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Antibacterial Activities, Chemical Composition, and Efficacy of Green Extract *Carica Papaya* Peel on Food Model Systems

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Abstract

This study investigated anti-bacterial activities, chemical composition, and extract efficacy of *Carica papaya* peel (CPPE) var. Sekaki/Hong Kong. Nine green solvents were used to extract the *Carica papaya* peel, and the extracts were subjected to anti-bacterial tests and assays against 14 bacteria. The most potent extract was then subjected to phenolic and flavonoid assays, gas chromatography-mass spectrometry (GC/MS) analysis, and efficacy study on food model systems. All CPPEs showed anti-bacterial activities, and pentane extract had moderate to high anti-bacterial activities against all 14 bacteria. Ethanol extract of *Carica papaya* peel (ECPPE) inhibited *C. perfringens, L. monocytogenes, B. subtilis, V. parahaemolyticus,* and *V. vulnificus* with a minimum inhibitory concentration (MIC) of 1.563 mg/ml; therefore, the ECPPE was selected as the most potent extract. The total phenolic (TPC) and flavonoid contents (TFC) of the CPPEs ranged between 6.20 to 58.75 mg GAE/g DW and 1.35 - 29.09 mg QE/g DW, respectively. Palmitic acid, linoleic acid, β-sitosterol, and stigmasterol in ECPPE may be potential anti-bacterial compounds that render anti-bacterial activities. This study evaluated the ECPPE effectiveness on carbohydrate, protein, fat, and fibre model systems via optical density measurement against *C. perfringens, L. monocytogenes, B. subtilis, V. parahaemolyticus,* and *V. vulnificus*. The result showed that the ECPPE could effectively inhibit the tested bacteria in low carbohydrate and high protein, fat, and fibre food model systems.

1. Introduction

Carica papaya is part of the *Caricaceae* family and is generally known as papaya (English), pepe (Bangladesh), mamao (Brazil), papeeta (Hindi), and a variety of other names across the world. It is a well-known fruit with beneficial nutrients obtained throughout the year. Due to the abundance of this fruit, 27 million tonnes of *Carica papaya* have been produced worldwide (Food and Agriculture Organization of the United Nations, 2022). Nevertheless, approximately 8.5% or 2.3 million tonnes of the peels were often discarded (Sagar *et al.*, 2018), although the peels were reported to render anti-bacterial activities against a broad spectrum of pathogenic bacteria. The extract of *Carica papaya* peel (CPPE) has been reported to inhibit the growth of *C. diphtheriae*, *S. pneumoniae*, *B. subtilis,* and *C. perfringens* (Sani *et al.*, 2017a) and *S. aureus, B. cereus* and *E. coli* (Asghar *et al.*, 2016).

Previous studies of anti-bacterial activities of CPPE underwent various extraction techniques, e.g., maceration, Soxhlet, ultrasonication, microwave, etc. Nevertheless, applying potentially hazardous chemicals that impose toxicities hinders the production of anti-bacterial compounds from the peels. To address this issue, the extraction of the anti-bacterial compounds via maceration using class III green solvents was carried out since these solvents have low toxicity for humans, do not need a health-based exposure limit, are renewable, and do not require a high cost of synthesis energy (Das *et al.*, 2017; Tarczykowska, 2017). The only negligible report is available on the anti-bacterial activities of *Carica papaya* peel extract from the green-solvent extraction.

Although the CPPE is rich in vitamins such as vitamin C, A, and E, minerals such as magnesium and potassium, folate and fibre, and proteolytic enzymes like papain and chymopapain, the bioactive compounds that render the anti-bacterial activities have not been exhaustively reported. Kadiri *et al.* (2016) reported the presence of phenolics, including carotenoids and flavonoids, while Sani *et al.* (2017a) identified fatty acids, esters, alkane, tocopherols, and sterols in the methanol CPPE; nevertheless, the chemical composition of *Carica papaya* peel extracted by the green solvents have never been reported. Sani *et al.* (2017a) also proposed that phenolics and 9,12,15-octadecatrienoic acid were the potential anti-bacterial compounds to inhibit the gram-positive and gram-negative bacteria. The gas chromatography-mass spectrometry (GC/MS) analysis on the CPPE was only carried out on volatile compounds. Hence, our study carried out the GC/MS analysis by derivatising the CPPE to facilitate the volatility of the non-volatile compounds. The derivatisation reduces the cost of chemical composition compared to the standard analysis by liquid chromatography mass-spectrometer, which requires expensive chemical standards to confirm the separated compounds (Sani *et al.*, 2020).

Common practice post determination of the anti-bacterial activities of plant extract is to investigate the efficacy of the plant extract on prepared model media to represent the food model system. This approach is carried out due to_anti-bacterial activities using the disc diffusion test, and minimum inhibitory and bactericidal concentrations assays were tested on tryptone soy broth, not on the food model or actual food systems (Sani *et al.*, 2017b). An example of the efficacy study of plant extract is the determination of bacterial growth in a mixture of the plant extract and bacterial-inoculated suspension in beef extract, starch, and sunflower oil which represented carbohydrate, protein, and fat model systems, respectively (Klangpetch & Noma, 2018). An anti-bacterial study of cinnamaldehyde on inoculated protein matrix with *S. typhimurium* indicated the extract's efficacy and interactions with the model media (Bouarab-Chibane, Forquet, *et al.*, 2018). Besides, the matrix complexity of the actual food system may affect the efficiency since the abundant nutrients may facilitate bacteria to repair their damaged cells prior to the inhibitory action of the anti-bacterial agents. Since CPPE efficacy has never been investigated in food model systems, this study provides information on CPPE efficacy in carbohydrate, protein, fat, and fibre model systems. Subsequently, this study may lead to selecting the best food model system that the CPPE could act as a potential food preservative in the actual food system.

Therefore, this study aimed to investigate (1) the anti-bacterial activities of CPPE using green solvents, (2) the chemical composition of the most potent CPPE, and (3) CPPE efficacy on food model systems. It is anticipated that this study could facilitate the quest for anti-bacterial compounds from plant by-products that could serve as a preservative in food products.

2. Materials and methods

2.1 Plant material

Throughout the study, *Carica papaya* fruits were bought from D'Lonek Sdn. Bhd. Organic Farm, located at Rembau, Negri Sembilan, Malaysia, and the *Carica papaya* plant was given a voucher for herbarium specimen numbered SK 2368/14 by the Herbarium of Institute of Bioscience, Universiti Putra Malaysia as part of the identification process. The peels were freeze-dried using a freeze dryer (Christ, Germany) with vacuum pressure at 0.04 mbar at -50°C, grounded using a grinder machine (800G Capacity Grinder, China), and kept in airtight jars until further used. All experiment procedures were conducted in dim light.

2.2 Extraction of phytochemicals

The *Carica papaya* peel was macerated using nine green solvents, i.e., acetone, ethanol, 1-butanol, 1-propanol, pentane, heptane, acetic acid, ethanol, and isobutyl acetate. An amount of 50 g dried powder of the peels of *Carica papaya* was weighed in the Schott bottle and mixed with 500 mL green solvent at 1:10 (w/v) of sample-to-solvent ratio. The maceration method was carried out by stirring the sample for 72 hours at 30°C. The extract of *Carica papaya* peel (CPPE) was filtered using Smith filter papers qualitative high speed 101 (Smith, USA) to a pre-weighed round bottom flask and concentrated by a rotary vacuum evaporator (Heidolph, Germany) at 40°C. The concentrated CPPE was weighed to determine the extraction yield and stored at 4°C until further use. Extraction was done in triplicates. The extraction yield (mg/g) on a dry weight basis was determined using the equation below:

Extraction yield (mg/g) = concentrated extract weight (mg)/dry weight of sample (g)

2.3 Disc diffusion test (DDT)

The anti-bacterial activity was tested using the disc diffusion method against 14 strains, including *Bacillus cereus* (ATCC 10875), *Bacillus subtilis* (ATCC 11774), *Clostridium perfringens* (ATCC 13124), *Corynebacterium diphtheria* (ATCC 13812), *Escherichia coli* (ATCC 11229), *Listeria monocytogenes* (ATCC 19111), *Proteus mirabilis* (ATCC 12453), *Salmonella enteritidis* (ATCC 13076), *Salmonella typhimuriu*m (ATCC 13311), *Shigella sonnei* (ATCC 29930), *Staphylococcus aureus* (ATCC 12600), *Streptococcus pneumoniae* (ATCC 10015), *Vibrio parahaemolyticus* (ATCC 17802), *Vibrio vulnificus* (ATCC 27562). These bacteria were cultured in sterile tryptone soy broths (TSB) (Oxoid, England) for 4 - 16 hours at 37°C to achieve an inoculum containing 10^6 – 10^8 CFU/mL (Sani *et al.*, 2017b). *C perfringens* strain was grown anaerobically in sterile TSB incubated at 37°C for 24 hours in an anaerobic chamber containing a gas mixture of 10% H_2, 5% CO_2, and 85% N_2 (Timbermont *et al.*, 2014). A volume of 100 µL of bacterial culture in TSB was spread onto a Mueller-Hinton agar (Oxoid, England) plate. A sterilised 6 mm diameter filter paper disc (Smith, US) was placed on the surface of the spread agar plate.

