A study of antimicrobial activity of dried and extrudate from bitter gourd fruit against foodborne pathogens

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Abstract

Momordica charantia L., bitter gourd (BG) is a common fruit found in tropical regions. Due to its benefits to human health its cultivation has started in non-tropical countries as well. This study aims to evaluate and screen the antimicrobial properties of hot air dried, spray dried, and extrudate of bitter gourd fruit on selected Gram-negative, Gram-positive bacteria and one strain of yeast through disk diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Time-kill curve was determined for tested microorganisms. *Candida albicans* was resistance to all bitter gourd dried fruit extracts in disk diffusion assay. However, *K. pneumoniae* and *S. epidermidis* were susceptible to all tested extracts. *B. cereus, E. coli, S. mutans, S. aureus and P. aeruginosa* recorded different potential level of growth inhibition towards extracts. The study results effectively concluded that bitter gourd ethanol extract of extrudate at 80°C and hot air dried have significant antibacterial effect recorded by having lowest MIC values. The time-kill needed to destroy the bacteria was within 4 h, except for *P. aeruginosa*. In conclusions, the crude extracts of bitter gourd fruit possessed bactericidal and bacteriostatic activities against foodborne bacteria.

Keywords: bitter gourd, hot air dried, spray dried, extrudates, antimicrobial activity

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Introduction

Rising bacterial resistance has necessitated the search for new antibacterial agents from plants to be able to combat newly resistant pathogenic bacteria (Mead, 2004). Therefore, plant extracts have recently become used to control microbial infections or even to control bacterial growth in food products (Goyal *et al.*, 2020). Therefore, natural antimicrobial compounds found in many edible plants can serve as substitute for the chemical based treatments as such chemicals sometimes leave harmful residues (Gil *et al.*, 2009; Mith *et al.*, 2014 ;Qin *et al.*, 2019; Kupnik *et al.*, 2021).

Momordica charantia L., known as bitter gourd (BG) or *peria katak* in Malaysia, is a tropical plant has been used in folklore medicine and in cooking as well (Grover and Yadav, 2004). However, many phytochemical compounds such as saponins, antioxidants, amino acids, and phenolic content are found in the fresh fruit extract (Anilakumar *et al.*, 2015; Amira *et al.*, 2013), it is often neglected in terms of overall consumption due to its bitter taste. However, BG is among the candidates for study in recent research for its antidiabetic, anticancer, anti-inflammatory, and anti-triglycerides properties (Harinantenaina *et al.*, 2006; Zulbadli *et al.*, 2011; Joseph and Jini, 2013). Saponins are one of the bioactive compounds that have been proven having numerous pharmaceutical properties. Considering that saponins are bioactive compound found in prepared extracts from BG with biological activities in humans, some studies have investigated crude saponins extracts in augmenting the glucose uptake and homeostasis, cholesterol lowering, and antimicrobial activity (Han *et al*., 2008; Sharma *et al*., 2009; Patel *et al*., 2010).

Therefore, more information was needed on the biological activity demonstrated of crude total saponins extracts from processed BG matrices. Thus, a few studies have been conducted on the extract of processed BG fruit to evaluate its antimicrobial activity. Saeed and Tariq (2005) observed antibacterial activity in extract of fresh fruit of *Momordica charantia* L. However, Jabeen and Khanum (2014) found the opposite. To investigate, this study provides information on antimicrobial activities based on the use of dried and extrudate of BG fruit against some of pathogenic microorganisms associated with safety. To the best of our knowledge, this work will be the first studying antimicrobial properties of processed BG fruit including spray dried and extrudates. It will provide information on extract concentration and time needed to kill selected eight standard food pathogens.

Materials and Methods

Plant material and the procedure for drying and extrusion

Dark green bitter gourd (BG) fruits in commercial mature (Subcontinent phenotype) were obtained from a local market (Pasar Borong) in Serdang, Selangor, Malaysia. The fruits were washed carefully with flow of distilled water, and the pulps were removed. The BG fresh fruits were weighed and parts were cut into 3 mm slices and dried in cabinet-oven dryer (SMA-112, Smoke Master, Japan) at 40ºC overnight. Then the dried BG slices were grinded using a mixer and divided into two parts; one was kept as hot air dried BG powder at room temperature for further analysis, when the second part used in extrusion-cooking process for preparing BG extrudates at a constant processing temperature (80, 100, 120°C) using a single screw extruder machine (KE-19/25 D, Brabender, Germany). Then, both BG hot air and extrudates were ground in a food mixer and sieved separately. The other part of fresh fruits was blended into juice and used to make spray dried powder at different inlet temperatures (90, 120, 147, 175 and 200°C) using a pilot scale spray dryer (Niro A/S, GEA, Germany). All particle sizes of the powders were ≤ 425 µm and labeled with its processing temperature. Five grams of each BG powders were separately extracted with 250 ml absolute ethanol via stirring using a stirrer for 2 h. The extraction yields of each BG powder after concentration using a rotary evaporator extraction and the content of saponins as one of the main extract components were determined using the method of Makkar *et al.* (2007) and calculated in terms of percentage. The extracts were then preserved at 4˚C for further use. The expriments was performed in duplicates.

