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Antibacterial and cytotoxic activities of different wild bitter gourd cultivars (*Momordica charantia* L. var. *abbreviata* Seringe)

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ABSTRACT. Wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA), which is normally smaller than cultivated bitter gourd (*Momordica charantia* L., MC), both belong to the family Cucurbitaceae. The fresh fruits of MC and MCA are frequently used as vegetables in Taiwan. Water (H) and methanolic (M) extracts of sixteen cultivars from Taiwanese indigenous MCA (10 mg/mL) were tested for their antibacterial activities toward the methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), and *Salmonella enterica* subsp. *enterica* (ATCC 19126), and for their cytotoxic activities toward human fibrosarcoma HT 1080 cells. None of the extracts showed inhibitory activity against methicillin-resistant *Staphylococcus aureus* or *Pseudomonas aeruginosa*, however, several H and M extracts (even H extracts that were heated at 100°C for 5 min) showed inhibitory activity against the growth of *Escherichia coli* and *Salmonella enterica*. For cytotoxic activities toward human fibrosarcoma HT 1080 cells, H extracts (1 mg/mL) from mature D, E, and F cultivars, and M extracts (1 mg/mL) from mature D and E cultivars showed similar cytotoxic activities compared to that of 10 μg/mL of doxorubicin. The use of MCA extracts as natural food additives to control food-borne pathogens and/or their development as health foods for chemoprevention in the future is discussed.

Keywords: Antibacterial activity; Cytotoxic activity; *Escherichia coli*; *Momordica charantia* L. var. *abbreviata* Seringe (MCA); *Salmonella enteric*.

INTRODUCTION

Wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA), which is normally smaller than cultivated bitter gourd (*Momordica charantia* L., MC), both belong to the family Cucurbitaceae. The fresh fruits of MC and MCA are frequently used as vegetables in Taiwan. MC has been used as a traditional medicine and is listed in the Chinese pharmacopoeia *Ben Cao Kong Mu*. The different parts of MC extracts are reported to have many pharmacological activities (Grover and Yadav, 2004), including anti-bacterial (Omoregbe et al., 1996), anti-inflammatory (Kobori et al., 2008), anti-viral (Lee-Huang et al., 1990), cytotoxic (Lee-Huang et al., 1995), hypoglycemic (Miura et al., 2001; Rathi et al., 2002; Kar et al., 2003), and triglyceride-lowering activities (Senanayake et al., 2004). MCA extracts have activated the peroxisomal proliferator-activated receptor α (Chao and Huang, 2003), and have exhibited antioxidant (Wu and Ng, 2008) and anti-inflammatory activities (Lii et al., 2009). Wu and Ng (2008) assayed and compared the antioxidant and anti-radical activities of separate hot-water and ethanol extracts of one MCA cultivar.

In order to inhibit food-borne pathogens and to extend shelf life, synthetic chemicals are often used as preservatives in food processing and storage. Consumer awareness concerning the potential health risks of synthetic food

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additives has renewed the interest in naturally-occurring alternatives. Therefore, food preservatives derived from plants and herbs are of growing interest, since plant compounds often possess antimicrobial properties that protect them from infection (Serra et al., 2008; Lou et al., 2010). Bacterial resistance to β-lactam antibiotics has shown a significant increase in recent years, which has been attributed to the spread of plasmid-mediated extended spectrum β-lactamases (Sanders and Sanders, 1987). In our report, we tested the water (H) and methanolic (M) extracts of sixteen indigenous wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA) cultivars in Taiwan for their antibacterial activities, including the methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), and *Salmonella enterica* subsp. *enterica* (ATCC 19126), and for their cytotoxic activities toward human fibrosarcoma HT 1080 cells. Our results showed that MCA extracts may be used as natural food additives for the control of foodborne pathogens and/or developed as chemopreventive health food in the future.