The CPPE was diluted to 0.1 g/mL with dimethyl sulfoxide (DMSO) (Fisher Scientific, UK) and filtered through a 0.22 µm cellulose membrane (Bioflow Lifescience, Malaysia). A volume of 10 µL of the extract filtrate was pipetted onto the filter paper disc to allow diffusion. The agar plates were incubated for 24 hours at 37°C. The anti-bacterial activity was present through the inhibition zone around the disc. A volume of 10 µL of 10 mg/mL tetracycline hydrochloride (Fisher Scientific, UK) and DMSO served as positive and negative controls, respectively. Tetracycline served as the positive control since it is one of the antibiotics which effectively inhibits a broad spectrum of bacteria (Liang *et al.*, 2019), while the DMSO had shown no anti-bacterial effect on the tested bacteria (Weed *et al.*, 2018). The DDT was carried out in triplicates. The inhibition zone was determined based on the following criteria after the deduction of the 6 mm diameter of the filter paper disc: Slight anti-bacterial activity (< 3 mm inhibition zone); Moderate anti-bacterial activity (3 mm ≤ inhibition zone < 4 mm); High anti-

bacterial activity (≥ 4 mm inhibition zone) (Sani *et al.*, 2017a). The CPPEs with high and moderate anti-bacterial activities were subjected to the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays.

2.4 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

This MIC assay was carried out according to a method described by Sani *et al.* (2017b). Two-fold serial dilution was carried out for the CPPE. First, a volume of 100 µL of sterilised TSB was added to each well. Next, 100 µL of 50 mg/mL of the CPPE was added to the first well. Then, each microtiter row with 100 µL was pipetted from the first well into the second well and repeated until the last well. A volume of 100 µL from the last well was discarded. Post the serial dilution, 90 µL of each well was mixed with 10 µL bacterial strains at 10^6 CFU/mL to produce 50 - 0.049 mg/mL. Tryptone soy broths (TSB) medium with DMSO was used as a positive control of growth as TSB was the growth medium for tested bacteria without the CPPE. The MIC assay of 10 mg/mL tetracycline hydrochloride in a TSB medium containing inoculated bacteria was carried out to verify the assay's effectiveness (Mogana *et al.*, 2020). The well turbidity was measured at an optical density of 600 nm by Microplate Reader (Thermo Scientific, UK) before (T_0) and after incubation (T_{24}) at 37°C. The turbidity value in each well was determined using criteria $T_{24} - T_0 \leq 0$, where $T_{24} = T_0$ or $T_{24} < T_0$, and the well concentration which turbidity that fulfilled these criteria was selected as the MIC (Sani *et al.*, 2022).

To determine the MBC, a loophole from each well that was equal and higher than MIC was streaked on MHA and incubated at 37°C for 24 hours. All determinations were performed in triplicate. The MBC was selected on plates with concentrations that showed no apparent growth. The CPPE with the lowest MIC and MBC were selected and subjected to chemical composition analysis by gas chromatography-mass spectrometry (GC/MS).

2.5 Quantification of total phenolic content (TPC)

The total phenolic content in the CPPE was determined via the Folin-Ciocalteu colourimetric method, according to Sani *et al.* (2022), with slight modification. An amount of 0.001 g of gallic acid (R&M chemicals, UK) was dissolved in 100 mL ethanol in a 100 mL volumetric flask. Seven working standards at 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/L, including ethanol as blank, were prepared, and a calibration curve of the working standards was plotted.

An amount of 0.05 g of CPPE was diluted in a 100 mL volumetric flask prior to analysis. A 1 mL diluted extract volume was mixed with 1 mL Folin-Ciocalteu reagent (Sigma-Aldrich, Switzerland) at 1:10 dilution in a 5 mL volumetric flask wrapped with aluminium foil and vortexed for 10 s. Then, the extract solution was incubated at 30°C for 5 minutes and mixed with 1 mL of 10% (w/v) sodium carbonate solution (Merck, USA). The solution was marked with ethanol and vortexed (ZX4 Advanced IR Vortex Mixer, Italy) for another 10 s. Then, the extract mixture was incubated in the dark for 30 minutes at 30°C. The absorbance of a blue-coloured aqueous layer was measured at 747 nm using a spectrophotometer (Thermo Scientific, UK) against ethanol as the blank. Readings were carried out in triplicate.

2.6 Quantification of total flavonoid content (TFC)

The CPPEs were measured for total flavonoid contents using a colourimetry assay following Lydia *et al.* (2016). Before the analysis, a calibration curve was developed by preparing a quercetin stock standard (Merck, Germany). A series of working standards entailing 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/L, including ethanol as blank, was prepared from the stock standard, and a standard curve was plotted prior to the TFC analysis for CPPE. The measurements were carried out in triplicates.

An amount of 0.05 g CPPE was diluted in a 100 mL volumetric flask. Next, a volume of 1.25 mL of the extract was mixed with 0.5 mL of 0.1 g/mL aluminium chloride solution (Friedemann Schmidt, Washington) and 0.5 mL of 1 M sodium acetate solution (Kollin Chemicals, UK) in a 5 mL volumetric flask, which was furthered wrapped with aluminium foil to reduce exposure to light. Then, the extract mixture was made-up to 5 mL with ethanol and vortexed for 10 seconds. Finally, the extract mixture was incubated at 30°C for 15 minutes, and its absorbance was measured using a spectrophotometer at 438 nm against ethanol as the blank.

2.7 Gas chromatography-mass spectrometry analysis

Based on the DDT, MIC, and MBC results, the ethanolic extract of *Carica papaya* peel (ECPPE) was selected and analysed for its chemical composition. An amount of 0.01 g of dried ECPPE was derivatised with 100 µL of pyridine (Sigma-Aldrich, Switzerland) and a mixture of 100 µL of bis-trimethylsilyl trifluoroacetamide (BSTFA) and 1% trimethylchlorosilane (TMCS) (Macherey-Nagel, Germany) to give 0.01 g/mL of extract concentration. The vial was heated for 30 minutes at 60°C before being analysed with a GC/MS.

The GC/MS analysis adopted the technique of Sani *et al.* (2020). By using the GC/MS of Agilent-Technologies 7890A entailing gas chromatography (GC) system equipped with an Agilent-Technologies 5975 mass selective (MS) detector (Agilent Technologies, USA), 1 µL of the derivatised extract was injected into the GC system. The ECPPE was heated at a 240°C inlet of GC injector and eluted by helium gas through a capillary HP-5ms column with 30 m length x 0.25 mm diameter and a film thickness of 0.25 µm. The compounds of ECPPE were separated via oven temperature ramping: (1) 70°C for 1 minute, then increased to 150°C at 15°C/min and held for 15 minutes, and (2) raised to 300°C at 15°C/min and held for 30 minutes. The separated compounds were eluted to the MS transfer line at 230°C, ionised at 70 eV, and detected by the MS set within the m/z 50-550 mass range. The extract compounds were identified based on a 90% similarity match of their mass spectra against the Mass Spectral Database of the National Institute of Standards and Technology (NIST 14).

