	0	0	0	–
Seed 1	6.72	6.38	6.27	6.46±0.23
Seed 2	9.09	8.76	8.54	8.80±0.28
Seed 3	9.22	8.57	8.95	8.91±0.33
Seed 4	4.82	4.68	4.71	4.74±0.07

Note:1 to 4 means the period from young to mature at developing stages in papaya.

Table 2
Anti-proliferative activity of BITC against H69 (n=5).

Concentration (μ mol/L)	Administration group A($\bar{x}\pm s$)	Inhibition rate(%)
1.25	0.488±0.015	3.42
2.50	0.433±0.014*	13.35
5.00	0.307±0.012**	40.77
10.00	0.151±0.019**	68.89
20.00	0.069±0.007**	86.69

Note: Compared with control group *P<0.05, **P<0.01.

Table 2 showed that BITC has better anti-proliferative activity against human lung cancer H69 cell. The MTT assay result of control group was 0.502±0.039. In the high-dose group , the inhibition rate of cell proliferation can reach more than 80%, which conformed with the result reported in the literature[5]. The IC_{50} was 6.5 μ mol/L. The result provided foundation for the further research of anti-tumor activity of BITC extract from papaya.

4. Discussion

BG is the precursor of BITC. Previous results reported that BG almost could not be detected in the mature papaya pulp[4]. We systematically investigated the contents of BG in different tissues[3] and the contents of BG in papaya pulp and papaya seed at different growth periods. BG exists in the pulp before maturation, but it can not be detected after its maturation, while the content of BG is the highest in papaya seed. The literature reported that acrinyl glucosinolates would transferred from walls to seeds in the maturation period of silique[8]. Combined the related gene expression in the biosynthesis and the results of content determination, we can infer that BG is also transferred to the seed during the maturation period of papaya and finally stored in the seed. The biological functions of β-glucosides in the tissues of plants are manifested as follows. Firstly, they usually take part in the growth of plants as signaling molecules or hormones. Secondly, they are component of sulfur pool as reserve substance. The third, they are related to the defence of plants. Some kinds of cyanogenic glycosides can degrade and produce some toxic substances to participate in the defensive reaction. That BG in the papaya is transferred to the seed at last is not only the maturation signal of fruit but also the storage of sulfur, and at the same time serves the function of seed protection.

Several studies[9-16] reported that BITC has a variety of anti-cancer activities, but these studies merely rested on *in vitro* cell tests. The results in this research demonstrated that BITC has better anti-proliferative activity against human lung cancer H69 cell . In the high-dose group , the inhibition rate of cell proliferation can reach more than 80%, which conformed with the result reported in the literature[6]. The result provided foundation for the further research of anti-tumor activity of BITC extract from papaya.

Conflict of interest statement

We declare that we have no conflict of interest.

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