Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):970-975

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In-vitro antibacterial activity of tomato glycosides

Jianguang Li* and Lu Zhang

College of Pharmacy, Xinjiang Medical University, Urumqi, China

---

ABSTRACT

Glycosides were extracted from green tomatoes with ethanol as the solvent. The extracted glycosides were purified by an HPD macroporous resin and a silica gel column, crystallised and then recrystallised to yield white powder. The minimal inhibitory concentration (MIC) of the extracted tomato glycosides against Escherichia coli was determined to be 3.54 mg/mL through agar diffusion test. The effects of pH, temperature and reaction time on the anti-bacterial activity of the tomato glycosides were investigated. The bacteriostatic effect of the tomato glycosides was stronger than that of 1.5% sodium benzoate. The tomato glycosides exhibited the strongest anti-bacterial effect at pH 7.

Key words: Tomato glycosides; Preparation; Anti-bacterial activity; MIC; Effect

---

INTRODUCTION

Tomato glycosides contain a D-xylose, a D-galactose and two molecules of glucose steroidal alkaloid glucoside; these constituents are also present in eggplants, potatoes and other solanaceous plants[1]. The structural formula of tomato glycosides is shown in Figure 1. Tomato glycosides reportedly exhibit insect-repellent, anti-tumour, anti-inflammatory, cholesterol-lowering and anti-diuretic effects. The effect also can be applied to cardiovascular, cholinesterase and related enzymes of calmodulin; tomato glycosides can also serve as an adjuvant of malaria vaccines[2-6].

Refago[6] found that glycosides are responsible for the capacity of green tomato to inhibit grey mould. Pingnlkar et al[7]. showed that tomato glycosides can restrain the growth of mononucleosis sex liszt fungus. Tomato glycosides also reportedly inhibit the growth of some pathogenic fungi, bacteria and viruses in plants or humans. The immune function of tomato glycosides is indispensable in humans and animals. Moreover, tomato glycosides demonstrate an anti-viral activity; that is, these compounds can restrain the growth of tobacco Mosaic virus in tomato plants. H.V.Thome revealed that tomato glycosides are effective against herpes simplex virus[8]; the sugar chain part and virus membrane receptor interaction in these glycosides have important contributions to this anti-viral effect[9,10].

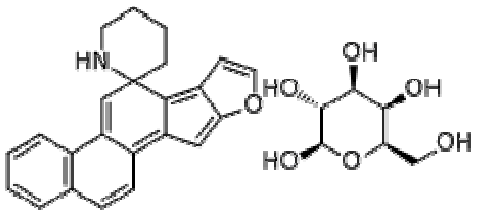

Figure 1 Structural formula of tomato glycosides

Tomato glycosides also exhibit strong anti-bacterial activity. In the present study, *Escherichia coli* was used as the experimental object. Agar diffusion test was performed to evaluate the anti-bacterial activity of tomato glycosides.

The results of this study provide a basis for developing natural anti-microbial products with high efficiency and low toxicity in Chinese herbal medicine[11-13].

EXPERIMENTAL SECTION

Raw materials

Fresh green tomatoes with some white parts were obtained from Shihezi, Xinjiang as the raw material.

Reagents

The reagents used in this study include tomato glycoside reference substance (Shanghai Industrial Development Co., Ltd., batch number 6-15-9), methanol (Chromatography Pure, America TENDA), anhydrous ethanol, chloroform, hydrochloric acid, sodium hydroxide, HPD-100 macroporous adsorption resin (Tianjin Bone Glue Factory), silica gel G, sulphuric acid, methanol, 1.5% sodium benzoate and NaCl. Reagents used above are pure homebred analysis. E. Mr Bush's coli (e.coli, Immune teaching and research section of xinjiang medical university), nutrient AGAR homemade(laboratory).