2.8 Anti-bacterial efficacy of ethanolic *Carica papaya* peel extract on food model systems

The ECPPE at MIC 1.56 mg/mL was used to examine its anti-bacterial potency by using carbohydrate, protein, fat, and fibre model systems against the *C. perfringens, L. monocytogenes, B. subtilis, V. parahaemolyticus,* and *V. vulnificus*. Individual stock medium mixed with 0.1% Tween 80 (Merck, country of origin) was prepared separately: (1) 50% potato starch solution (Sigma-Aldrich, Ireland) in deionised water; (2) 100% beef

extract solution (Sigma-Aldrich, Ireland) in deionised water; (3) 100% palm oil solution (Delima Oil Products Sdn. Bhd., Malaysia) in deionised water; and (4) 100% vegetable extract solution (Pacific Foods, US) in deionised water. These media represented carbohydrate, protein, oil, and fibre food model systems. The prepared stock solutions were autoclaved at 121°C for 15 min. A series of 5% - 90% beef extract, palm oil, and vegetable extract solutions, and 5% - 45% potato starch solution was prepared from the sterile stock solution, and 180 µL of each solution was transferred to 96-well microplates.

A total of 156 mg of nitrogen-blown down ECPPE was mixed with sterilised deionised water and marked to a final volume of 5 mL to form a 31.2 mg/mL stock ECPPE solution. In each well of the 96-well microplate, 10 µL stock ECPPE solution was mixed with 10 µL TSB containing 10^6 CFU/mL of the tested bacteria and 180 µL potato starch, beef extract, palm oil, and vegetable extract solutions. This solution mixture produced the final 1.56 mg/mL ECPPE equal to the MIC. Media solutions inoculated with respective bacteria served as the positive control, while a mixture of crude extract, Tween 80, and deionised water served as the negative control. All tested solutions in 96 well-microplates were incubated at 37°C for 24 hours and subjected to optical density measurement. The growth inhibition in the test wells was determined using the below formula (Sani *et al*., 2022):

$$\text{Growth inhibition, \%} = [1-(\text{OD test well}/\text{OD of positive control well})] \times 100$$

2.9 Statistical analysis

The data for triplicate samples collected from the green-solvent extraction was measured as a mean ± standard deviation. In addition, a one-way analysis of variance (ANOVA) of Turkey's test was calculated to determine the significant difference between the means at a 95% confidence level ($p < 0.05$). Pearson correlation was carried out to evaluate the correlations among the yield, TPC, and TFC using XLSTAT-Pro (2019) statistical software (Addinsoft, Paris, France).

Table 1: Disc diffusion test of *Carica papaya* peel extract against seven gram-positive bacteria

Carica papaya peel extract	Inhibition zone on gram-positive bacteria[1,2,3] (mm)						
	C. perfringens	*C. diphtheria*	*L. monocytogenes*	*B. subtilis*	*B. cereus*	*S. aureus*	*S. pneumoniae*
Ethanol	3.67 ± 0.00^{ab}B	na	3.33 ± 0.00^{b}B	3.0 ± 0.00^{ab}B	2.67 ± 0.00^{bcd}A	2.0 ± 0.00^{ab}A	3.67 ±0.00^{a}B
Acetone	na	na	4.56 ± 0.19^{bcd}C	na	2.111 ± 0.51^{bc}A	5.0 ± 0.33^{b}C	4.0 ± 0.33^{a}C
Butanol	na	3.67 ± 0.58^{b}B	4.44 ± 0.38 ^{bcd}C	3.78 ± 0.38^{b}B	2.444 ± 1.02^{bc}A	4.33 ± 0.33^{b}C	4.67 ± 0.33^{a}C
Propanol	na	3.67 ± 0.58^{b}B	4.78 ± 0.38^{cd}C	3.33 ± 0.00^{ab}B	4.11 ± 0.84^{d}C	4.0 ± 0.00^{b}C	3.56 ± 0.51^{a}B
Pentane	4.11 ± 0.96^{b}C	4.11 ± 0.51^{b}C	5.33 ± 0.33^{d}C	3.89 ± 0.38^{b}B	4.0 ± 0.00^{d}C	4.56 ± 0.51^{b}C	4.11 ± 0.69^{a}C
Heptane	1.444 ± 1.92^{ab}A	3.33 ± 0.67^{b}B	3.89 ± 0.51^{bc}B	3.22 ± 0.19^{ab}B	Na	4.0 ± 0.58^{b}C	3.89 ± 0.38^{a}B
Ethyl acetate	3.22 ± 0.19^{ab}B	2.556 ± 0.51^{ab}A	5.33 ± 0.58^{d}C	3.44 ± 0.51^{ab}B	3.11 ± 0.51^{bcd}B	4.56 ± 0.19^{b}C	3.0 ± 0.67^{a}B
Positive control[4]	23.5 ± 0.68^{c}C	17.5 ± 0.48^{c}C	23.5 ± 0.25^{e}C	20.1 ± 0.54^{c}C	17.7 ± 0.70^{e}C	22.4 ± 0.46^{c}C	17.1 ± 0.45^{b}C
Negative control[5]	na	na	Na	na	na	na	na

[1]Means ± SD was computed from triplicate measurements. The different superscripts indicate a significant difference in the inhibition zone among extracts ($p < 0.05$).
[2]na - No Anti-bacterial activity (inhibition zone of sample < 1 mm).
[3]Different capital letters indicated ranges of Anti-bacterial activities; A: Slight Anti-bacterial activity (inhibition zone of sample < 3 mm), B: Moderate Anti-bacterial activity (3 mm ≤ inhibition zone of sample < 4 mm), and C: High Anti-bacterial activity (inhibition zone of sample ≥ 4 mm).
[4]Tetracycline hydrochloride was used as the positive control.

3.0 Results and discussion

3.1 Disc diffusion test for anti-bacterial screening

The DDT was used to screen the anti-bacterial activity of the nine CPPEs against gram-positive and gram-negative bacteria. The anti-bacterial evaluation was made based on numbers of inhibited bacteria and numbers of inhibition zone as outlined by Sani *et al.* (2017a), whereby the IZ classification fell into three ranges: inhibition zone < 3 mm for slight anti-bacterial activity; 3 mm ≤ inhibition zone < 4 mm for moderate anti-bacterial activity and ≥ 4 mm inhibition zone for high anti-bacterial activity.

Table 1 shows the acetic acid CPPE as the least effective extract in suppressing the gram-positive bacteria compared to other peel extracts since it only inhibited the *growth of C. diphtheria and S. pneumoniae*. On the other hand, pentane, ethyl acetate, and isobutyl acetate CPPE inhibited all seven gram-positive bacteria. Of these nine extracts, the ranking of CPPE that rendered number of high (H) and moderate (M) anti-bacterial activities as follows: pentane (6H, 1M) > propanol (3H, 3M) > isobutyl acetate (3H, 3M) > ethyl acetate (2H, 4M) > butanol (3H, 2M) > heptane (1H, 4M) > ethanol (3M) > acetone (3H).

Table 2: Disc diffusion test of *Carica papaya* peel extract against seven gram-negative bacteria

Carica papaya peel extract	Inhibition zone on gram-negative bacteria 1,2,3 (mm)						
	P. mirabilis	*V. vulnificus*	*V. parahaemolyticus*	*S. enteritidis*	*S. typhimurium*	*S. sonei*	*E. coli*
Ethanol	na	3.33 ± 0.00^{a}B	3.33 ± 0.00^{a}B	3.33 ± 0.00^{bc}B	na	1.33 ± 0.00^{ab}A	na
Acetone	na	Na	2.222 ± 1.07^{a}A	2.556 ± 1.84^{bc}A	4.67 ± 0.00^{c}C	3.22 ± 1.54^{cd}B	2.444 ± 1.71^{ab}A
Butanol	3.44 ± 0.38^{ab}B	2.889 ± 1.07^{a}A	3.67 ± 0.33^{a}B	4.22 ± 1.02^{bc}C	2.778 ± 0.84^{bc}A	3.44 ± 0.69^{cd}B	4.78 ± 0.51^{d}C
Propanol	4.0 ± 0.33^{b}C	3.56 ± 0.38^{a}B	3.22 ± 0.19^{a}B	3.33 ± 0.58^{b}B	2.556 ± 1.17^{abc}A	3.44 ± 0.96^{cd}B	3.78 ±0.38^{bcd}B
Pentane	3.89 ± 0.51^{b}B	4.11 ± 0.51^{a}C	4.0 ± 0.58^{a}C	4.56 ± 0.51^{c}C	3.67 ± 0.33^{abc}B	3.44 ± 1.02^{cd}B	3.89 ±0.38^{bcd}B
Heptane	3.78 ± 0.19^{b}B	2.778 ± 0.19^{a}A	3.11 ± 0.19^{a}B	4.0 ± 0.33^{bc}C	3.56 ± 0.19^{bc}B	2.444 ± 1.26^{bcd}A	3.78 ± 0.19^{bcd}B
Ethyl acetate	2.66 ± 0.33^{ab}A	2.778 ± 0.19^{a}A	3.22 ± 0.51^{a}B	3.0 ± 0.58^{bc}B	2.333 ± 0.33^{abc}A	2.0 ± 0.00^{bc}A	4.0 ± 0.88^{cd}C
Isobutyl acetate	3.78 ± 0.38^{b}B	3.56 ± 0.19^{a}B	4.0 ± 0.00^{a}C	4.0 ± 1.00^{bc}C	3.78 ± 0.51^{bc}B	3.67 ± 0.33^{d}B	3.89 ±0.38^{bcd}B
Acetic acid	Na	1.333 ±0.19^{a}A	Na	Na	Na	na	2.556 ± 0.51^{bc}A
Positive control[4]	19.4 ± 0.54^{c}C	22.9 ± 0.36^{b}C	22.1 ± 0.28^{b}C	35.4 ± 2.07^{d}C	22.4 ± 0.43^{d}C	8.7 ± 0.35^{e}C	20.0 ± 0.46^{e}C
Negative control[5]	na	na	na	na	Na	na	na