MATERIALS AND METHODS

Materials

Bacterial strains including the methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), and *Salmonella enterica* subsp. *enterica* (ATCC 19126) and human fibrosarcoma HT 1080 cells were obtained from the Bioresources Collection and Research Center (BCRC), Food Industry Research and Development Institute (Hsinchu, Taiwan). Nutrient broth and Nutrient agar were from Difco (Becton Dickison Microbiology Systems, MD, USA). Other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of MCA extracts

Sixteen cultivars of fresh MCA (Figure 1) were provided by Researcher Jong-Ho Chuang (Hualien District Agricultural Research and Extension Station, Hualien, Taiwan). After washing, the flesh of each de-seeded MCA (200 g) was cut into strips and blended with 200 mL distilled water. After centrifugation at 12,500 ×*g* for 30 min, the supernatants were saved as crude water extracts which were lyophilized as H extracts. Each de-seeded MCA (200 g) was cut into strips and blended with 200 mL methanol, then left for three days. After filtering, the residue was extracted with methanol using the same procedure, twice. The filtrates were collected, concentrated by rotary vacuum evaporator and then lyophilized as M extracts.

Bacterial strain cultivation and growth inhibition

The methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), and *Salmonella enterica* subsp. *enterica* (ATCC 19126) were activated from lyophilized powders by nutrient broth, cultured on the nutrient agar following the manufacture's guidelines, then stored at 4°C. A single colony from each bacterial strain was then cultured in nutrient broth at 37°C for 18 h, counted and diluted to 2×10^6 CFU/mL broth. For growth inhibitory experiments, the total 200 μL of cultured solution contained 50 μL of each bacterial broth, 20 μL of MCA sample (10 mg/mL) and 130 μL of nutrient broth, which was placed on a 96-well microplate and cultured at 37°C for 0, 6, 12, 18 and 24 h. The changes in absorbance at 600 nm were recorded using an ELISA reader (TECAN Sunrise microplate reader, Männedorf, Switzerland) for growth turbidity. For heat-stable or heat-labile water extracts of MCA against *Escherichia coli* (ATCC 10536) and *Salmonella enterica* subsp. *enterica* (ATCC 19126), water extracts (H extracts) of MCA were preheated at 100°C for 5 minutes and then performed as mentioned above.

Cytotoxic activity of MCA against human fibrosarcoma HT 1080 cells

The human fibrosarcoma HT 1080 cell line was cultured in Dulbecco's modified eagle medium (DMEM, GibcoBRL, USA) containing 10% fetal bovine serum (FBS, GibcoBRL, USA), adjusted to 1×10^5 cell/mL, seeded in a 24-well plate (500 μL/well), then cultured in a 5% CO_2 humidified incubator at 37°C for 48 h with or without MCA sample additions. Two hours before the end of culturing, 5 μL of 12 mg/mL resaruzin was added to each well, the ratio of $A_{570nm}/A_{600\ nm}$ was measured using an ELISA reader (TECAN Sunrise microplate reader, Männedorf, Switzerland), and the cytotoxic activity was calculated with the equation: $(Blank_{A570nm/A600\ nm}\text{-}Sample_{A570nm/A600\ nm}) \div (Blank_{A570nm/A600\ nm}\text{-}Medium_{A570nm/A600\ nm})$.

RESULTS AND DISCUSSION

The color pictures of the 16 indigenous MCA cultivars we used for our study in Taiwan are shown in Figure 1. The cultivars A and D both contained mature (green flesh, AG and DG) and ripe (yellow flesh, AY and DY) MCA, and thus a total 18 different kinds of MCA were used in this experiment. The recovery of MCA extracts (H and M extracts) is shown at the bottom of each figure panel (Figure 1) as a percentage, and was around 2%. Except for the F, H, I, J, and N cultivars, the M extracts had a higher recovery rate than did the H extracts.