Instrument

The equipment used in this study include an Agilent1200RRLC-Agilent6410 series triple level 4 pole mass spectrometer, a XDB-C18 column (2.1 mm × 50 mm, 1.8 μm), an electronic balance (Sartorius BP211D, 0.01 mg), a RE-52 rotary evaporation apparatus (Shanghai Anting Electronic Instrument Factory), a PHILIPS beater, a four fluorine 0.20 μm disposable filter head (Dr, Germany), a vertical steam pressure steriliser (ShenAn LDZX-401 Shanghai Medical Instrument Factory), a thermostatic incubator (HN303-3 Nantong Lugu South Scientific Instrument) and a clean bench (SW-CJ-IBV Sujing Group Suzhou Antai Technology).

Preparation

Green tomatoes were extracted with anhydrous ethanol as the solvent at a temperature of 60 °C, an extraction time of 2.5 h and a solid–liquid ratio of 1:5. Ultrasonic processing was performed for 30 min. The extraction process was conducted twice. The extracted tomato glycosides were filtered, added with 5% NaOH solution (pH > 8), allowed to stand overnight, precipitated and then centrifuged. Acetone precipitation was conducted, followed by chloroform extraction for three times. The extracts were dissolved in methanol. The insoluble filtrate was discarded. Finally, the soluble filtrate was dried to obtain raw tomatoes glycosides.

An HPD-100 resin was used for purification. The sample size was 6 BV. The eluent used was 95% ethanol, and the elution volume was 3 BV. The tomato eluent was placed on a silica gel column to dry into white powder. The tomato glycosides were subjected to macroporous resin adsorption and elution, weighed, mixed with methanol solution and silica gel, placed on a rotary evaporator to dry and then placed on the column.

Approximately 55 g of silica gel was activated at 160 °C for 2 h and placed on a wet packing column (3.5 cm × 70 cm). The mobile phase systems for the elution were chloroform–methanol–28% ammonia (75:20:4, v/v/v) and chloroform–methanol–10% ammonia (70:30:4, v/v/v). The elution velocity was 7 mL/min for a 50 mL sample. TLC was used to separate the tomato glycosides. After terminating the elution, the eluent was dried through rotary evaporation, weighed, dissolved in methanol and then filtered to separate the insoluble fraction. The tomato glycosides were mixed with 80% ethanol elution, heated at 70 °C heat to dissolve, filtered and then cooled to room temperature. The sample was slowly crystallised in a refrigerator at 4 °C. After complete crystallisation, the supernatant fluid was washed with methanol, filtered and then dried. Crystallisation was repeated twice until tomato glycerides were obtained in the form of white powder.

Content determination

The HPLC/MS/MS analysis conditions were as follows: mobile phase, CH_3OH:10 mol/L NH_4Ac solution (containing 0.1% HCOOH)=area (V1, V2), SIM, ESI (+), XDB-C18 column (2.1 mm × 50 mm, 1.8 μm); column temperature, 40 °C; sample quantity, 3 μL; and flow rate, 0.2 mL/min. The drying temperature was 350 °C, and the drying air flow was 9 L/min. The conditions for preparing the standard solution of tomato glycosides were as follows: atomising air pressure,40 psi; capillary, 4000 V; and voltage, 135 V.

Anti-bacterial activity

Configuration of reference substance solution

Approximately 10.10 mg of tomato products was dissolved in methanol and placed in a 100 mL volumetric flask to obtain the reference substance of tomato glycosides.

Configuration of sample solution
Up to 0.1662 g of the tomato glycosides in white powder form was dissolved in methanol and placed in a 10 mL volumetric flask. The sample solution of tomato glycosides had a concentration of 14.15 mg/mL. The positive control was 1.5% sodium benzoate solution.

Activation of strains
Agar slant tubes were incubated at 35 °C to 37 °C for 18 h to 24 h and then stored in a refrigerator at 4 °C until use.

Preparation of medium plate
Culture medium was prepared and placed in an electric furnace at a constant temperature (50 °C to 60 °C) to prevent the medium from solidifying. Approximately 15 mL to 20 mL of the medium was poured in Petri dishes and then subjected to high-pressure sterilisation.