[1]Means ± SD was computed from triplicate measurements. The different superscripts indicate a significant difference in the inhibition zone among extracts ($p < 0.05$).
[2]na - No Anti-bacterial activity (inhibition zone of sample < 1 mm).
[3]Different capital letters indicated ranges of Anti-bacterial activities; A: Slight Anti-bacterial activity (inhibition zone of sample < 3 mm), B: Moderate Anti-bacterial activity (3 mm ≤ inhibition zone of sample < 4 mm), and C: High Anti-bacterial activity (inhibition zone of sample ≥ 4 mm).
[4]Tetracycline hydrochloride was used as the positive control.
[5]Dimethyl sulfoxide was used as the negative control.

This ranking indicated that pentane was the most potent CPPE against gram-positive bacteria.

Table 1 also exhibited the sensitive gram-positive bacteria against the nine CPPEs. The *L. monocytogenes,* followed by *S. pneumoniae,* were the most sensitive bacteria against the CPPE, while *C. perfringens* was the least sensitive bacteria. Of these seven bacteria, the ranking of sensitivity against the CPPEs with number of high (H) and moderate (M) anti-bacterial activities as follows: *L. monocytogenes* (6H, 2M) > *S. pneumoniae* (4H, 4M) > *S. aureus* (7H) > *B. subtilis* (7M) > *C. diphtheria* (1H, 4M) > *B. cereus* (2H, 2M) > *C. perfringens* (1H, 2M).

Similarly, acetic acid showed the least effective CPPE against all gram-positive bacteria, as it showed inhibition on *V. vulnificus and E. coli* only (Table 2). Meanwhile, butanol, propanol, pentane, heptane, ethyl acetate, and isobutyl acetate CPPEs inhibited all seven gram-negative bacteria strains. The ranking of CPPEs that produced number of high (H) and moderate (M) anti-bacterial activities as follows: pentane (3H, 4M) > isobutyl acetate (2C, 5M) > propanol (1H, 5M) > butanol (2H, 3M) > heptane (1H, 4M) > ethyl acetate (1H, 2M) > ethanol (3M) > acetone (1H, 1M). This ranking indicated that pentane was the most potent CPPE against gram-negative bacteria.

Table 2 also showed the susceptible gram-negative bacteria against the nine CPPEs. Based on the result, *S. enteritidis* and *V. parahaemolyticus* were the most susceptible bacteria, while *S. typhimurium* and *V. vulnificus* were the least susceptible *V. parahaemolyticus* were the most susceptible bacteria, while *S. typhimurium* and *V. vulnificus* were the least susceptible bacteria. The ranking of sensitivity against the CPPEs that render number of high (H) and moderate (M) anti-bacterial activities as follows: *S. enteritidis* (4H, 3M) > *V. parahaemolyticus* (2H, 5M) > *E. coli* (2H, 4M) > *P. mirabilis* (1H, 4M) > *S. sonnei* (5M) > *S. typhimurium* (1H, 3M) = *V. vulnificus* (1H, 3M).

Based on the cumulative moderate and high anti-bacterial activities, the gram-positive bacteria were more sensitive to the CPPEs. Pentane extract was the most potent CPPE against gram-positive and gram-negative bacteria. This finding contradicted Sani *et al.* (2017a) work since they found that methanol CPPE exhibited the most moderate and high anti-bacterial activities compared to other solvents. The authors also discovered the slight anti-bacterial activity of ethanol and acetone CPPEs against *S. pneumoniae, B. cereus, S. aureus, V. parahaemolyticus, S. enteritidis,* and *S. sonnei* compared to our findings which showed moderate anti-bacterial activities. However, the ethanol and ethyl acetate CPPEs by Asghar *et al.* (2016) reported high anti-bacterial activities against *S. aureus, B. cereus,* and *E. coli* compared to our findings. Nevertheless, these authors did not mention the deduction of filter disc diameter (6 mm) in this study prior to determining the inhibition zone. The absence of information on deduction or inclusion of filter disc diameter (6 mm) in some studies during the measurement of inhibition zones leads to the possibly inaccurate comparison of the anti-bacterial activities of CPPEs

with other extracts.

Other than that, Egbuonu *et al.* (2016) also mentioned that ethanolic extract of *Carica papaya* peel showed a larger zone of inhibition of 17.33 mm and 15.00 mm for *E. coli* and *S. aureus,* respectively, compared aqueous extract with 12.33 mm and 8.00 mm for *E. coli* and *S. aureus* respectively. The ethanol extract of *Vasconcellea pubescens* pulp showed anti-bacterial activities of 9.42, 10.2, and 9.23 mm for *S. aureus, E. coli*, and *B. cereus*, respectively (Vega-Gálvez *et al*., 2021).

In comparing the anti-bacterial activities of CPPEs to other plant peels, ethanol CPPE had anti-bacterial activities against *B. cereus, S. aureus,* and *S. pneumoniae* compared to ethanol extract of *Musa acuminata* peel since no inhibition was found against these bacteria (Subramaniam *et al*., 2020). No anti-bacterial activity was observed in ethanol extract of the cocoa peel against *S. aureus* (Aguilar-Méndez *et al*., 2020).

However, Oikeh *et al.* (2020) reported that the ethanol extract of *Citrus sinensis* peel showed high anti-bacterial activity against *S. aureus, P. aeruginosa* and *E. coli* with 4, 6, and 8 mm inhibition zones, respectively. Also, *Citrus limon* peels ethanol extract showed high anti-bacterial activity against *E. coli* (Henderson *et al*., 2017). Yassin *et al.* (2021) discovered that acetone extract of pomegranate peel has high anti-bacterial activity against *S. aureus, E. coli,* and *S. typhimurium* with 15 to 20 mm inhibition zones. Nevertheless, these authors did not explain the deduction nor the inclusion of filer disc diameter (6 mm) before determining the inhibition zone, leading to a possibly inaccurate comparison of the anti-bacterial activities of CPPEs with other extracts.

Since DDT is acknowledged as a preliminary test and could not provide information on the concentration that renders the anti-bacterial potency (Sowhini *et al*., 2020), the CPPEs had high and moderate anti-bacterial activities subjected to the determination of MIC and MBC.

3.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC)

Broth microdilution was used to evaluate the minimum inhibitory concentration (MIC) and minimum bactericidal inhibition (MBC) of the CPPEs that exhibited the DDT results' moderate and high anti-bacterial activity. Based on Table 3, the *C. perfringens*, *L. monocytogenes, V. vulnificus, V. parahaemolyticus,* and *B. subtilis* were sensitive to the lowest ethanol CPPE concentration (1.56 mg/mL), which this concentration has been recorded as the MIC. Likewise, 1.56 mg/mL was the MIC for butanol CPPE that effectively inhibited the *C. diphtheria, L. monocytogenes, S. pneumoniae,* and *E. coli* growths. These results also denoted that the ethanol CPPE was the most potent extract in this study since it could inhibit bacterial growths at the lowest concentration at 1.56 mg/mL compared to other CPPEs. The *C. perfringens, L. monocytogenes, V. vulnificus, V. parahaemolyticus,* and *B. subtilis* were selected as the indicator microorganisms based on their sensitivities towards the ethanol CPPE at the MIC.