Although the use of antibiotics has greatly reduced the incidence of infectious diseases, their extensive uses in therapy or as growth promoters in animal food has led to the appearance of drug-resistant bacteria (Normanno et al., 2007), which is a major public health issue worldwide. Penicillin-resistant *Staphylococcus aureus* (PRSA) was found in the early 1950s (Finland, 1955), and 90% to 95% of clinical *S. aureus* strains throughout the world are resistant to penicillin (Sakoulas and Moellering Jr., 2008). Methicillin-resistance was first described in 1961, the year Methicillin was marketed (Jevons, 1961). In the past 2 de-

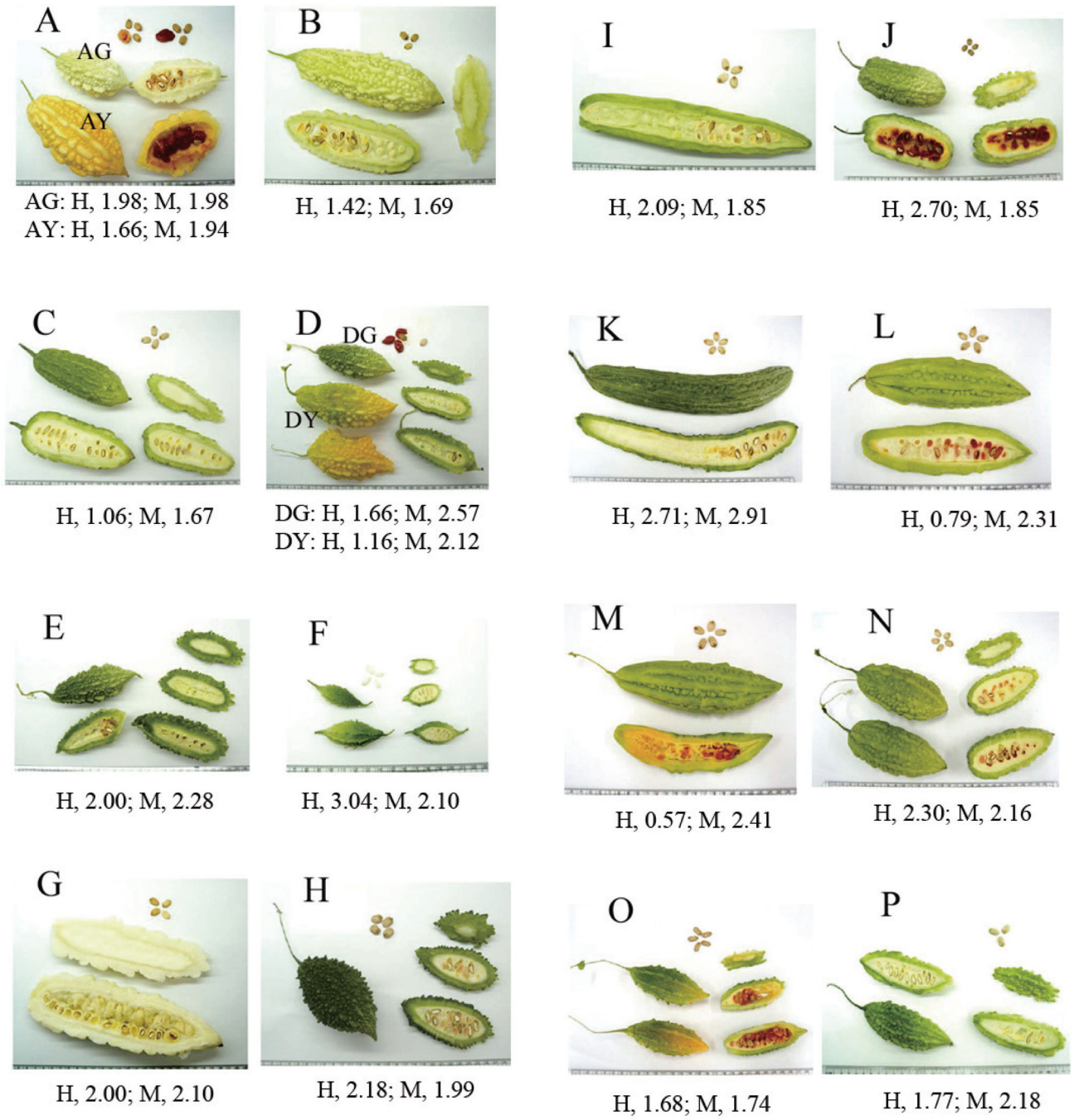

Figure 1. Photographs of different of wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe, MCA) cultivars used in experiments. There were sixteen cultivars (A to P) of MCA, cultivars of A and D contained the mature (AG and DG, green flesh) and ripe (AY and DY, yellow flesh) samples. The recovery (%) of each extract is shown at the bottom (H, water extracts; M, methanolic extracts).

