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Research Article

Inhibition effect against 20 bacteria and 4 cell lines of methanol and water extract from pawpaw (*Asimina triloba* [L.] Dunal) cultivated in Korea

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Abstract Pawpaw (*Asimina triloba* [L.] Dunal) is a subtropical plant belonging to the Annonaceae family and is different from papaya. Except for the fruit, the remaining parts are used for medicinal purposes, but little is known about them in Korea. In this study, antibacterial activities of pawpaw extracts derived using methanol and water solvents on roots, twigs, and leaves were investigated by determining the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against 20 bacterial strains. Most of the extracts produced an inhibition zone at 50 mg/disc against all tested microorganisms. In particular, pawpaw twigs and root methanolic extracts (PTM and PRM, respectively) showed potent antibacterial effects superior to those of other samples. The anticancer activities of the extracts were investigated using a cytotoxicity assay against 4 cancer cell lines (human fibrosarcoma cells HT-1080, human cervical cancer cells HeLa, human liver cancer cells HepG2, and human gastric cancer cells AGS). PTM showed the strongest inhibition activity against HT-1080 (IC_{50}=64.57 μg/mL), HepG2 (IC_{50}=68.99 μg/mL), and AGS (IC_{50}=70.48 μg/mL) cells. PRM exhibited the highest antiproliferative activity for HeLa cells, with an IC_{50} value of 126.17 μg/mL. These findings suggest that the pawpaw twigs and root extracts have medicinal value and can be further investigated as biomaterials in various fields.

Keywords *Asimina triloba*, pawpaw, antibacterial activity, anticancer activity

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1. Introduction

Plants are a major source of first-line medicine for local communities around the world (Mbosso et al., 2015), and two-thirds of the world's population uses traditional therapies due to their efficacy and the high cost of pharmaceutical products (Tagboto and Townson, 2001). Approximately 6% of the world's plants have been screened for their bioactivity, and only 15% of these have been evaluated for phytochemicals such as phenolic compounds, alkaloids, and terpenoids, which are used to develop antioxidant, anticancer, antimicrobial, and anti-inflammatory pharmaceuticals (Verpoorte, 2000). Many antimicrobial and anticancer medicines now on the market contain plant-derived natural material. Research is continuing into the wide range of traditionally therapeutic plants not yet investigated.

Pawpaw (*Asimina triloba* [L.] Dunal) is a commercially important member of the Annonaceae family that includes custard apple, cherimoya, sweetsop, and soursop (Brannan et al., 2015). It was historically harvested in the wild but is now cultivated as an orchard crop in several regions of America, Italy, Portugal, Belgium, China, Japan, and Korea (Brannan et al., 2015; Nam et al., 2017). It can be grown without pesticides (Ferreira et al., 2011) and is tolerant of a range of climates (Callaway, 1993). However, pawpaw is not well known to people, and although it has been steadily cultivated by farmers since 2015, it is difficult to commercialize it.

Since pawpaw is associated with a number of health benefits, various studies have been investigated its antioxidant, anticancer, and trematocidal activities (Brannan and Salabak, 2009; Farag, 2009; Kobayashi et al., 2008; McLaughlin, 2008; Nam et al., 2018; Nam et al., 2021; Ratnayake et al., 1993). In particular, acetogenin contained in the pawpaw is known to have strong anticancer activity, and a medicine made from pawpaw extract containing 50 types of acetogenin is also on sale. However, domestic research on the anticancer activity of pawpaw is minimal and limited to specific cancer cells. In addition, there have been few studies on antibacterial effects according to various pawpaw parts. Therefore, we aimed to investigate effective antibacterial and anticancer activities of materials derived from pawpaw as a promising source of phytochemicals.

2. Materials and methods

2.1. Preparation of samples and reagents

Pawpaw trees (n=150; 2-3 years old, 1-2 m height) were obtained from a farm in Okchon, Korea (average annual temperature of 13.0℃, average annual relative humidity of 66.7%, average annual wind velocity of 1.9 m/s; total annual rainfall 1,458.7 mm). A voucher specimen was authenticated by Dr. Otto Jahn (United States Department of Agriculture/ Agricultural Research Service) and was deposited in the herbarium by the U.S. National Plant Germplasm System. After cleaning the trees, the leaves, twigs, and roots were separated and immediately washed with cold water to remove any soil, dust, or insects. The samples were then lyophilized using a pilot plant freeze dryer (LP100, Ilshin Lab Co., Daejeon, Korea), pulverized with a grinder (Blixer, Robot Coupe USA, Jackson, MS, USA), and passed through a 40-mesh sieve to remove large debris. Samples were stored in a deep freezer at -70℃ until use.

Mueller Hinton Agar (MHA) and kanamycin were purchased from Difco (Sparks, MD, USA). Roswell Park Memorial Institute (RPMI) and Dulbeco's Modified Eagle's Medium (DMEM) were obtained from Gibco (Grand Island, NY, USA), and fetal bovine serum (FBS) and streptomycin-penicillin were purchased from Welgene (Seoul, Korea). Tetrazolium-based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was supplied from Sigma-Aldrich (St. Louis, MO, USA). All solvents and chemicals used were of analytical grade.

2.2. Preparation of sample extracts

Water and methanol, which are most commonly used when measuring various biological activities, show different activities depending on the properties of the functional compounds. Briefly, water can extract aqueous compounds, and water-methanol mixed solvent can extract both aqueous and nonaqueous compounds. Accordingly, pawpaw leaf, twig, and root were extracted in distilled water or 80% methanol in at least triplicate to obtain pawpaw leaf, twig, and root distilled water extracts (PLW, PTW, and PRW, respectively) and methanolic extracts (PLM, PTM, and PRM, respectively). Briefly, distilled water or 80% methanol was added to each sample at a ratio of 1:10 (w/v) with continuous agitation on a horizontal shaker (BS-21, Jeio Tech, Daejeon, Korea) for 24 h at 25℃. The extracts were centrifuged at 11,325 ×*g* for 20 min at 4℃ and the supernatant was recovered using vacuum filtration. Sample extracts were concentrated using a rotary evaporator (R-210, Buchi, Flawil, Switzerland) and lyophilized. The dried extracts were weighed to calculate the yield and stored at -70℃ until further analysis.