Interestingly, pentane extract inhibited the *C. perfringens, C. diphtheria, L. monocytogenes, P. mirabilis, S. enteritidis, S. sonnei,* and *S. aureus* growths at 12.5 mg/mL only. However, it has been reported to produce the highest number of high anti-bacterial activity in DDT compared to other CPPEs. This occurrence may be due to the non-polar pentane CPPE having low miscibility with DMSO and TSB in the MIC assay compared to the polar ethanol and methanol CPPEs; hence, producing a cloudy solution that increased the MIC value (Sowhini *et al*., 2020).

The possible reason for the different results is that the pentane extract was not immiscible in DMSO solvent (Merck, 2019), which could lead to the production of a cloudy solution and increase the growth of bacteria as MIC value. For the DDT approach only as a preliminary test, the reaction could be different with MIC. This could be challenging to generate a new anti-bacterial agent using non-polar solvent extract.

Compared to other works of CPPEs and other fruit peel extracts, the green CPPEs in this study had higher potency based on the lower MIC than other peel extracts. Our acetonic CPPE had a lower MIC (7.81 mg/mL) against *S. aureus* than the similar CPPE reported by Orhue & Momoh (2013) at 24 mg/mL. The ethanol CPPE at MIC of 1.56 mg/mL was more potent against *L. monocytogenes* than the ethanol extract of *Punica granatum* and *Citrus sinensis* peels at MIC of 10 mg/mL and 150 mg/mL, respectively (Hanafy *et al*., 2021). Besides, the acetone CPPE had better potency (MIC = 7.813 mg/mL) to inhibit the *S. aureus* than the acetone extract of *Citrus sinensis* since the latter had a higher MIC (40 mg/mL) (Gupta *et al*., 2021).

Nevertheless, the green CPPEs were reported to have lower potency than previous researchers' works based on the higher MIC. The acetone CPPE had a higher MIC (31.25 mg/mL) against *S. pneumoniae* than the 5.63 mg/mL of MIC of a similar CPPE (Sani *et al*., 2017a). The ethyl acetate CPPE also rendered higher MIC than the ethyl acetate extract of banana peel at 0.52 mg/mL, 0.79 mg/mL, and 0.78 mg/mL MIC against *E. coli, S. aureus,* and *L. monocytogenes*, respectively (Saleem & Saeed, 2020).

Table 3 also shows the MBC of the green CPPEs, which ranged from 1.56 mg/mL to 62.5 mg/mL against the selected bacteria. Of these extracts, the lowest MBC was 1.56 mg/mL by the ethanol CPPE against *L. monocytogenes*, *V. vulnificus*, *V. parahaemolyticus, B. subtilis*, and butanol CPPE against *S. pneumoniae* and *E. coli*. Since the MBC of these CPPEs was equal to the MIC, the ethanol and butanol CPPEs could act as inhibitory and killing agents on these bacteria. The MIC and MBC result also denoted that ethanol CPPE was this study's most potent green CPPE. The extraction yield and total phenolic and flavonoid contents were evaluated to identify which chemical compounds in the green CPPEs may influence the anti-bacterial activities.

3.3 Extraction yield, total phenolic, and total flavonoid contents

To study the potential chemical constituents that may render the anti-bacterial activities in the green CPPEs, their extract yield, total phenolic (TPC), and total flavonoid contents (TFC) were determined in Table 4. The correlations between yield, TPC, and TFC were also evaluated.

The yield of the green CPPEs ranged between 14.46 mg/g to 68.72 mg/g. Of these green CPPEs, propanol CPPE showed the highest yield (68.72 mg/g), while heptane CPPE (14.46 mg/g) had the lowest yield. The polar solvent CPPEs entailing polar protic and polar aprotic solvent CPPEs exhibited the highest yield (26.47 to 68.72 mg/g) compared to non-polar solvent CPPEs (14.46 mg/g to 35.63 mg/g). This finding was in accordance with several studies that stated that polar protic solvents such as propanol, butanol, and ethanol effectively

Table 3: Minimum inhibitory and bactericidal concentrations of *Carica papaya* peel extracts

Bacteria	Minimum inhibitory and bactericidal concentrations of *Carica papaya* peel extract (mg/mL)[1,2]										
	Ethanol	Acetone	Butanol	Propanol	Pentane	Heptane	Ethyl acetate	Isobutyl acetate	Acetic acid	Positive control[3]	Negative control[4]
C. perfringens	1.56 (50)	nd	nd	nd	12.5 (25)	nd	12.5 (25)	25 (50)	nd	0.16	0
C. diphtheria	nd	nd	1.56 (12.5)	25 (50)	12.5 (25)	12.5 (50)	nd	nd	nd	0.16	0
L. monocytogenes	1.56 (1.56)	25 (25)	1.56 (12.5)	12.5 (25)	12.5 (12.5)	12.5 (12.5)	12.5 (12.5)	25 (25)	nd	0.04	0
P. mirabilis	nd	nd	25 (50)	12.5 (50)	12.5 (12.5)	12.5 (12.5)	nd	nd	nd	0.16	0
V. vulnificus	1.56 (1.56)	nd	nd	6.25 (50)	50 (50)	nd	nd	nd	nd	0.63	0
V. parahaemolyticus	1.56 (1.56)	nd	12.5 (12.5)	12.5 (12.5)	25 (25)	25 (25)	6.25 (12.5)	25 (50)	nd	0.31	0
S. enteritidis	12.5 (12.5)	nd	6.25 (12.5)	12.5 (12.5)	12.5 (25)	12.5 (25)	25 (50)	50 (50)	nd	0.16	0
S. typhimurium	nd	50 (50)	nd	nd	25 (25)	25 (25)	nd	nd	nd	0.63	0
S. sonei	nd	25 (25)	3.13 (25)	25 (50)	12.5 (12.5)	nd	nd	nd	nd	0.16	0
S. aureus	nd	7.81 (15.6)	3.13 (3.13)	12.5 (25)	12.5 (25)	6.25 (6.25)	12.5 (25)	25 (25)	nd	0.08	0
S. pneumoniae	12.5 (12.5)	31.3 (62.5)	1.56 (1.56)	25 (50)	25 (25)	12.5 (12.5)	25 (50)	25 (25)	nd	0.16	0
E. coli	nd	nd	1.56 (1.56)	12.5 (25)	25 (25)	15.63 (62.5)	25 (50)	25 (25)	nd	0.16	0
B. subtilis	1.56 (1.56)	nd	25 (50)	12.5 (50)	25 (50)	12.5 (12.5)	12.5 (50)	12.5 (12.5)	nd	0.16	0
B. cereus	nd	nd	nd	12.5 (25)	25 (25)	nd	25 (50)	12.5 (25)	nd	5	0

[1]nd - Not determined due to slight anti-bacterial activity in disk diffusion test.
[2]Value in parenthesis is the minimum bactericidal concentration (MBC).
[3]10 mg/mL tetracycline hydrochloride was used for verification of MIC assay.
[4]Tryptone soy broth was used as the negative control.

Table 4: Yield, total phenolic and flavonoid contents of *Carica papaya* peel extracts

Solvent polarity	*Carica papaya* peel extact	Yield (mg/g sample)[1,4,7]	TPC (mg GAE/g DW)[1,2,3,4,8]	TFC (mg QE/g DW)[1,5,6,7,8]
Polar protic	Ethanol	33.54 ± 0.05^{d}	19.94 ± 0.10^{e}	9.44 ± 0.03^{c}
	Butanol	40.26 ± 0.14^{f}	15.55 ± 0.83^{d}	12.85 ± 0.08^{d}
	Propanol	68.72 ± 0.04^{h}	13.43 ± 0.30^{c}	9.33 ± 0.11^{c}
	Acetic acid	62.15 ± 0.12^{g}	6.20 ± 0.55^{a}	1.69 ± 0.17^{a}
Polar aprotic	Isobutyl acetate	26.47 ± 0.02^{b}	47.93 ± 0.29^{g}	24.98 ± 0.50^{g}
	Ethyl acetate	33.60 ± 0.26^{d}	17.72 ± 0.17^{e}	13.69 ± 0.32^{e}
	Acetone	27.89 ± 0.03^{c}	6.74 ± 0.03^{b}	1.35 ± 0.11^{b}
Non-polar	Heptane	14.46 ± 0.06^{a}	58.75 ± 0.31^{h}	29.09 ± 0.43^{h}
	Pentane	35.63 ± 0.16^{e}	25.27 ± 0.78^{f}	18.69 ± 0.03^{f}

[1]Means ± SD was from triplicate measurement. Different superscripts showed significantly different ($p < 0.05$) value.
[2]GAE: Gallic acid equivalent; DW: Dry weight.
[3]Calibration curve equation for TPC and coefficent determination (R^2) were $y = 0.0196x + 0.0429$ and $R^2 = 0.9939$, respectively.
[4]Pearson correlation of yield versus TPC was -0.6610.
[5]QE: Quercetin equivalent; DW: Dry weight.
[6]Calibration curve equation for TFC and coefficent determination (R^2) were $y = 0.0086x + 0.0028$ and $R^2 = 0.9979$, respectively.
[7]Pearson correlation of yield versus TFC was -0.6011.
[8]Pearson correlation of TPC versus TFC was 0.9476.

extrracted phytochemicals from plant cells. Sani *et al.* (2017a) also reported that polar solvent CPPEs such as water and acetone had the highest yield than non-polar CPPEs such as petroleum ether.