cades, *S. aureus* (MRSA) has become a major nosocomial pathogen, and the therapeutic options for MRSA infection are very limited since most MRSA strains are resistant not only to β-lactams but also to multiple antimicrobial agents (Maple et al., 1989; Shiota et al., 1999). The β-lactam family of antibiotics includes many of the most commonly used antibacterials in clinical medicines. The majority of clinically useful β-lactams belong to either the penicillin (penam) or the cephalosporin (cepems) group (Tyczkowska et al., 1994). One of the major mechanisms of resistance to β-lactams was the expression of β-lactamases which hydrolyzed the β-lactam ring. The β-lactamases (EC 3.5.2.6), such as penicillinase and cephalosporinase, which degraded penam and cepems, respectively, have been found widely in both gram-positive and gram-negative bacteria (Livermore, 1995). In order to inhibit food-borne pathogens and to extend shelf life, synthetic chemicals are often used as preservatives in food processing and storage.

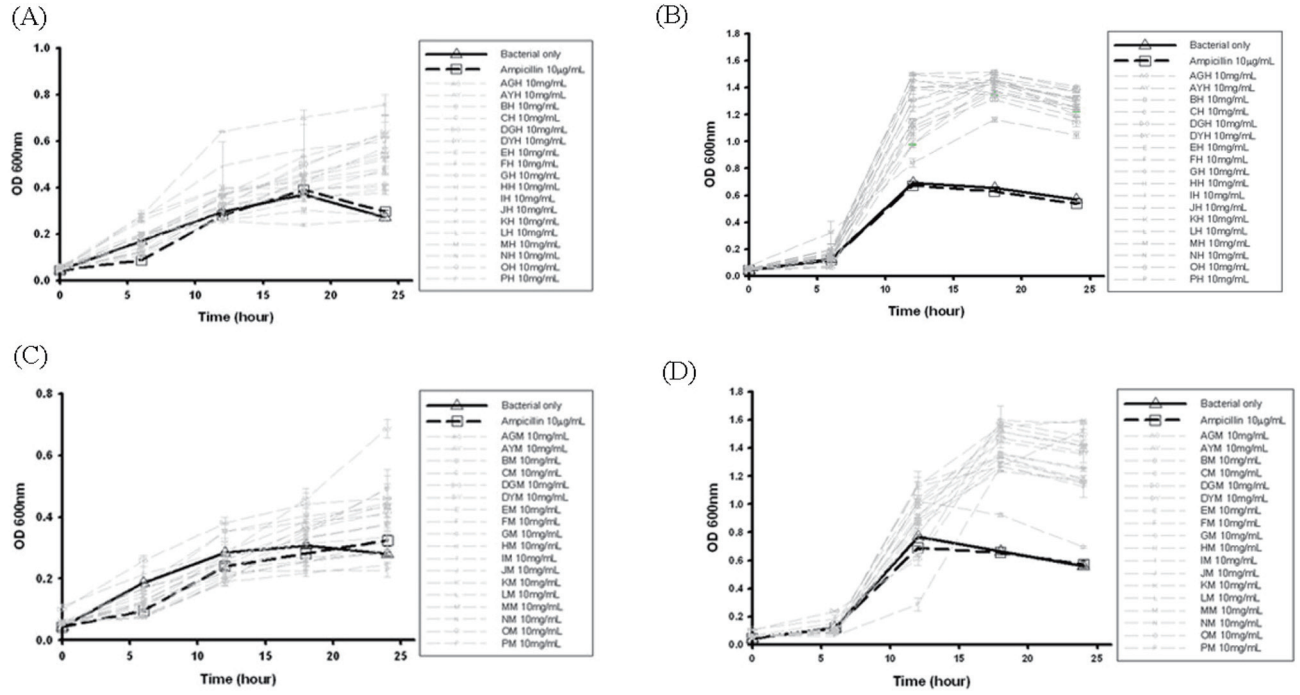

Figure 2. The water extracts (A, B) and methanolic extracts (C, D) of sixteen wild bitter gourd cultivars at a concentration of 10 mg/mL were used to inhibit the growth of (A, C) methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591) and (B, D) *Pseudomonas aeruginosa* (ATCC 27853) using spectrophotometry to detect changes in turbidity. Ampicillin (10 μg/mL) was used as a positive control.

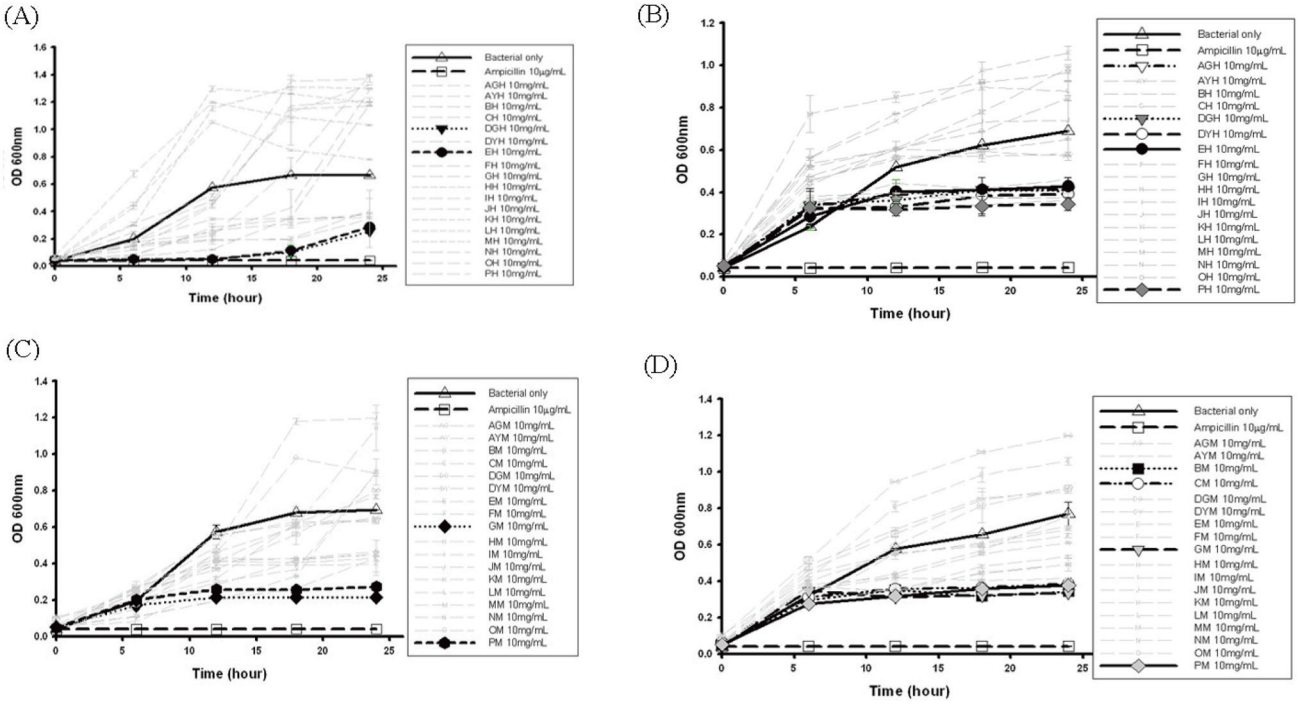

Figure 3. The water extracts (A, B) and methanolic extracts (C, D) of sixteen wild bitter gourd cultivars at a concentration of 10 mg/mL were used to inhibit the growth of (A, C) *Escherichia coli* (ATCC 10536) and (B, D) *Salmonella enterica* subsp. *enterica* (ATCC 19126) using spectrophotometry to detect changes in turbidity. Ampicillin (10 μg/mL) was used as a positive control.