Preparation of bacterial suspension
Bacterial suspension was prepared by inoculating *E. coli* in 9 mL of sterile saline with shaking.

Controlled trials
Perforate qualitative filter papers 5 mm in diameter were placed in Petri dishes and sterilised at 120 °C for 2 h. The sterile filter papers were immersed in the tomato glycoside test solution, 1.5% sodium benzoate or sterile water for 24 h. Under sterile operation conditions, 0.2 mL of the prepared bacterial suspension was uniformly poured in Petri dishes containing the medium. The immersed filter papers were then placed on the medium. The Petri dishes were divided into three parts labelled with test liquid filter, 1.5% sodium benzoate filter and blank control (sterile water filter). The Petri dishes were incubated at 35 °C to 37 °C for 18 h to 24 h. Then, the diameters of inhibition were measured.

Determination of minimal inhibitory concentration (MIC)
The tomato glycosides were double diluted with methanol into the following concentrations: 14.15, 7.08, 3.54, 1.77, 0.89 and 0.44 mg/mL. A 0.5 mL aliquot of each solution was added to the culture medium by using a sterile pipette. Approximately 0.1 mL of bacterial suspension was coated on the plate using a pipetting gun, followed by incubation at 35 °C to 37 °C for 18 h to 24 h.

Another dilution series was prepared; the solution without inoculum served as the control. The lowest concentration that inhibited bacterial growth was the MIC. Repeat the above step for six times.

Influence of different conditions
Different pH values
The pH of the tomato glycoside test solution was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by adding HCl or NaOH. The sterile filters were immersed in the tomato glycoside test solutions with different pH values for 24 h, coated with 0.1 mL of the medium and then incubated at 35 °C to 37 °C for 18 h to 24 h. The inhibition zone diameter was measured, and the effect of pH on the anti-bacterial activity of the tomato glycosides was determined. The test was repeated thrice.

Different temperatures
Approximately 5 mL of the tomato test solution was placed in small beakers and then subjected to a water bath treatment at different temperatures (20 °C, 40 °C, 60 °C, 80 °C and 90 °C) for 15 min. The sterile filters were soaked in the solutions in the beakers for 24 h, coated with 0.1 mL of the bacterial suspension medium, and then incubated at 35 °C to 37 °C for 18 h to 24 h. The inhibition zone diameter was measured. The above operation was repeated three times, and the effect of temperature on the anti-bacterial activity of the tomato glycosides was determined.

Different times
A 1 mL aliquot of the tomato glycoside test solution was mixed with 0.1 mL bacterial suspension with shaking. The filter papers were immersed into the mixture for 1, 3, 5, 8, 10 and 12 h. One to six hybrid filters were coated with 0.1 mL of the bacterial suspension medium and then incubated at 35 °C to 37 °C for 18 h to 24 h. The inhibition zone diameter was measured. The above operation was repeated three times.

RESULTS AND DISCUSSION

Preparation
Establishment of the standard curve
Approximately 10.10 mg of the tomato reference substance was dissolved in methanol and then placed in a 100 mL

of volumetric flask to obtain the tomato glycoside reference substance. The reference substance was dissolved with methanol into the following concentrations: 0.0505, 0.1010, 0.2020, 0.3030 and 0.5050 mg/mL. The linear equation was Y = 1249.8 X − 0.6733, r = 0.9998, where Y is the peak area and X is the tomato glycoside concentration. The linear concentration range was 0.0505 mg/mL to 0.5050 mg/mL. The HPLC/MS/MS diagram is shown in Figures 2 and 3.