Table 4 also shows the TPC and TFC ranges between 6.20 to 58.75 mg GAE/g DW and 1.35 to 29.09 mg QE/g DW, respectively. The TPC and TFC had strong and proportional correlations based on the Pearson correlation value of 0.9476, indicating that green CPPEs with high will likely possess high TFC and vice versa. This finding was anticipated since they are abundant in plants (Sani *et al.*, 2017b).

The heptane and acetic acid CPPEs had the highest and lowest TPC, respectively. Likewise, heptane and acetone had the highest and lowest TFC, respectively. Interestingly, the non-polar solvents, i.e., heptane and pentane CPPEs, had higher TPC and TFC than polar protic CPPEs, i.e., ethanol, butanol, and propanol CPPEs. This finding was in line with Suleria *et al.* (2020), which had low TPC (3.13 mg GAE/g) and TFC (1.06 mg QE/g) in polar solvent CPPEs, but was lower TPC and TFC than our result. Nawaz *et al.* (2020) also found higher TPC and TFC in non-polar solvent extracts of bean seeds than in the polar solvent extracts.

Nevertheless, the high TPC and TFC in non-polar CPPEs contradicted the claim that polar solvents could extract a higher amount of phenolic compounds from plant cells in free and glycosides forms than non-polar solvents (Nawaz *et al.*, 2020). This was evident by the highest TPC and TFC in ethanol and acetone CPPEs by Sani *et al.* (2017b) compared to non-polar solvent CPPEs. Since the ethanol CPPE in their study rendered the highest anti-bacterial activities, phenolics in the ethanol CPPE were deemed, possible anti-bacterial compound groups.

Contrary to our finding, there was insufficient evidence to infer that anti-bacterial compounds in the CPPEs are primarily attributable to phenolics and flavonoids, although they were reported to be bacteriostatic and bactericidal against microorganisms in other studies (Bouarab-Chibane *et al.*, 2019). This was due to the TPC and TFC variations in different polarities of the green CPPEs, which denoted that these compound groups may not be the only responsible compounds that inhibit the growth of bacteria, especially in ethanol CPPE that had the highest number of moderate and high anti-bacterial activities, and lowest MIC and MBC. Hence, an investigation of the chemical composition of the ethanol CPPE by GC/MS was carried out to identify the potential anti-bacterial compounds in the CPPE.

3.4 *Carica papaya* peel composition by GC/MS

Based on the previous result, the ethanolic extract of *Carica papaya* peel (ECPPE) exhibited higher anti-bacterial activity than other extracts. Therefore, this extract was selected to identify the anti-bacterial compound using a gas chromatography-mass spectrometer (GC/MS).

Forty-seven compounds were identified from the ECPPE with more than 90% similarity against the NIST 14 library's mass spectra, as shown in Table 5, while Figure 2 shows the chromatogram of the ECPPE. The ECPPE mainly consisted of sugar constituents (30.61%), fatty acid (13%), sterol (12.88%), and alkane (1.6%). The highest percentage area with β-D-allopyranose (9.54%), D-fructofuranose (9.19%), β-sitosterol (8.9%), ethyl 9,12,15-octadecatrienoate (7.16%), and silane (6.16%). Sugar constituents such as sugars, sucrose, glycosides, glycofuranoses, and glycopyranoses were inactive glycosides and naturally bonded to phenolics. Also, since the extraction of ECPPE did not undergo acid or alkaline hydrolysis prior to the maceration, the sugar constituents remained attached to the phenolics via glycosidic bonds. They broke down into volatiles trimethylsilyl (TMS) form during the derivatisation (Sani *et al.*, 2020). Subsequently, the derivatising agent BSTFA acts as a trimethylsilyl donor that transforms the hydroxyl groups of acids and phenols into TMS ethers or TMS esters. Additionally, trimethylchlorosilane (TMCS) catalysed the formation of functional groups of secondary alcohols and amines (Moldoveanu & David, 2019). Derivatisation of ECPPE facilitated the volatility of the non-volatile compounds,

Table 5: Chemical composition of ethanol extract of *Carica papaya* peel

No.	Retention time (min)	Coumpound[1,2]	Area (%)
1	5.310	N,N-Bis(trimethylsilyl)trifluoroacetamidine	1.57
2	5.669	2,2,4,7,7-pentamethyl-3,6-Dioxa-2,7-disilaoctane	0.32
3	6.280	D-(-)-lactic acid TMS ether	1.87
4	6.372	TMS acetic acid	0.32
5	6.945	TMS (+/-)-3-hydroxybutyric acid	1.13
6	7.266	TMS propanedioic acid	0.12
7	7.686	TMS benzoic acid	0.24
8	7.900	TMS glycerol	3.42
9	8.061	Trimethyl-silanol phosphate (3:1)	0.64
10	8.145	1,2,3-tris(trimethylsiloxy)-butane	0.22
11	12.294	Tetrakis-TMS meso-erythritol	0.15
12	12.692	5-oxo-1-TMS L-proline	0.87
13	17.804	TMS dodecanoic acid	0.23
14	20.418	Tetrakis-TMS 2-deoxy-galactopyranose	0.00
15	24.109	2-methyl-1,4-bis[(trimethylsilyl)oxy]-3-(2-propenyl)-cyclopentane	0.36
16	24.376	Eicosanoic acid	0.05
17	26.883	Pentakis-TMS D-(-)-fructofuranose	9.19
18	27.395	Pentakis-O-TMS glucopyranose	2.11
19	27.441	Tetra-TMS lyxose	1.31
20	27.640	Pentakis(trimethylsilyl) ether, trimethylsilyloxime D-allose (isomer 1)	2.11
21	27.991	Pentakis-TMS β-D-allopyranose	9.54
22	28.312	1,2,3,4,6-pentakis-O-TMS α-D-mannopyranose	1.15
23	28.526	TMS hexadecanoic acid	3.51
24	28.885	Oleanitrile	1.11
25	28.931	(Z,Z) 9,12-octadecadienoic acid methyl ester	0.88
26	29.000	(Z,Z,Z) 9,12,15-octadecatrienoic acid methyl ester	0.94
27	29.420	Ethyl 9,12,15-octadecatrienoate	7.16
28	30.177	TMS α-linolenic acid,	1.20
29	30.551	TMS nonadecanoic acid	1.38
30	31.346	2',3',5'-tris-O-TMS uridine	3.45
31	31.407	2,3,5,6,7-pentakis-O-(trimethylsilyl)-γ-lactone D-glycero-D-gulo-heptonic acid	0.53
32	31.568	Methyl 2,3,4,6-tetrakis-O-TMS α-D-glucopyranoside	2.03
33	32.164	TMS docosanoic acid	3.42
34	32.232	Bromazepam	0.90
35	32.301	Tetrakis-TMS adenosine	1.86
36	32.423	Octakis-TMS sucrose	1.60
37	32.454	1,3,4,6-tetrakis-O-(trimethylsilyl)-beta-D-fructofuranosyl 2,3,4,6-tetrakis-O-TMS α-D-glucopyranoside	1.42
38	33.799	δ-TMS tocopherol	1.28
39	33.891	TMS pentacosanoic acid	1.39
40	34.609	ɣ-TMS tocopherol	4.09
41	34.884	5(2-Dimethylamino-1-phenyl)-vinyl-1,2,4-thiadiazol	0.67
42	35.220	Hexatriacontane	0.70
43	36.038	O-TMS (+)-α-tocopherol	4.00
44	36.451	(+/-)-α-tocopherol acetate	0.52
45	37.742	[[(3β,24R)-ergost-5-en-3-yl]oxy]trimethyl-silane	6.16
46	38.124	TMS stigmasterol	3.98
47	39.164	TMS β-sitosterol	8.90

[1]Compounds identified at 90% similarity match against the standard mass spectra in the NIST 14 library and comparison with the retention index of literature.
[2]TMS = trimethylsilyl derivatives.

typically separated and identified using liquid chromatography. Due to the lack of mass spectrometry information and the requirement to utilise eluents with different polarities, derivatisation and GC/MS analysis have become alternative approaches to identifying non-volatile compounds (Sani *et al.*, 2020).