2.3. Microorganisms

The test strains for the agar diffusion assay were obtained from the Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea) and Korean Culture Center of Microorganisms (KCCM) (Seoul, Korea), and included gram-positive bacteria (*Bacillus cereus* KCCM 40935, *Bacillus subtilis* KCTC 3135, *Clostridium perfringens* KCTC 3269, *Corynebacterium xerosis* KCTC 3435, *Listeria monocytogenes* KCTC 13064, methicillin-resistant *S. aureus* KCCM 40510, *Propionibacterium acnes* KCTC 3314, *Staphylococcus aureus* KCCM 12103,

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Staphylococcus epidermidis KCCM 40416, and *Streptococcus mutans* KCCM 40105) and gram-negative bacteria (*Campylobacter jejuni* KCTC 5327, *Cronobacter sakazakii* KCTC 2949, *Escherichia coli* KCTC 2441, *E. coli* O157:H7 KCCM 11862, *Proteus vulgaris* KCTC 2512, *Pseudomonas fluorescens* KCTC 12453, *Salmonella typhimurium* KCTC 2514, *Vibrio fluvialis* KCTC 2473, *Vibrio parahaemolyticus* KCCM 11965, and *Yersinia enterocolitica* KCCM 41657) (Table 1). After passaging twice in tryptic soy broth medium (Difco), the strains were activated in MH broth (MHB) at 37℃ for 24 h. Bacterial suspensions of each strain were prepared in sterile water and stored at 4℃ for 24 h.

2.4. Determination of antimicrobial activity using agar diffusion assay

The antimicrobial activity of *A. triloba* extracts was evaluated using a modified version of the Kirby-Bauer disc diffusion method (Bauer et al., 1966; Rios et al., 1988). The extract concentrations were adjusted with water or 80% methanol to 100, 250, 500, 750, and 1,000 mg/mL and sterilized by passage through a 0.45 μM syringe filter. A 50 μL volume of these extracts was injected onto a 6 mm sterile disc (Advantec Toyo Roshi Kaisha, Tokyo, Japan) and the solvent was evaporated under an aseptic hood, yielding discs containing 5, 12.5, 25, 37.5, and 50 mg crude extract. A standard kanamycin disc (30 μg/disc) served as positive control and distilled water or 80% methanol served as negative controls for the antimicrobial test.

A double layer of medium was used to determine the inhibition zone of bacteria. The bottom and top layers of MHA contained 1.5% and 0.75% agar, respectively. The turbidity of the bacterial suspension was adjusted to 0.5

Table 1. Bacterial strains used to assess antimicrobial activity

Bacterial strain	Medium	Origin	
Gram-positive bacteria			
Bacillus cereus	Muller Hinton agar	KCCM 40935	ATCC 14579
Bacillus subtilis	Muller Hinton agar	KCTC 3135	ATCC 6051
Clostridium perfringens	Reinforced Clostridial Medium	KCTC 3269	ATCC 13124
Corynebacterium xerosis	Muller Hinton agar	KCTC 3435	ATCC 373
Listeria monocytogenes	Brain Heart Infusion agar	KCTC 13064	ATCC 15313
Methicillin-resistant *Staphylococcus aureus*	Muller Hinton agar	KCCM 40510	ATCC 33591
Propionibacterium acnes	Muller Hinton agar	KCTC 3314	ATCC 6919
Staphylococcus aureus	Muller Hinton agar	KCCM 12103	ATCC 12600
Staphylococcus epidermidis	Muller Hinton agar	KCCM 40416	ATCC 14990
Streptococcus mutans	Brain Heart Infusion agar	KCCM 40105	ATCC 25175
Gram-negative bacteria			
Campylobacter jejuni	Heart Infusion agar	KCTC 5327	ATCC 33560
Cronobacter sakazakii	Muller Hinton agar	KCTC 2949	ATCC 29544
Escherichia coli	Muller Hinton agar	KCTC 2441	ATCC 11775
Escherichia coli O157:H7	Muller Hinton agar	KCCM 11862	ATCC 14193
Pseudomonas fluorescens	Muller Hinton agar	KCTC 12453	ATCC 13525
Proteus vulgaris	Muller Hinton agar	KCTC 2512	ATCC 13315
Salmonella typhimurium	Muller Hinton agar	KCTC 2514	ATCC 13311
Vibrio fluvialis	3% NaCl Nutrient agar	KCTC 2473	ATCC 33809
Vibrio parahaemolyticus	3% NaCl Nutrient agar	KCCM 11965	ATCC 17802
Yersinia enterocolitica	Muller Hinton agar	KCCM 41657	ATCC 23715

McFarland standard. The bottom layer was spread in the dish and the molten top agar was mixed with 100 μL bacterial suspension and immediately poured on the bottom layer. Sample filter paper discs were placed on the dish followed by incubation at 37℃ for 20 h. Antimicrobial activity was determined by measuring the diameter of the clear zone (mm) for triplicate samples.

2.5. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC, defined as the lowest concentration of antimicrobial substance that can inhibit microbial growth (Edziri et al., 2012; Sharma et al., 2012), was determined using broth microdilution for 20 pathogens using 96-well micro test plates (SPL Life Sciences, Seoul, Korea). Briefly, a bacterial suspension was diluted to 1.5×10^8 cfu/mL using MHB and 100 μL was seeded in a 96-well plate, followed by treatment with an equal amount of sample extract (0.006 mg/mL to 100 mg/mL). The plate was then incubated at 37℃ for 20 h. The optical density of the culture was measured at 600 nm using VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). MBC was investigated for each well showing growth inhibition. Bacterial cells from the MIC test plate were sub-cultured on an agar plate by pouring them onto the agar surface and incubating at 37℃ for 20 h. The concentration at which no bacterial growth occurred on the plate was considered the MBC (Akinyemi et al., 2005).

2.6. Cell culture

RAW 264.7 cell lines and human cancer cell lines from the Korean Cell Line Bank (KCLB; Seoul, Korea) were used to assess the anticancer effects of pawpaw extracts: RAW 264.7 (KCLB No. 40071), HT-1080 fibrosarcoma (KCLB No. 10121), HeLa cervical cancer (KCLB No. 10002), HepG2 hepatocellular carcinoma (KCLB No. 88065), and AGS stomach cancer (KCLB No. 21739). Cells were cultured in RPMI or DMEM containing 10% FBS and 100 unit/mL streptomycin-penicillin at 37℃ and 5% CO_2.