Microbial strains tested

Antimicrobial studies were conducted in vitro on *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 15692 were obtained from the American Type Culture Collection (Rockville, MD, USA). *Staphylococcus aureus* KCCM 11764 was obtained from Korean Culture Center of Microorganisms (Seoul, South Korea). *Bacillus cereus* ATCC 33019, *Klebsiella pneumoniae* ATCC 13733, *Streptococcus mutans* MT 8148, *Escherichia coli* O157:H7, and *Staphylococcus epidermidis* ATCC 12228. All microbial strains were obtained from the laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia.

Antimicrobial assays

Antimicrobial tests were performed at Laboratory of Natural Products, Microbiology Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang. For preparation for the treatments, 100 mg of extract was dissolved in 1 ml dimethyl sulfoxide (DMSO) to obtain a stock solution. Throughout this study, 1% and 9% (v/v) of the plant extract in deionized water were used. The expriment was run in duplicates.

Disc susceptibility assay

The pathogenic bacteria were cultured on Mueller Hinton agar (MHA) (Difco, USA) for 24 h at 37 ºC in an incubator. Whereas, the yeast was cultured on Sabouraud Dextrose agar (SDA) (Difco, USA) for at 35ºC for 24 h. The inoculum was prepared by direct colony suspension method CLSI M7-A6 (2003) and verification of the purity of inoculum was performed. The agar plates were inoculated with the test microorganisms and test disks 6 mm (Oxoid Ltd, UK) were placed on it carefully. The 20 µl/disk of each extract was loaded onto the antimicrobial test disks. The negative control was of dimethyl sulfoxide (DMSO). 500 ppm concentration of chlorhexidine (Sigma–Aldrich, Switzerland) used as a positive control of observation tested microbes fail to grow around paper discs containing antimicrobial drugs. A clear zone of inhibition was measured by an antibiotic zone scale in millimeters.

Minimum inhibitory concentration *(*MIC*)* assay

The determination of MIC of the BG ethanol extracts was carried out using the method of (CLSI, 2003). Briefly, 1% and 9% concentration of the extracts in deionized water were prepared and 100 µL was introduced into 100 µl of MHA broth in a microtiter plate 96- well in column 12. The lowest concentration of the BG extract was in column 3, whereas, inoculum of 5 $\times 10^6$ CFU/ml was added in column 2 and broth only in column 1. The plates were covered and incubated for 24 h at 37°C. The invisible growth in the broth was defined as the MIC.

Minimum bactericidal concentration *(*MBC*)* assay

The MBC of the plant extracts on different bacterial tested was determined by removing an aliquot the media from each well and seeded it on a MHA agar plate (Rukayadi *et* hwang,2006; 2009; 2010). After incubation for 24 h, the concentration that showed no

visible growth on agar plates was determined as a MBC point. Therefore, the calculation of the ratio MBC/MIC of the extracts has permitted to determine their antibacterial power (Youssouf *et al.*, 2015).