Consumer awareness over the potential risks of synthetic food additives to human health have renewed the interest in using naturally occurring alternatives. Therefore, MCA extracts were screened for antimicrobial properties that could serve to protect consumers from microbial infection (Serra et al., 2008; Lou et al., 2010).

Figure 2 shows the antibacterial activities of H extracts (A and B) and M extracts (C and D) from different MCA cultivars against the methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591) (Figure 2A and 2C) and *Pseudomonas aeruginosa* (ATCC 27853) (Figure 2B and 2D) using the changes of turbidities in the cultures. The ampicillin (10 μg/mL) was used as a control. Basically, the ampicillin did not have effects on the methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (ATCC 33591) (Figure 2A and 2C) and *Pseudomonas aeruginosa* (ATCC 27853), and the turbidity curves of H (Figures 2A and 2B) and M extracts (Figures 2C and 2D) were above that of bacteria only, which meant that there were no effects on these two bacterial strains at concentrations of 10 mg/mL. There are previous reports concerning the use of plant crude extracts (Aqil et al., 2005, 2006) in combination with fewer amounts of antibiotics for anti-bacterial activities, especially for antibiotic-resistant bacteria, compared to antibiotics alone (Schmidt et al., 2008). Further investigations into the synergy of fractions or purified natural compounds from MCA and antibiotics for antibiotic-resistant bacteria.

Figure 3 showed the antibacterial activities of H extracts (A and B) and M extracts (C and D) from different MCA cultivars against the *Escherichia coli* (ATCC 10536) (Figure 3A and 3C) and *Salmonella enterica* subsp. *enterica* (ATCC 19126) (Figure 3B and 3D) using the changes of turbidities in the cultures. The ampicillin (10 μg/mL) was used as a control. Basically, the ampicillin have antibacterial effects on the *Escherichia coli* (ATCC 10536) (Figure 3A and 3C), and *Salmonella enterica* subsp. *enterica* (ATCC 19126) (Figure 3B and 3D). The turbidity curves of H extracts (DGH and EH, Figure 3A) or M extracts (GM and PM, Figure 3C) showed a promise antibacterial activities against *Escherichia coli* (ATCC 10536) at concentrations of 10 mg/mL; and the turbidity curves of H extracts (AGH, DGH, DYH, EH, and PH, Figure 3B) or M extracts (BM, CM, GM, and PM, Figure 3D) showed a promise antibacterial activities against *Salmonella enterica* subsp. *enterica* (ATCC 19126) at concentrations of 10 mg/mL. Khan and Omoloso (1998) showed that the water extracts of MC fruit exhibited antibacterial activity toward *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus albus*, *Salmonella typhi*, *Streptococcus faecalis*, and *Micrococcus roseus*. Our present results showed that antibacterial activities were dependent on MCA cultivars. The M extracts from GM and PM (Figures 3C and 3D) showed inhibitory activities against food-borne pathogens of *Escherichia coli* (ATCC 10536) and *Salmonella enterica* subsp. *enterica* (ATCC 19126). The H extracts from DGH and EH (Figures 3A and 3B)