2.7. Cytotoxicity assay

RAW 264.7 or 4 human cancer cell lines ($2\text{-}4\times10^4$ cells/well) were treated with various concentrations (0, 2.5, 10, 50, 100, 200 μg/mL) of pawpaw extract in 96-well micro test plates. The treated plates were incubated for 24 h or 48 h at 37℃ in a 5% CO_2 incubator (BB 15, Thermo Fisher Scientific, Langenselbold, Germany). Following this, 20 μL MTT solution (5 mg/mL) was added to the wells and allowed to react for 2 h. The medium was removed by suctioning and 100 μL dimethyl sulfoxide was added to each well. Optical density at 540 nm was measured on a microplate reader (Infinite M200, Tecan, Männedorf, Switzerland) (Nam et al., 2018). The cytotoxicity of pawpaw extracts was expressed as the inhibition concentration (IC) of the pawpaw extracts that yielded 50% growth inhibition of human cancer cells.

2.8. Statistical analysis

Data were analyzed using SPSS v. 18.0 (SPSS Inc., Chicago, IL, USA) and are presented as mean±SD. Mean differences were evaluated using one-way analysis of variance with Duncan's multiple range test, and considered significant at $p<0.05$.

3. Results and discussion

3.1. Antibacterial activity of pawpaw extracts

Antibacterial activities of six pawpaw extracts (PLW, PTW, PRW, PLM, PTM, and PRM) were evaluated using agar diffusion assay at concentrations of 5, 12.5, 25, 37.5, and 50 mg/disc in 20 strains of bacteria, including pathogenic microorganisms.

The antibacterial activity of each part of the pawpaw was very diverse (Tables 2 and 3). PLW showed inhibitory activity against *B. cereus*, *C. perfringens*, *C. xerosis*, and *S. aureus* among gram-positive bacteria (6.33-17.33 mm clear zone), and exhibited inhibitory activity against *C. sakazakii*, *E. coli*, *E. coli* O157:H7, *P. fluorescens*, *P. vulgaris*, *S. typhimurium*, *V. fluvialis*, *V. parahaemolyticus*, and *Y. enterocolitica* among gram-negative bacteria (6.00-15.33 mm clear zone). PTW showed inhibitory activity against *B. cereus*, *B. subtilis*, *C. perfringens*, Methicillin-resistant *S. aureus*, and *S. aureus* among gram-positive bacteria (6.33-19.00 mm clear zone), and exhibited inhibitory activity against *E. coli*, *E. coli* O157:H7, *P. fluorescens*, *S. typhimurium*, *V. fluvialis*, *V. parahaemolyticus*, and *Y. enterocolitica* among gram-negative bacteria (6.00-15.67 mm clear zone). PRW no exhibited inhibitory effect on gram-positive bacteria except for *B. cereus*, *B. subtilis*, and *C. perfringens*, and showed broad

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Table 2. Antimicrobial activity of *Asimina triloba* extracts against gram-positive bacteria

Strain	Concentration (mg/disc)	Diameter of clear zone (mm)[1]						
		PLW[2]	PTW	PRW	PLM	PTM	PRM	Kanamycin (30 μg/disc)
Bacillus cereus	5	6.33±0.58[3)b4)]	–[5]	–	6.33±0.58[b]	8.00±1.00[a]	8.67±0.58[a]	23.17±1.25
	12.5	8.33±0.58[b]	–	–	7.67±0.58[b]	11.00±0.00[a]	11.33±0.58[a]	
	25	9.67±0.58[b]	6.33±0.58[c]	7.00±1.00[c]	9.67±0.58[b]	13.33±0.58[a]	12.67±0.58[a]	
	37.5	10.67±0.58[b]	7.67±0.58[d]	9.00±0.00[c]	11.33±0.58[b]	14.67±0.58[a]	14.67±0.58[a]	
	50	11.67±0.58[c]	10.00±0.00[d]	10.33±0.58[d]	13.00±1.00[b]	16.33±0.58[a]	16.67±0.58[a]	
Bacillus subtilis	5	–	–	–	–	–	10.00±0.00[a]	31.00±0.84
	12.5	–	–	–	–	9.33±0.58[b]	12.00±0.00[a]	
	25	–	–	–	–	12.33±0.58[b]	13.33±0.58[a]	
	37.5	–	6.33±0.58[b]	7.33±0.58[b]	–	15.33±1.15[a]	15.67±0.58[a]	
	50	–	7.67±0.58[d]	9.00±0.00[c]	–	17.33±0.58[b]	20.00±0.00[a]	
Clostridium perfringens	5	–	11.00±0.00[c]	11.33±0.58[c]	12.33±0.58[b]	19.67±0.00[a]	19.00±1.00[a]	8.39±0.50
	12.5	8.00±0.00[e]	12.33±0.58[d]	14.00±1.00[c]	14.00±1.00[c]	24.00±1.00[a]	20.33±0.58[b]	
	25	11.00±0.00[e]	15.67±0.58[d]	18.33±0.58[c]	18.67±1.15[c]	28.33±0.58[a]	23.67±0.58[b]	
	37.5	12.67±0.58[e]	17.00±1.00[d]	21.00±1.00[c]	20.80±1.00[c]	29.33±0.58[a]	26.00±1.00[b]	
	50	14.00±0.00[e]	19.00±1.00[d]	24.67±1.53[c]	20.33±0.58[d]	30.33±0.58[a]	28.00±1.00[b]	
Corynebacterium xerosis	5	–	–	–	–	7.33±0.58[a]	–	34.67±0.69
	12.5	–	–	–	–	9.33±0.58[a]	9.00±0.00[a]	
	25	8.67±0.58[c]	–	–	–	14.33±0.58[a]	12.00±0.00[b]	
	37.5	12.67±0.58[c]	–	–	–	15.00±0.00[b]	16.33±0.58[a]	
	50	17.33±0.58[b]	–	–	–	16.33±0.58[c]	20.67±0.58[a]	
Listeria monocytogenes	5	–	–	–	–	–	–	20.61±1.29
	12.5	–	–	–	–	7.33±0.58[a]	6.33±0.58[b]	
	25	–	–	–	–	8.67±0.58[a]	8.00±0.00[b]	
	37.5	–	–	–	–	10.33±0.58[a]	9.00±0.00[b]	
	50	–	–	–	–	13.33±0.58[a]	9.67±0.58[b]	
Methicillin-resistant *S. aureus*	5	–	–	–	–	–	–	-
	12.5	–	–	–	–	9.00±0.00[a]	–	
	25	–	–	–	9.33±0.58[b]	13.00±1.00[a]	6.67±0.58[c]	
	37.5	–	7.33±0.58[d]	–	13.67±1.15[b]	18.33±0.58[a]	10.00±0.00[c]	
	50	–	8.67±0.58[d]	–	16.00±1.00[b]	21.00±0.00[a]	13.33±0.58[c]	
Propionibacterium acnes	5	–	–	–	–	–	–	19.94±0.87
	12.5	–	–	–	–	–	–	
	25	–	–	–	–	–	6.33±0.58[a]	
	37.5	–	–	–	–	–	9.00±0.00[a]	
	50	–	–	–	–	–	12.00±0.00[a]	