Of the 13% fatty acids, the GC/MS identified hexadecanoic acid (3.51%), docosanoic acid (3.42%), pentacosanoic acid (1.39%), nonadecanoic acid (1.38%), and α-linolenic acid (1.2%). Palmitic acid (hexadecanoic acid) and linoleic acid ((Z, Z)-9,12-Octadecadienoic acid) exhibited anti-bacterial activity as reported by inhibiting the enoyl-acyl carrier protein reductase activity, a carrier protein that controls the bacteria's fatty acid synthesis (Canli *et al.*, 2017). These compounds are also reported to inhibit *B. subtilis, L. monocytogenes, S. enteritidis,* and *S. typhimurium* growths. According to Johny *et al.* (2019), α-linolenic acid was potent against gram-positive and gram-negative bacterial strains. Besides that, linoleic acid has also been an effective anti-bacterial agent against *S. aureus, Helicobacter pylori, V. parahaemolyticus,* and *Mycobacterium*.

The GC/MS analysis also identified β-sitosterol (8.9%) and stigmasterol (3.98%) are plant sterol (phytosterol) that belong to the phenolics. This abundance of phytochemicals maintains the structure and function of the cell membrane as well as exerts anti-bacterial activities against *S. aureus*, *E. coli* (Ajijolakewu *et al.*, 2021), *S. thyphimurium*, *B.cereus,* and *V. vulnificus* (Sani *et al.*, 2021).

The ɣ-tocopherol (4.09%), α-tocopherol (4.00%), δ-tocopherol (1.28%), and α-tocopherol acetate (0.52%) were also present in our ECPPE. As part of the phenolic groups, they possess high antioxidant properties with an effective anti-bacterial effect. This was evident since the tocopherols act as an effective anti-bacterial agent against multi-resistant bacteria such as *P. aeruginosa, E. coli,* and *S. aureus* (Ghimire *et al.*, 2017). They have also been utilised to treat infections and reduce biofilm formation caused by specific gram-positive or gram-negative bacteria (Gamna & Spriano, 2021).

The phenolics, flavonoids, and other compounds such as fatty acids in the ECPPE may exert their anti-bacterial potency based on GC/MS analysis. Sani *et al.* (2022) found that the phenolics, elaidic acid, and palmitoleic acid from *Carica papaya* seed synergistically inhibited *S. enteritidis*, *B. cereus*, *V. vulnificus,* and *P. mirabilis*. Additionally, isolated cis- vaccenic acid from the *Carica papaya* seed had been proven to render anti-bacterial activities against the same bacteria (Sani *et al.*, 2021).

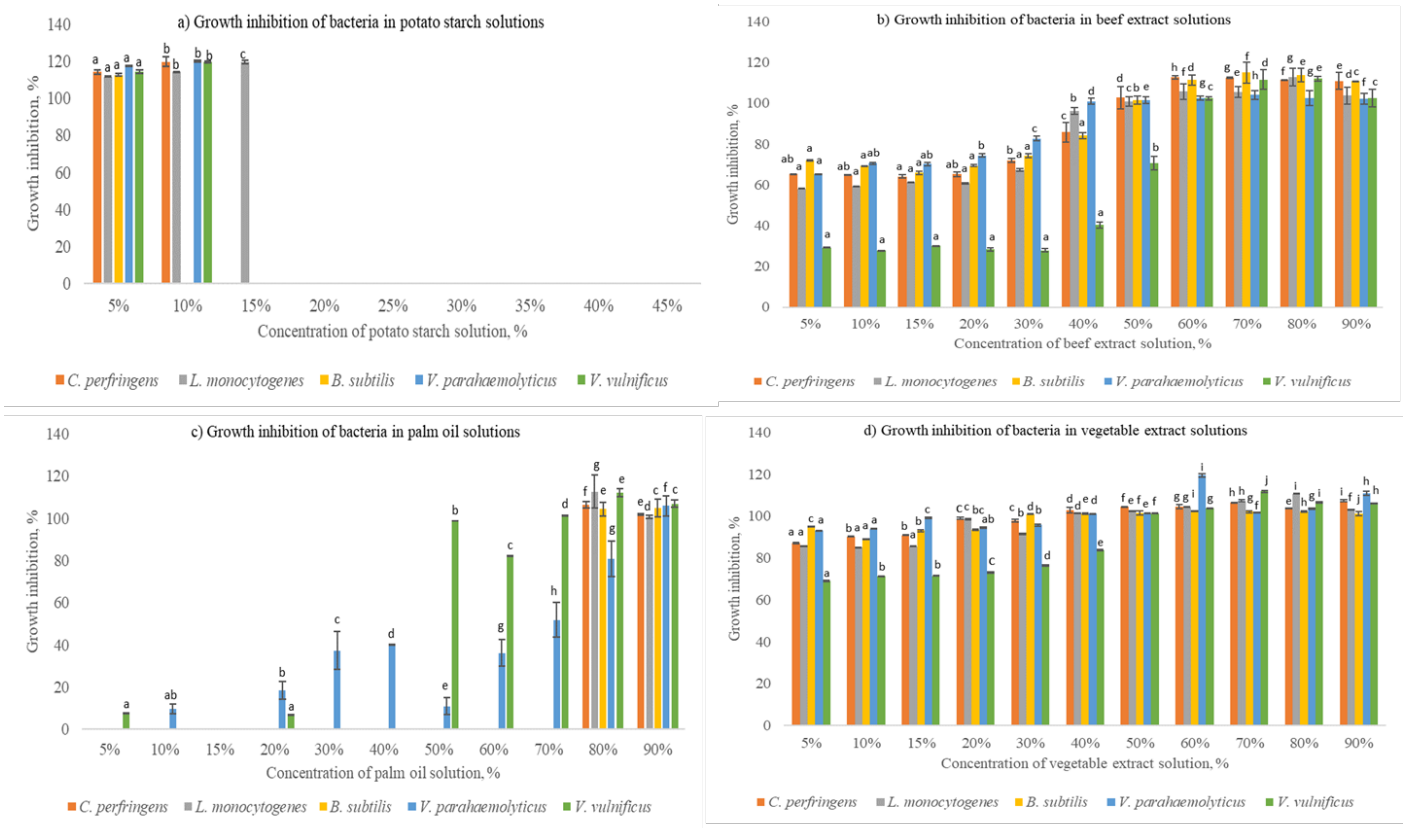


Figure 1: Growth inhibition of *C. perfringens, L. monocytogenes, B. subtilis*, *V. parahaemolyticus* and *V. vulnificus* in (a) potato starch, (b) beef extract, (c) palm oil and (d) vegetable extract solutions treated with ethanol extract of *Carica papaya* peel. The different letters indicate a significant difference in the growth inhibition among the extract concentrations ($p < 0.05$)

3.5 Anti-bacterial efficacy of ethanol *Carica papaya* peel extract on food model systems

The ECPPE efficacy against *C. perfringens, L. monocytogenes, B. subtilis*, *V. parahaemolyticus,* and *V. vulnificus* in a food model system entailing carbohydrate, protein, fat, and fibre was evaluated. Various concentrations of potato starch, beef extract, palm oil, and vegetable extract solutions represented these food model systems to mimic the actual food system. This study proposed potential actual food systems for further application based on growth inhibition that exceeded 100% in these food model systems.