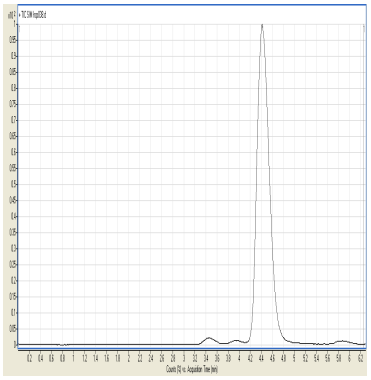

Fig. 2 HPLC-MS/MS chart of standard tomato glycosides

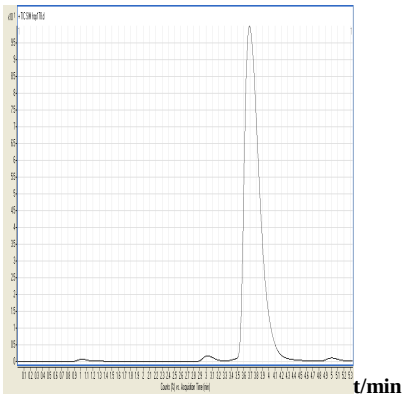

Fig. 3 HPLC-MS/MS chart of tomato glycosides after recrystallisation

Preparation

Tomato glycosides are soluble in ethanol and methanol but almost insoluble in water; however, dissolving tomato glycosides in methanol results in optic nerve damage.[10] Thus, anhydrous ethanol was used to extract glycosides from green tomatoes. Tomato glycosides are alkaline and insoluble in acetone; thus, acetone precipitation was used to remove water. Tomato glycosides slightly dissolve in chloroform. Thus, chloroform was used to remove chlorophyll and fat-soluble impurities from the tomato glycosides. The capacities of chloroform and n-hexane to remove chlorophyll were compared. Results showed that the tomato glycosides were insoluble in n-hexane. Trace amounts of tomato glycosides were lost after dissolving in chloroform; however, this loss is negligible. The capacity of chloroform to remove chlorophyll was better than that of n-hexane. Hence, chloroform was selected to extract the tomato glycosides. The dissolution of the precipitate containing the tomato glycosides in methanol produced light green powder. The results of HPLC/MS/MS showed that the content of tomato glycosides was 12.73%. The final tomato yield was 80.03%. The supply of green tomato is abundant, and the solvents ethanol, acetone, chloroform and methanol can be recycled. Therefore, the large-scale production of the tomato glycosides has certain feasibility.

Anti-bacterial activity

Tomato glycosides and sodium benzoate contrast experiment

Table 1 Bacteriostatic effect of tomato glycosides and sodium benzoate

(inhibition zone diameter: mm)

Selected strain	Samples of tomato glycosides /bacteriostatic zone diameter	Sodium benzoate /bacteriostatic zone diameter	Sterile water /bacteriostatic zone diameter
E. coli	7.88 mm	6.30 mm	5.00 mm

Tomato glycosides **Sodium benzoate** **Sterile water**

Fig. 4 Image of bacteriostatic effect

The bacteriostatic ring diameter can directly reflect the bacteriostatic activity of a drug (Figure 4). As shown in Table 1, the bacteriostatic ring diameter of 14.15 mg/mL tomato glycosides was larger than that of sodium benzoate.

This result indicated that the bacteriostatic activity of 14.15 mg/ml tomato glycosides was stronger than that of sodium benzoate.

MIC of tomatine against *E. coli*

Table 2 MIC of tomato glycosides

Selected strain	Tomato glycoside dilution (mg/mL)					
	14.15	7.08	3.54	1.77	0.89	0.44
E. coli	-	-	-	+	+	+

Note: − said sterile growth, + bacterial growth

As shown in Table 2, *E. coli* growth occurred when the concentration of the tomato glycosides decreased to 1.77 mg/mL. Therefore, the MIC of the tomato glycosides was 3.54 mg/mL in the experiment.

Effects of different conditions
Effects of different pH values

Table 3 Effect of different pH values
(inhibition zone diameter: mm)

pH	Bacteriostatic circle diameter/mm	
	Mean	Variance
3	6.30	0.03
4	7.24	0.07
5	7.46	0.06
6	7.88	0.17
7	10.96	0.07
8	10.38	0.09
9	9.52	0.10

As shown in Table 3, pH affects the bacteriostatic activity of tomato glycosides. At pH 3 to 6, the bacteriostatic ring diameter of the tomato glycosides ranged from 6 mm to 8 mm. At pH 7, the bacteriostatic ring diameter reached the maximum of 10.96 mm, indicating that the best bacteriostatic activity was reached at this pH level. Tomato glycosides precipitate under alkaline conditions. They exhibit strong bacteriostatic activity and good thermal stability under neutral conditions.[11]