(continued)

Strain	Concentration (mg/disc)	Diameter of clear zone (mm)[1)]						
		PLW[2)]	PTW	PRW	PLM	PTM	PRM	Kanamycin (30 μg/disc)
Staphylococcus aureus	5	–	–	–	–	–	6.33±0.58[a]	24.28±0.75
	12.5	8.00±0.00[a]	–	–	8.33±0.58[a]	6.67±0.58[b]	8.33±0.58[a]	
	25	8.67±0.58[b]	7.00±0.00[c]	–	11.33±0.58[a]	9.00±0.00[b]	11.33±0.58[a]	
	37.5	9.33±0.58[b]	9.00±0.00[b]	–	12.33±0.58[a]	12.67±0.58[a]	13.00±0.00[a]	
	50	12.00±1.00[b]	10.33±0.58[c]	–	13.67±0.58[a]	14.67±0.58[a]	14.67±0.58[a]	
Staphylococcus epidermidis	5	–	–	–	–	–	–	21.50±0.92
	12.5	–	–	–	–	6.33±0.58[b]	7.50±0.50[a]	
	25	–	–	–	6.67±0.58[b]	11.00±0.00[a]	10.83±0.76[a]	
	37.5	–	–	–	8.67±0.58[b]	12.00±0.00[a]	12.00±0.00[a]	
	50	–	–	–	10.00±0.00[b]	13.33±0.58[a]	13.67±0.58[a]	
Streptococcus mutans	5	–	–	–	–	–	–	12.72±0.46
	12.5	–	–	–	–	–	6.33±0.58[a]	
	25	–	–	–	–	–	9.67±0.58[a]	
	37.5	–	–	–	–	–	12.33±0.58[a]	
	50	–	–	–	–	–	15.00±0.00[a]	

[1)]The diameter of the paper disc was 6 mm.
[2)]PLW, pawpaw leaf water extract; PTW, pawpaw twig water extract; PRW, pawpaw root water extract; PLM, pawpaw leaf methanolic extract; PTM, pawpaw twig methanolic extract; PRM, pawpaw root methanolic extract.
[3)]Values are mean±SD (n=3).
[4)]Means with different superscript letters within a row differ significantly at $p<0.05$ by Duncan's multiple range test.
[5)]– indicates a zone of no inhibition.

inhibitory activity against some gram-negative bacteria (6.00-20.50 mm clear zone). PLM showed inhibitory effect on gram-positive bacteria including *B. cereus*, *C. perfringens*, Methicillin-resistant *S. aureus*, *S. aureus*, and *S. epidermidis* (6.33-20.80 mm clear zone) and exhibited effect on gram-negative bacteria including *V. fluvialis* and *Y. enterocolitica* (6.00-12.33 mm clear zone). PTM showed inhibitory activity against all gram-positive bacteria except for *P. acne*s and *S. mutans*, and exhibited inhibitory effect on gram-negative bacteria including *C. jejuni*, *P. vulgaris*, *V. fluvialis*, *V. parahaemolyticus*, and *Y. enterocolitica*, ranged from 6.00-25.67 mm clear zone). PRM, at 50 mg/disc, exhibited potent, broad-spectrum antibacterial activity against all tested gram-positive and -negative strains, producing inhibition zones ranging from 9.67-29.50 mm. It was also the only sample that inhibited the growth of *P. acnes* and *S. mutans*. *P. acnes* is a pathogen that prefers anaerobic conditions and is related to dermal acne (Kirschbaum and Kligman, 1963). *S. mutans* is another anaerobic pathogen which contributes to dental caries (Persson et al., 1998). Consequently, pawpaw is under study for improving the quality of toothpaste. Based on these observations, PRM has potential applications as an active ingredient in cosmetics or toothpaste. *C. perfringens* was inhibited intensely by all pawpaw extracts. In particular, PTM showed stronger inhibitory activity than that of other extracts, presenting a clear zone of 30.33 mm at 50 mg/disc. *C. perfringens* is a spore-forming pathogen and is one of the most common causes of food poisoning in the United States (Kenneth, 1994). As such, PTM extract may be applicable in food preservatives.

Gram-positive bacteria were more inhibited by pawpaw methanol extracts than water extracts, and gram-negative bacteria were more inhibited by water extracts than methanol extracts. For example, PLW, PTW, and PRW had no

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Table 3. Antimicrobial activity of *Asimina triloba* extract against gram-negative bacteria