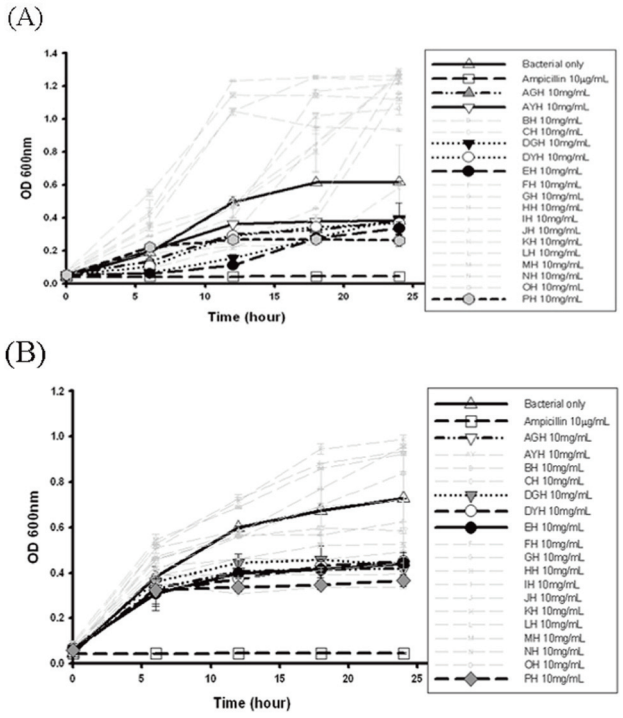

Figure 4. The water extracts of sixteen wild bitter gourd cultivars at a concentration of 10 mg/mL were heated at 100°C for 5 min and were then used to inhibit the growth of (A) *Escherichia coli* (ATCC 10536) and (B) *Salmonella enterica* subsp. *enterica* (ATCC 19126) using spectrophotometry to detect changes in turbidity. Ampicillin (10 μg/mL) was used as a positive control.

showed inhibitory activities against *Escherichia coli* (ATCC 10536) and *Salmonella enterica* subsp. *enterica* (ATCC 19126).

For food processing utilizations, the H extracts were preheated at 100°C for 5 min then separate *Escherichia coli* (ATCC 10536) (Figure 4A) and *Salmonella enterica* subsp. *enterica* (ATCC 19126) (Figure 4B) antibacterial assays were performed. The heat treatment did not affect the antibacterial activity toward both bacterial strains of food-borne pathogens, which meant that the components responsible for antibacterial activity were heat-stable in H extracts (Figure 4). Surprisingly, some H extracts, including AGH, AYH, and DYH, recovered antibacterial activities toward *Escherichia coli* (ATCC 10536), after heat treatments (Figure 4A). Some of the interfering substances were destroyed after being heated at 100°C for 5 min, which may have enhanced the antibacterial activity toward *Escherichia coli* (ATCC 10536) and may thus be beneficial for food processing utilizations. There were few reports concerning purified compounds from MC or MCA for antibacterial activity toward food-borne pathogens. Further purification and identification of the active compounds may be valuable.

Figure 5 shows the cytotoxic activity of H (Figure 5A) and M extracts (Figure 5B) of MCA cultivars toward human fibrosarcoma HT 1080 cells using a resaruzin assay.