Effect of different temperatures

Table 4 Effect of different temperatures
(inhibition zone diameter: mm)

Temperature (°C)	Inhibition zone diameter: mm	
	Mean	Variance
20	7.86	0.06
40	8.02	0.13
60	8.24	0.08
80	8.80	0.15
90	8.94	0.09

As shown in Table 4, the bacteriostatic activity of the tomato glycosides increased with increasing temperature. A significant difference in bacteriostatic activity was observed between 60 °C to 80 °C. The concentration of tomato glycosides increased and their anti-bacterial activity enhanced with methanol evaporation.

Effect of different times

Table 5 Effect of different times
(inhibition zone diameter: mm)

Operation time (h)	Inhibition zone diameter: mm	
	Mean	Variance
1	7.04	0.07
3	8.10	0.06
5	8.56	0.11
8	9.04	0.12
10	9.52	0.09
12	10.02	0.13

As shown in Table 5, the bacteriostatic ring diameter of the tomato glycosides increased with time. This result indicated that the anti-bacterial activity of the tomato glycosides increased with time.

CONCLUSION

A strong bacteriostatic activity on a microorganism indicates that the microorganism is sensitive to drugs. Results showed that the bacteriostatic activity of 14.15 mg/mL tomato glycosides was better than that of sodium benzoate. The bacteriostatic activity of the tomato glycosides was strong under neutral pH. In addition, better bacteriostatic effect was observed with increased temperature and prolonged time. The tomato glycosides demonstrated an inhibitory effect on the bacteria in tomato. Under a neutral environment, the tomato glycosides exhibited stronger anti-bacterial activity, better thermal stability and greater inhibitory effect than sodium benzoate.[12]

Acknowledgments
This work was supported by the Natural Science Foundation of Xinjiang Uygur Autonomous Region (No. 2012XJMUBSQD06). We thank Test and Analysis Center of Xinjiang Medical University for providing us with the experiment platform and technical support.

REFERENCES

[1]Jiang J. W., Xiao J. X. **1986**, 3.
[2]Mendel Friedman T., Fitch E., Yokoyama W. E. *J. Food Chemical Toxicology*. **2003**, 38: 549-553.
[3]Yang X. W., Ran F. X., Wang R. Q. *J. Modern Chinese Medcine*, **2007**, 9(1): 8-13.
[4]Tao Y. X., Liu H. H., Wang Z. M. *J. Natural Product Research and Development*, **2006**, 18: 438-440.
[5]Wu K. J., Gong P. Y., Ruan Y. M. *J. Acta Entomologica Sinica.* **2006**, 49(3): 421-427.
[6]Faucher A. Monnet R. C. R. Herd. Seances Acad. *J. Sci, Ser.D* **1967**, 264: 2247-2249.
[7]Pingulkar K., Kamat A., Bongirwar D. *J. Food Sci. Nutr*. **2001**, 52: 15-23.
[8]Duan J. Y., Zhang J. H. *J. Acta Botanica Boreali-occidentalia Sinica,* **1995**, 28(3): 107-109.
[9]McCance D. J., Drysdale R. B. *Physiol. Plant Pathol* **1975**, 7: 221-230.
[10]Wang Y. Z., Zhu X., Bian S. Z. *J. Journal of Xinjiang Agricultural University*. **2000**, 23(2): 35-37.
[11]Mourboul Ablise, Zhang D. C., Rena Kasim. *J.Xinjiang Med. Univ.* **2012**, 02, 125-146.
[12]Mourboul Ablise, Wang Y. B., Xu F. Y. *J.Xinjiang Med. Univ.* **2013**, 36, 1744-1748.
[13]Li L. L., Zhang Y. M., Wang X. F. *J.Xinjiang Med. Univ.* **2005**, 02, 98-101.