Strain	Concentration (mg/disc)	Diameter of clear zone (mm)[1)]						
		PLW[2)]	PTW	PRW	PLM	PTM	PRM	Kanamycin (30 μg/disc)
Campylobacter jejuni	5	–[3)]	–	–	–	–	–	20.72±1.27
	12.5	–	–	–	–	6.33±0.58[4)a5)]	–	
	25	–	–	–	–	8.67±0.58[a]	9.00±0.00[a]	
	37.5	–	–	–	–	10.00±0.00[b]	10.83±0.29[a]	
	50	–	–	–	–	11.00±0.00[b]	13.00±1.00[a]	
Cronobacter sakazakii	5	–	–	–	–	–	–	22.50±0.42
	12.5	–	–	–	–	–	–	
	25	6.50±0.87[a]	–	–	–	–	6.33±0.58[a]	
	37.5	7.17±0.29[b]	–	–	–	–	9.33±0.58[a]	
	50	9.83±0.29[b]	–	–	–	–	10.67±0.58[a]	
Escherichia coli	5	–	–	–	–	–	–	19.78±1.59
	12.5	–	–	–	–	–	6.33±0.58[a]	
	25	6.33±0.58[c]	7.33±0.58[b]	6.33±0.58[c]	–	–	10.33±0.58[a]	
	37.5	8.00±0.00[b]	8.33±0.29[b]	8.00±0.00[b]	–	–	11.67±0.58[a]	
	50	8.50±0.50[b]	8.83±0.29[b]	8.83±0.29[b]	–	–	14.67±0.58[a]	
Escherichia coli O157:H7	5	7.00±0.00[a]	–	–	–	–	6.33±0.58[b]	–
	12.5	8.00±0.00[b]	6.33±0.58[c]	–	–	–	9.00±0.00[a]	
	25	9.33±0.58[b]	8.67±0.58[b]	7.00±1.00[c]	–	–	11.67±1.53[a]	
	37.5	10.33±0.58[bc]	9.67±0.58[c]	11.00±1.00[b]	–	–	13.67±0.58[a]	
	50	13.00±1.00[b]	11.00±1.00[c]	13.67±0.58[b]	–	–	16.00±1.00[a]	
Pseudomonas fluorescens	5	–	–	–	–	–	–	19.00±0.59
	12.5	–	–	–	–	–	–	
	25	6.00±0.00[b]	6.00±0.00[b]	–	–	–	8.00±1.00[a]	
	37.5	8.00±0.00[b]	7.67±0.58[b]	6.67±0.58[c]	–	–	12.67±0.58[a]	
	50	9.00±0.00[b]	8.67±0.58[b]	9.33±0.58[b]	–	–	15.33±0.58[a]	
Proteus vulgaris	5	–	–	–	–	–	–	24.39±0.78
	12.5	–	–	–	–	–	9.00±0.00[a]	
	25	–	–	6.00±0.00[b]	–	–	12.00±1.00[a]	
	37.5	6.00±0.00[d]	–	7.67±0.58[c]	–	9.00±0.00[b]	15.00±1.00[a]	
	50	7.00±0.00[d]	–	9.00±0.00[c]	–	11.00±0.00[b]	17.33±1.15[a]	
Salmonella typhimurium	5	–	–	–	–	–	–	22.39±0.92
	12.5	6.67±0.58[a]	–	–	–	–	–	
	25	8.33±0.58[b]	7.33±0.58[bc]	6.67±0.58[c]	–	–	11.00±1.00[a]	
	37.5	9.67±0.58[b]	10.00±0.00[b]	8.33±0.58[c]	–	–	12.67±0.58[a]	
	50	11.33±0.58[b]	11.33±0.58[b]	9.67±0.58[c]	–	–	14.00±1.00[a]	

(continued)

Strain	Concentration (mg/disc)	Diameter of clear zone (mm)[1]						
		PLW[2]	PTW	PRW	PLM	PTM	PRM	Kanamycin (30 μg/disc)
Vibrio fluvialis	5	6.00±0.00^{b}	–	–	–	6.67±0.58^{a}	–	17.25±0.45
	12.5	8.00±1.00^{b}	–	–	–	9.00±0.00^{a}	–	
	25	9.67±0.76^{a}	8.00±1.00^{b}	6.33±0.58^{c}	8.00±1.00^{b}	10.00±0.00^{a}	8.33±0.58^{b}	
	37.5	10.67±0.58^{b}	13.33±0.58^{a}	8.00±1.00^{c}	10.33±0.58^{b}	11.00±0.00^{b}	11.00±0.00^{b}	
	50	12.00±0.00^{c}	15.67±1.15^{a}	10.33±0.58^{d}	12.33±0.58^{c}	12.00±0.00^{c}	14.00±0.00^{b}	
Vibrio parahaemolyticus	5	–	–	7.17±0.29^{b}	–	9.00±0.50^{a}	9.17±0.29^{a}	19.11±0.76
	12.5	–	–	9.33±0.58^{c}	–	11.67±0.58^{b}	14.17±0.29^{a}	
	25	8.00±0.00^{d}	–	12.00±0.50^{c}	–	16.33±0.58^{b}	17.33±0.58^{a}	
	37.5	10.17±0.29^{d}	8.17±0.29^{e}	15.33±0.58^{c}	–	21.83±0.76^{b}	24.50±0.50^{a}	
	50	11.50±0.50^{d}	9.33±0.58^{e}	20.50±0.50^{c}	–	25.67±0.58^{b}	29.50±0.50^{a}	
Yersinia enterocolitica	5	–	–	–	–	–	8.00±0.00^{a}	26.56±0.92
	12.5	6.00±0.00^{b}	–	6.00±0.00^{b}	–	6.00±0.00^{b}	10.00±0.00^{a}	
	25	8.00±0.00^{c}	6.33±0.58^{d}	7.67±0.58^{c}	6.00±0.00^{d}	9.00±0.00^{b}	13.00±0.00^{a}	
	37.5	10.33±0.58^{b}	8.33±0.58^{cd}	9.00±0.00^{c}	8.00±0.00^{d}	10.17±0.76^{b}	19.33±0.58^{a}	
	50	15.33±0.58^{b}	9.67±0.58^{d}	10.33±0.58^{cd}	9.33±0.58^{d}	11.33±0.58^{c}	21.67±0.58^{a}	

[1]The diameter of the paper disc was 6 mm.
[2]PLW, pawpaw leaf water extract; PTW, pawpaw twig water extract; PRW, pawpaw root water extract; PLM, pawpaw leaf methanolic extract; PTM, pawpaw twig methanolic extract; PRM, pawpaw root methanolic extract.
[3]– indicates a zone of no inhibition.
[4]Values are mean±SD (n=3).
[5]Means with different superscript letters within a row differ significantly at $p<0.05$ by Duncan's multiple range test.

antibacterial activity against 4 gram-positive bacteria (*L. monocytogenes*, *P. acnes*, *S. epidermidis*, and *S. mutans*) that were inhibited by PLM, PTM, or PRM. However, they had antibacterial activity against 4 gram-negative bacteria (*E. coli*, *E. coli* O157:H7, *P. fluorescens*, and *S. typhimurium*) that were not inhibited by PLM and PTM. According to a previous study, 4 extracts (n-hexane, methanol, ethanol, and distilled water) from guava contain various phytochemicals including phenols, tannins, saponins, terpenoids, flavonoids, and glycosides. Among guava extracts, a distilled water extract was the only one containing saponins (Biswas et al., 2013). In addition, saponin content of dandelion stem was higher in water extract than in methanol extract (Mir et al., 2013). Saponin fractions obtained from *Gymnema sylvestre* and *Eclipta prostrata* were more efficacious against gram-negative bacteria than gram-positive bacteria (Gopiesh Khanna and Kannabiran, 2008). Therefore, we suggest that the greater efficacy of pawpaw water extract against gram-negative bacteria may be due to saponins contained in the water extract.