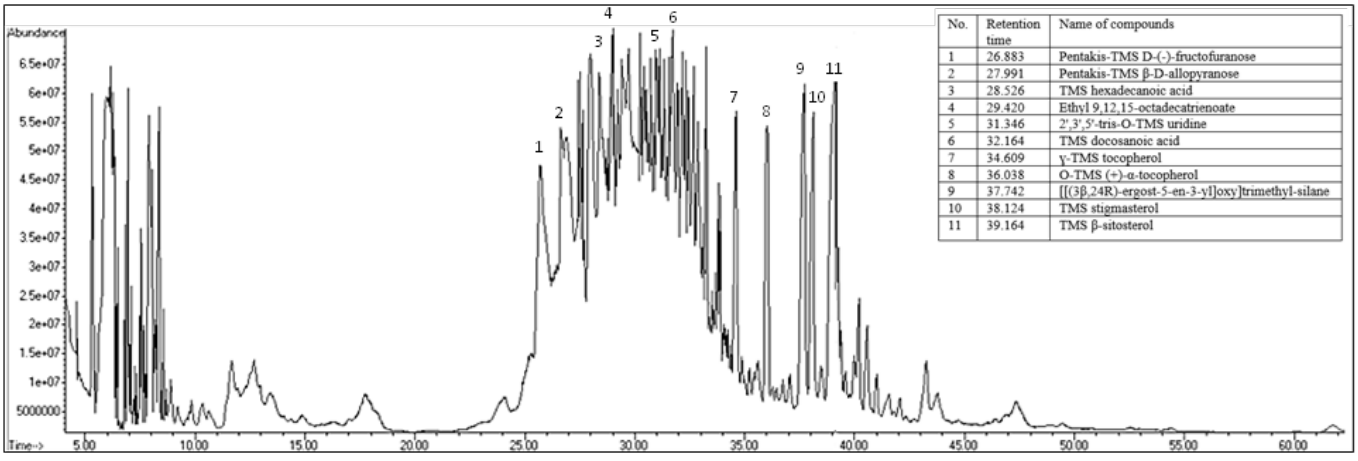
Based on the Figure 1 (a), the ECPPE showed significant ($p < 0.05$) growth inhibition > 100% of all tested bacteria in 5% of potato starch solution. As the concentration of potato starch solution increased, the ECPPE was significantly ($p < 0.05$) effective in inhibiting *C. perfringens, L. monocytogenes, V. parahaemolyticus,* and *V. vulnificus* in 10% potato starch solution and *L. monocytogenes* in 15% potato starch solution. These results indicated that a high concentration of the starch gave a lower inhibitory effect of ECPPE due to the increment of (1) complexity carbohydrate matrix; (2) starch's protective function on potential electrostatic interactions; and (3) starch viscosity that exhibited restrictive behaviour against selected bacteria (Ma, 2015). Hence, the anti-bacterial efficacy of ECPPE could be improved in a simple carbohydrate matrix. Also, the starch may be bound to hydroxyl groups of the sterols, i.e., β-sitosterol, stigmasterol, and tocopherols (Zhang *et al.*, 2014), thus, reducing the inhibitory effect of ECPPE.

Figure 1 (b) shows the efficacy of ECPPE in 11 concentrations of beef extract solutions. All tested bacteria were resistant against the ECPPE in 5% to 30% beef extract solutions while they had significant ($p < 0.05$) growth inhibition > 100% in 60% to 90% beef extract solutions. Specifically, the extract showed *V. parahaemolyticus*'s growth inhibition > 100% in 40% to 90% beef extract solutions. Therefore, it was proposed that the ECPPE effectively inhibited the selected bacteria in a food matrix of high protein concentrations. These findings were in accordance with the efficacy of oregano and cinnamaldehyde against *L. monocytogenes* and *S. typhimurium* (Bouarab-Chibane, Forquet, *et al.*, 2018) in high concentrations of the protein matrix, respectively, due to hydrophilic property of the beef extract solutions might interact electrostatically with the ECPPE to promote its dissolution in this medium (Klangpetch & Noma, 2018).

The ECPPE efficacy in the fat model system was represented by growth inhibition of the bacteria in palm oil solutions in Figure 1 (c). The ECPPE exhibited growth inhibition > 100% significantly ($p < 0.05$) in 80% and 90% palm oil solutions against all tested bacteria except *V. parahaemolyticus,* which had 80% growth inhibition in the 80% palm oil solution. Of the tested bacteria, only *V. vulnificus* had growth inhibition > 100% significantly ($p < 0.05$) in the 70% palm oil solution. This finding suggested that the ECPPE could suppress the selected bacteria in a high concentration of fat system, which supported the inhibition of L. *monocytogenes* in sunflower oil concentrations by thyme and reduced lag time of *S. typhimurium* by cinnamaldehyde (Klangpetch & Noma, 2018). However, these findings contradicted the claim of the protective mechanism against bacteria by fat components and low water content in food from the anti-bacterial action of plant extract (Ma, 2015). A possible explanation for these results could be due to the lipophilicity of the abundant fatty acids and their esters in the CPPE towards the fat model system that disrupted the bacterial' lipoprotein structure and allowed permeability within the bacterial cells (Casillas-Vargasa *et al.*, 2021; Mostafa *et al.*, 2018).

Figure 1 (d) exhibited the ECPPE efficacy in vegetable extract solution representing the fibre model system. The *B. subtilis* was the most sensitive bacteria to the ECPPE in 30% vegetable extract solutions compared to other bacteria. The ECPPE showed potent anti-bacterial activity (percentage inhibition > 100%) against all selected bacteria in 50% to 90% vegetable extract solutions, indicating that the ECPPE had a significant ($p < 0.05$) anti-bacterial effect in high fibre concentration. This finding refuted the claim that non-extractable polyphenol in fibre affected bacteria metabolism and thus increased bacteria growth (Rivas *et al.*, 2021) since the free polyphenols of the vegetable solutions in this study may facilitate the inhibition of tested bacteria.

In conclusion, the ECPPE could effectively inhibit the tested bacteria even in low carbohydrate concentrations and high protein, fat, and fibre concentrations. Such characteristics in food model systems may suit the investigation of ECPPE efficacy in actual food systems containing low carbohydrate and high protein-based media such as meat, poultry, and dairy



No.	Retention time	Name of compounds
1	26.883	Pentakis-TMS D-(-)-fructofuranose
2	27.991	Pentakis-TMS β-D-allopyranose
3	28.526	TMS hexadecanoic acid
4	29.420	Ethyl 9,12,15-octadecatrienoate
5	31.346	2',3',5'-tris-O-TMS uridine
6	32.164	TMS docosanoic acid
7	34.609	γ-TMS tocopherol
8	36.038	O-TMS (+)-α-tocopherol
9	37.742	[[(3β,24R)-ergost-5-en-3-yl]oxy]trimethyl-silane
10	38.124	TMS stigmasterol
11	39.164	TMS β-sitosterol

Figure 2: Chromatogram of ethanol extract of *Carica papaya* peel

products, high fat-content products such as mayonnaise, butter, and margarine, and high fibre food such as legumes, lettuce and barley (Govers, 2017).

4. Conclusion

It could be concluded that nine green solvents could be employed as alternative solvents to extract anti-bacterial compounds from the *Carica papaya* peel. The ECPPE was the most effective solvent in this study as it had successfully rendered anti-bacterial activities against a broad range of gram-positive and gram-negative bacteria such as *C. perfringens, L. monocytogenes, V. vulnificus, V. parahaemolyticus,* and *B. subtilis*. The presence of palmitic acid, linoleic acid, β-sitosterol, and stigmasterol in the ECPPE indicated that the *Carica papaya* peel could be a valuable source of bioactive compounds beneficial for the food industry. Although the study for this paper focused on the CPPE using a single extraction method, further investigations by using various extraction methods should be conducted to obtain the best method to extract the anti-bacterial compounds. The fractionation and purification of the active compounds shall be carried out to identify the actual anti-bacterial compounds in the ECPPE. Further application of the ECPPE to actual food systems is required to investigate the potential interactions between ECPPE and naturally occurring food compositions against the bacteria.

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Data availability

Data is available upon request.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions statement

Nurul Aimi Amanina Mohamad Asri - Writing original draft; Muhamad Shirwan Abdullah Sani – Conceptualisation, data curation, and reviewing the manuscript; Rashidi Othman – Methodology and reviewing the manuscript; Noor Faizul Hadry Nordin – Methodology and reviewing the manuscript; Mohd Nasir Mohd Desa - Methodology and reviewing the manuscript.

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