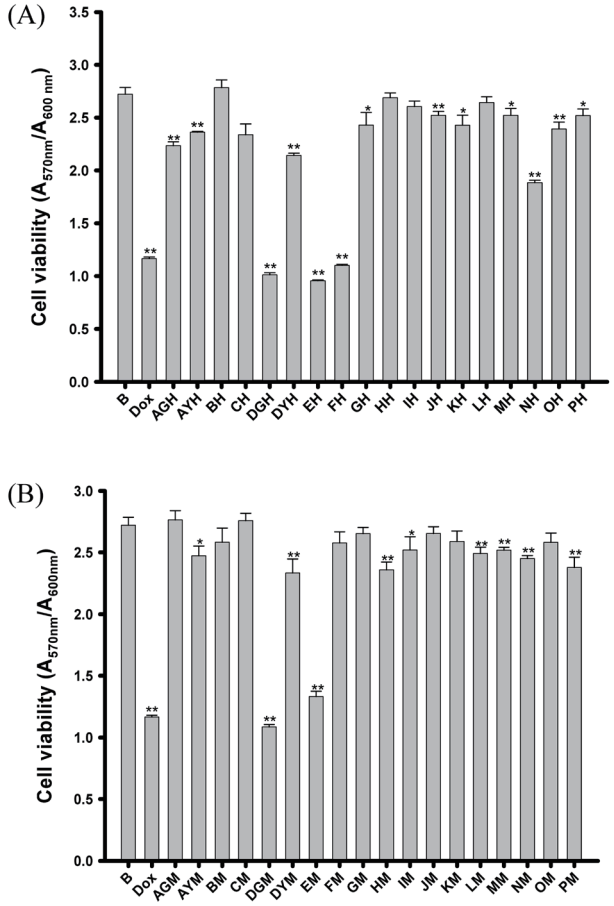

Figure 5. The cytotoxic activities of water extracts (A) and methanolic extracts (B) of sixteen wild bitter gourd cultivars at a concentration of 1 mg/mL against human fibrosarcoma HT 1080 cells by resazurin method and expressed as cell viability (A_{570nm}/A_{600nm}). Doxorubicin (10 μg/mL) was used as a positive control.

The basic theory of the resaruzin dye is that the blue color and the non-fluorescent resaruzin are absorbed into cells and reduced by living cells to a pink color and stronger fluorescent resorufin (O'Brien et al., 2000). Using doxorubicin (10 μg/mL) as a positive control, it was found that DGH, EH and FH (Figure 5A) and DGM and EM extracts (Figure 5B) showed comparably cytotoxic activities toward human fibrosarcoma HT 1080 cells. It was noted that H and M extracts from mature cultivar D had more cytotoxic activities than those from ripe ones. It was calculated that the half-inhibition concentration of H extracts and M extracts from DG were 0.37 and 0.55 mg/mL, respectively. It was reported that MAP 30 isolated from MC seeds exhibited cytotoxic activities toward different carcinoma cell lines (Lee-Huang et al., 1995). The α-momorcharin (a glycoprotein) isolated from MC seeds also showed cytotoxic activities toward cultured carcinoma cell lines (Tsao et al., 1990). Our present results show that the H and M extracts from deseeded MCA flesh exhibited cytotoxic activities toward human fibrosarcoma HT 1080 cells. Further work with purified active compounds for anticancer activity assays are necessary.

In conclusion, some MCA cultivars, especial for DGH and EH, showed antibacterial and cytotoxic activities toward human fibrosarcoma HT 1080 cells. In the future, MCA extracts could be used as natural food additives to control food-borne pathogens and/or be developed into chemopreventive health foods.

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不同品種山苦瓜抗細菌與細胞毒性之研究

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山苦瓜 (*Momordica charantia* L. var. *abbreviata* Seringe, MCA) 通常比常見之苦瓜 (*Momordica charantia* L., MC) 小，屬於葫蘆科植物。本實驗以花蓮區農業改良場提供之 16 種不同品種山苦瓜，分別以冷水（水抽物）與甲醇（甲醇抽取物）抽取，以濃度 10 mg/mL 進行抗細菌試驗，測試菌種包括抗藥性金黃色葡萄球菌 (ATCC 33591)、綠膿桿菌 (ATCC 27853) 、大腸桿菌 (ATCC 10536) 與沙門氏桿菌 (ATCC 19126)；以濃度 1 mg/mL 對 HT 1080 細胞進行細胞毒性評估。結果顯示，所有的山苦瓜抽取物對於抗藥性金黃色葡萄球菌 (ATCC 33591) 與綠膿桿菌 (ATCC 27853) 的生長都沒有抑制效果；部分品種之水抽物與甲醇抽取物具有抑制大腸桿菌 (ATCC 10536) 與沙門氏桿菌 (ATCC 19126) 的生長；即使將水抽物以 100°C 加熱五分鐘，亦有抑制上述兩種細菌生長。在 HT 1080 細胞細胞毒性方面，品種 D (成熟，綠皮)、E 與 F 的水抽物及品種 D (成熟，綠皮) 與 E 的甲醇抽取物具有顯著細胞毒性，與對照組藥物 doxorubicin (10 μg/mL) 相當。以上的結果顯示，山苦瓜抽取物未來也許可以開發為天然抑制食品病原細菌的食品添加物或是做為抑制癌細胞生長的保健品。

關鍵詞：抗細菌活性；細胞毒性 ; 大腸桿菌；山苦瓜；沙門氏桿菌。