The greater antibacterial effects in methanol extracts is consistent with previous reports from sweetsop (*Annona squamosa*), a co-familial species with pawpaw (Patel and Kumar, 2008), possibly because bioactive compounds have greater solubility in alcohol than in water. Our previous study indicated that PLM, PTM, and PRM had higher phenolic and flavonoid contents than PLW, PTW, or PRW (Nam et al., 2017). In addition, other studies have shown that high phenolic and flavonoid contents are associated with greater antibacterial effect (Duman et al., 2009; Pascoal et al., 2014); as such, the antimicrobial activities of pawpaw may be related to these compounds.

3.2. MIC and MBC of pawpaw extracts

To assess the antimicrobial activity of the six pawpaw extracts, we measured the MIC and MBC values (Table 4).

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Table 4. Minimum inhibitory concentration (MIC, mg/mL) and minimum bactericidal concentrations (MBC, mg/mL) of *Asimina triloba* extract against gram-positive and gram-negative bacteria

Strain	PLW[1)]			PTW			PRW			PLM			PTM			PRM		
	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio
Bacillus cereus	25	25	1.0	50	–[2)]	–	50	50	1.0	12.5	–	–	1.56	6.25	4.0	3.13	50	16.0
Bacillus subtilis	25	25	1.0	–	–	–	6.25	12.5	2.0	50	–	–	12.5	50	4.0	1.56	3.13	2.0
Clostridium perfringens	50	–	–	25	100	4.0	25	100	4.0	3.13	50	16.0	0.78	3.13	4.0	1.56	3.13	2.0
Corynebacterium xerosis	12.5	–	–	50	–	–	25	50	2.0	6.25	12.5	2.0	0.78	50	64.1	1.56	3.13	2.0
Listeria monocytogenes	25	–	–	50	–	–	–	–	–	25	–	–	3.13	100	31.9	25	–	–
Methicillin-resistant *S. aureus*	–	–	–	100	–	–	–	–	–	50	–	–	12.5	12.5	1.0	6.25	100	16.0
Propionibacterium acnes	25	–	–	100	–	–	–	–	–	100	–	–	–	–	–	12.5	–	–
Staphylococcus aureus	–	–	–	100	–	–	100	–	–	50	50	1.0	6.25	50	8.0	12.5	25	2.0
Staphylococcus epidermidis	–	–	–	100	–	–	–	–	–	12.5	100	8.0	6.25	12.5	2.0	1.56	6.25	4.0
Streptococcus mutans	–	–	–	100	–	–	–	–	–	25	50	2.0	100	–	–	0.78	25	32.1
Campylobacter jejuni	–	–	–	–	–	–	–	–	–	50	–	–	25	100	4.0	25	50	2.0
Cronobacter sakazakii	50	50	1.0	50	–	–	–	–	–	50	–	–	–	–	–	12.5	–	–
Escherichia coli	25	–	–	50	–	–	–	–	–	50	–	–	–	–	–	25	–	–
Escherichia coli O157:H7	12.5	–	–	25	–	–	–	–	–	50	–	–	–	–	–	25	100	4.0
Pseudomonas fluorescens	50	50	1.0	100	-	–	100	100	1.0	–	–	–	50	–	–	12.5	50	4.0
Proteus vulgaris	12.5	12.5	1.0	25	–	–	25	–	–	50	–	–	12.5	50	4.0	12.5	50	4.0
Salmonella typhimurium	12.5	25	2.0	100	–	–	–	–	–	–	–	–	–	–	–	12.5	25	2.0
Vibrio fluvialis	12.5	100	8.0	25	–	–	25	–	–	25	–	–	25	50	2.0	12.5	25	2.0
Vibrio parahaemolyticus	50	–	–	25	25	1.0	6.25	6.25	1.0	–	–	–	3.13	25	8.0	3.13	6.25	2.0
Yersinia enterocolitica	3.13	12.5	4.0	12.5	–	–	–	–	–	12.5	–	–	6.25	–	–	12.5	50	4.0

[1)]PLW, pawpaw leaf water extract; PTW, pawpaw twig water extract; PRW, pawpaw root water extract; PLM, pawpaw leaf methanolic extract; PTM, pawpaw twig methanolic extract; PRM, pawpaw root methanolic extract.
[2)]–, no inhibition.

MIC and MBC of PLW against gram positive bacteria were 12.5-50 mg/mL, and 25 mg/mL, respectively, and MIC and MBC against gram negative bacteria were 3.13-50 mg/mL, and 12.5-100 mg/mL, respectively. PTW showed MIC values of ranged from 25-100 mg/mL against gram positive bacteria, and ranged from 12.5-100 mg/mL against gram negative bacteria. MIC and MBC of PRW against gram positive bacteria were ranged from 6.25-100 mg/mL, and 12.5-100 mg/mL, respectively, and MIC and MBC against gram negative bacteria were ranged 6.25-100 mg/mL, and 6.25-100 mg/mL, respectively. PLM exhibited MIC values of ranged from 3.13-100 mg/mL against gram positive bacteria, and ranged from 12.5-50 mg/mL against gram negative bacteria. MIC and MBC of PTM against gram positive bacteria were ranged from 0.78-100 mg/mL, and 3.13-100 mg/mL, respectively, and MIC and MBC against gram negative bacteria were ranged 3.13-50 mg/mL, and 25-100 mg/mL, respectively. MIC and MBC of PRM against gram positive bacteria were ranged from 0.78-25 mg/mL, and 3.13-100 mg/mL, respectively, and MIC and MBC against gram negative bacteria were ranged 3.13-25 mg/mL, and 6.25-100 mg/mL, respectively. Briefly, it was confirmed that the MIC and MBC values of PRM were the lowest for most bacteria.

In particular, the highest efficacy against *S. mutans* was observed in PRM, which had MIC of 0.78 mg/mL. PTM had the second-highest antibacterial activity, and showed inhibition against 15 strains, though not against *P. acnes*, *E. coli*, *E. coli* O157:H7, *S. typhimurium*, or *E. sakazakii*. The highest antibacterial activity was observed against *C. xerosis* and *C. perfringens*, with MIC values of 0.78 mg/mL, respectively. PLM inhibited the growth of all bacterial strains except for *S. typhimurium*, *P. fluorescens*, and *V. parahaemolyticus*. PLW had a relatively strong antibacterial effect compared with other water extracts, especially against *Y. enterocolitica*, with an MIC value of 3.13 mg/mL. PTW and PRW showed the lowest antibacterial activities against most of strains. As indicated in the diffusion test, bacterial growth was more effectively inhibited by methanol than by water extracts. Briefly, the most potent antibacterial activity was attributable to PRM, with MBC values against *B. subtilis, C. xerosis,* and *C. perfringens* of 3.13 mg/mL, respectively, which is lower than other samples. The MBC value of PTM against *C. perfringens* was also 3.13 mg/mL, indicating strong inhibition of bacterial growth.

The MBC/MIC ratio is termed the MIC index, and can be used evaluate whether an extract has a bactericidal (MIC index ≤4) or bacteriostatic (4<MIC index<32) effect (Benamrouche et al., 2016; Hellal et al., 2017). The MIC index of PRM showed the strongest antibacterial activities, ranging from 2.0 to 32.1 for different bacteria strains. Its MIC index was <4 in 13 strains. PRM exhibited a bacteriostatic effect on *B. cereus* and methicillin-resistant *S. aureus* with an MIC index of 16.0, respectively, indicating that although it may inhibit their growth, it is not capable of killing them. Meanwhile, PTM showed bactericidal effects on *B. cereus, B. subtilis,* methicillin-resistant *S. aureus, S. epidermidis, C. perfringens, C. jejuni, V. fluvialis,* and *P. vulgaris* with MIC indices ranging from 1.0 to 4.0. However, it showed no bactericidal or bacteriostatic effects against *C. xerosis*, despite its low MIC value of 0.78 mg/mL. As such, PTM can be considered as having mild action against *C. xerosis*.

Patel and Kumar (2008) reported that the MIC values of petroleum ether, chloroform, methanol, and water extracts from *A. squamosa* against *S. aureus* were above 1,100, 1,100, 530, and 1,100 μg/mL, respectively. In addition, Edziri et al. (2012) showed that the MIC and MBC values of methanol and water extracts from Tunisian vegetables against *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis* ranged from 0.312 to 10 mg/mL. The MIC values of *Trilepisium madagascariense* stem bark extracts obtained in various solvents against gram-positive and -negative bacteria ranged from 0.78 to 25 mg/mL (Teke et al., 2011). *Annona reticulata* had high antimicrobial activities against *B. subtilis*, *E. faecalis*, *S. aureus*, and *E. coli* with MIC and MBC values of 1-3 mg/mL, but its antimicrobial activities were different from one another or had no inhibitory effect (Sangeetha et al., 2016). Accordingly, we suggest that the antimicrobial activities in plants are affected by plant species, bacterial strain, and extracting solvents.

3.3. In vitro cell cytotoxicity and anticancer activity of pawpaw extracts

Cytotoxicity of the six pawpaw extracts was evaluated in RAW 264.7 cell lines and 4 human cancer cell lines (HT-1080, HeLa, HepG2, and AGS) using the MTT assay. All pawpaw extracts showed more than 80% cell viability against the RAW 264.7 cell lines (data not shown). All the pawpaw extracts showed inhibitory activity against cancer cells in a concentration-dependent manner (Fig. 1). There was no effect

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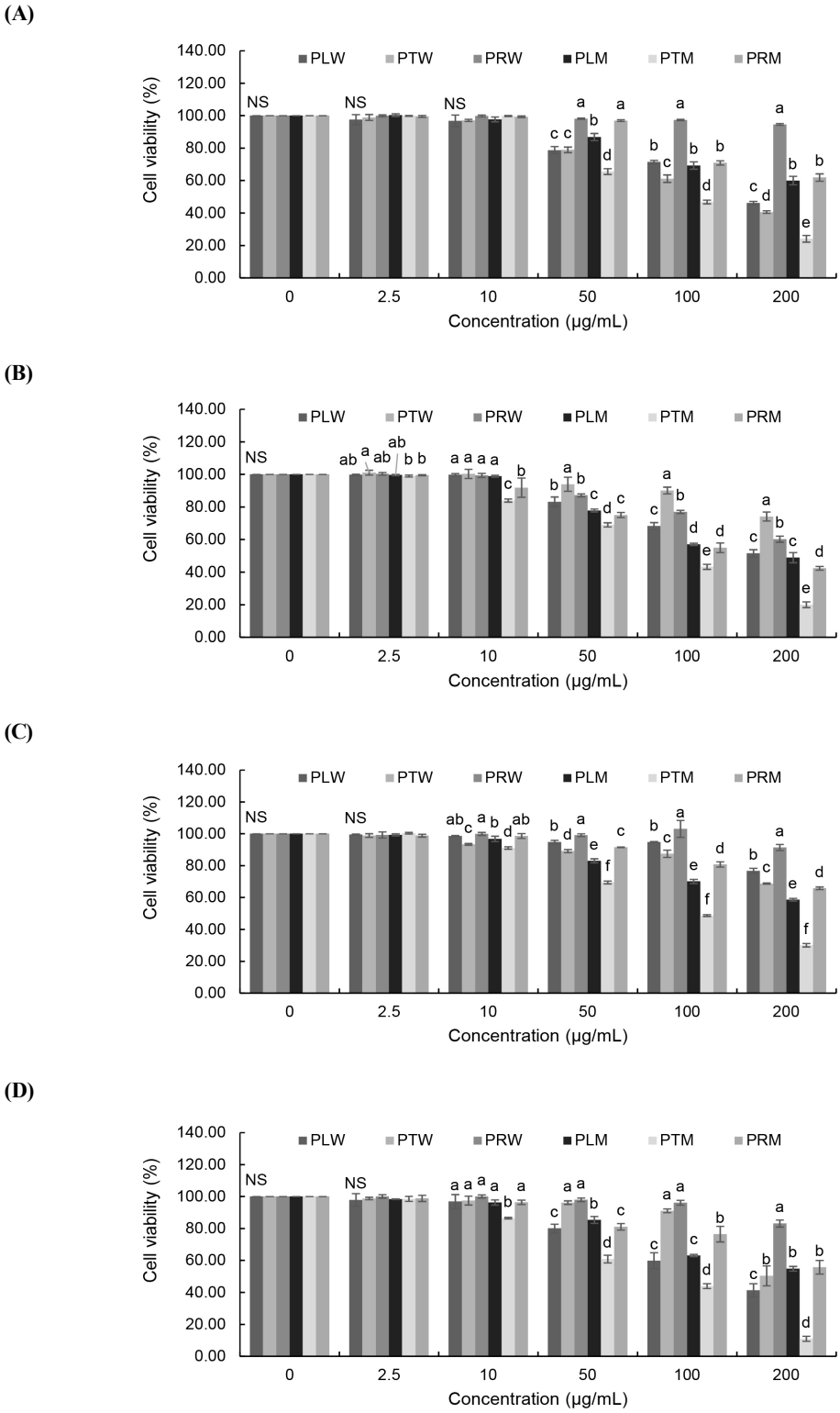

Fig. 1. Anticancer activity of *Asimina triloba* extracts against HT-1080 (A), HeLa (B), HepG2 (C), and AGS (D) cell lines. PLW, pawpaw leaf water extract; PTW, pawpaw twig water extract; PRW, pawpaw root water extract; PLM, pawpaw leaf methanolic extract; PTM, pawpaw twig methanolic extract; PRM, pawpaw root methanolic extract. Values are mean±SD (n=3). Means with different letters on the bars within the same concentration differ significantly at $p<0.05$ by Duncan's multiple range test. NS, not significant.

below 10 μg/mL concentration, but an inhibitory effect was observed at higher concentrations. In particular, in the case of HT-1080, PTM at a concentration of 50 μg/mL showed a viability of 65.52%, and at a concentration of 100 μg/mL, a viability of 46.79%. At a concentration of 200 μg/mL, an inhibitory effect of more than 80% was observed. HeLa, HepG2, and AGS were also confirmed to be the most active with PTM at a concentration of 200 μg/mL showing viability of 19.95%, 30.06%, and 10.94%, respectively. These results indicated that PTM has the highest inhibitory effect against 4 human cancer cell lines.

The IC_{50} values for anticancer activity are shown in Table 5. PTM had the strongest inhibitory effect against all cell lines except for HeLa cells, with IC_{50} values ranging from 64.57 to 128.60 μg/mL. The anticancer activity of PTM against fibrosarcoma HT-1080 was the highest, at twice that of the compound with the second-highest activity. In addition, five samples (PLW, PTW, PRW, PLM, and PRM) showed only 50% growth inhibition of HepG2 cells at a concentration of 200 μg/mL, while PTM inhibited this line at an IC_{50} value of 68.99 μg/mL. AGS stomach cancer cells were significantly inhibited by PLW and PTM, with respective IC_{50} values of 135.68 and 70.48 μg/mL. Cervical cancer HeLa cells were inhibited by methanol extracts, while the anti-proliferative activity of water extracts had negligible IC_{50} values of more than 200 μg/mL. As such, we can regard the cytotoxicity of PTM against HT-1080, HepG2, and AGS cell lines as the highest among the extracts.

The extract of *A. squamosa* pulp showed a weak influence on the HepG2 hepatocellular carcinoma cell line, with an IC_{50} value of 99 μg/mL (El-Darier and Abdelhady, 2017). Hashemi et al. (2017) demonstrated that ethanol extract of black tea inhibited the proliferation of AGS gastric cancer cells with an IC_{50} value of 264.3 μg/mL. Compared to these results, PTM had potent anti-proliferative activity. In addition, previous studies investigating the effects of pawpaw extracts have reported that the twig extract had higher acetogenin content and stronger pesticidal effect than extracts derived from other parts of the plant (McLaughlin, 2008; Ratnayake et al., 1993), and anticancer activity has been widely attributed to the Annonaceous acetogenin (McLaughlin, 2008; Nam et al., 2018). Accordingly, we suggest that the anticancer activity of PTM is related to acetogenin.

4. Conclusions

PRM was most effective in inhibiting the growth of all tested bacteria, and PTM was the most potent inhibitor of HT-1080 fibrosarcoma, HepG2 hepatocellular carcinoma, and AGS stomach cancer cell lines. This study is the first to demonstrate the antibacterial and anticancer activities of different parts of Korean pawpaw extracts. Although additional studies are needed to identify the precise compounds responsible for these effects, our findings indicate that Korean pawpaw extracts have potential medicinal value for the treatment of infections and cancer.

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Conflict of interests

The authors declare no potential conflicts of interest.

Table 5. IC_{50} values (μg/mL) for anticancer activity of *Asimina triloba* extracts against cultured human cancer cells

Cell lines	PLW[1)]	PTW	PRW	PLM	PTM	PRM
HT-1080	184.84±3.83[2)b3)]	153.81±7.02[c]	>200[a]	>200[a]	64.57±1.46[d]	>200[a]
HeLa	>200[a]	>200[a]	>200[a]	199.14±5.89[b]	128.60±2.93[c]	126.17±10.46[c]
HepG2	>200[a]	>200[a]	>200[a]	>200[a]	68.99±0.77[b]	>200[a]
AGS	135.68±7.17[b]	>200[a]	>200[a]	>200[a]	70.48±4.11[c]	>200[a]

[1)]PLW, pawpaw leaf water extract; PTW, pawpaw twig water extract; PRW, pawpaw root water extract; PLM, pawpaw leaf methanolic extract; PTM, pawpaw twig methanolic extract; PRM, pawpaw root methanolic extract.
[2)]Values are mean±SD (n=3).
[3)]Means with different superscript letters within a row differ significantly at $p<0.05$ by Duncan's multiple range test.

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Ethics approval

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