Determination of time-kill curve

Time-kill curve procedures were conducted according to National Committee for Clinical Laboratory Standards guidelines. CLSI M26-A (1999) was the reference method. The bacteria suspensions were prepared in 1 ml of MHA broth by touching three to five single colonies from a less than 24h-old culture plate. 10 µL of the resulting suspensions were added to 10 ml of MHA broth without extract, providing the starting inoculum of approximately 10^6 CFU/ml. The bitter gourd ethanol extracts (hot air dried and extrudate at 80°C) were diluted with the MHB medium containing inoculum to obtain one milliliter final volume of concentrations of 0× MIC, 1/2× MIC, 1× MIC, 2× MIC, 4× MIC, and 8× MIC for each bacterial species according to MIC results. For *Bacillus cereus, Klebsiella pneumoniae, Streptococcus mutans,* and *Staphylococcus epidermidis* the time-kill curve employed BG extrudate at 80°C extract. Hot air dried BG extract was used for *S. aureus, P. aeruginosa* and *E. coli.* Cultures were incubated at 37°C. A pipettor was used to remove 10 µl from each MIC concentration time points (0, 1, 2, and 4 h), which was transferred to micro-centrifuge tube and serially diluted 1:100 in 1% PBS (phosphate buffered saline). 10 µl of the dilution spread plated onto MHA in duplicate. The graph of $\log_{10}$ CFU/mL against time was plotted. Bactericidal activity was defined as a killing of 99.9% (≥3 $\log_{10}$ drop in CFU/ml) of the final inoculum size. Bacteriostatic activity was defined as maintenance of or reduction of < 99.9% (<3-$\log_{10}$ drop in CFU/ml) in the final inoculum concentration.

Results and Discussion

The findings showed several of potential levels of antimicrobial properties of Hot air dried BG, spray dried and extrudates ethanol extracts. Examining the selected pathogens found that *Candida albicans* was resistance to all prepared BG powder extracts in disk diffusion test (Tables 1, 2, and 4). The results for the yeast were consistent with findings recorded by kumar *et al.*,2010, and in contrast with reported in other studies (Mwambete, 2009; Chia and Yap, 2011; Ozusaglam and Karakoca, 2013; Rakholiya *et al.*, 2014; Yeo *et al.*, 2014).

The antimicrobial activity of ethanolic extract of hot air dried powder of bitter gourd is shown in Table 1. In Gram-positive bacteria, the extract at concentration of 10 mg/ml showed activity against all tested bacteria except with *S. aureus*, which needed 90 mkg/ml to be susceptible to extract. In Gram-negative bacteria, the extract showed activity against all tested bacteria.

The antimicrobial activity of ethanolic extract of spray dried powder of bitter gourd is shown in Table 2. In all tested bacteria, the extract at a concentration of 10 mg/ml showed no activity. However, the 90 mg/ml extract concentration for all spray dried extracts showed activity against all bacteria screened, except for P. aeruginosa. Moreover, S. aureus was resistant to the spray dried material for 120, 147, and 175°C extracts.

Table 1. Disk diffusion assay results of hot air dried BG samples

Microorganisms	Inhibition zone (mm)	Chlorhexidine (control)	MIC (mg/ml)	MBC (mg/ml)
B. cereus	10	14	2.5	2.5
C. albicans	R	10	-	-
E. coli	10	11	2.5	5
K. pneumoniae	12	13	2.5	2.5
P. aeruginosa	10	12	5	5
S. aureus	10	12	5.625	11.25
S. epidermidis	12	12	1.25	1.25
S. mutans	10	13	2.5	5

R, resistance (no inhibition zone was observed).

Table 2. Disk diffusion assay results of spray dried BG samples

Microorganisms	Inhibition zone (mm)					Control
	90°C	120°C	147°C	175°C	200°C	Chlorhexidine
B. cereus	10	10	10	10	10	14
C. albicans	R	R	R	R	R	10
E. coli	10	10	10	10	10	11
K. pneumoniae	11	12	13	13	13	13
P. aeruginosa	R	R	R	R	R	12
S. aureus	11	R	R	R	10	12
S. epidermidis	11	12	11	12	12	12

S. mutans	11	10	10	10	10	13

R, resistance (no inhibition zone was observed

The antimicrobial activity of ethanolic extract of extrudates powder of bitter gourd is shown in Table 4. In all bacteria screened, the extrudate at 80°C extract at a concentration of 10 mg/ml showed activity against all bacteria screened, except for *P. aeruginosa* and *S. aureus*. On other hand, *P. aeruginosa* and *S. aureus* were susceptible to all extrudates dried extracts only at concentrations of 90 mg/ml. The extrudate at 100°C extract at a concentration of 10 mg/ml showed activity only against *E. coli* and *B. cereus* screened. However, antibacterial activity needed 90 mg/ml of the extract to be achieved against the rest of screened bacteria, except for *S. epidermidis*, which was not susceptible to the extract at both concentrations. The extrudate at 120°C extract at a concentration of 10 mg/ml showed activity only against *S. epidermidis*; however, the antibacterial activity needed 90 mg/ml of the extract to be achieved against the rest of bacteria screened.

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values showed a good level of growth inhibition of seven bacterial strains tested in Tables 1, 3, and 4 showed for hot air dried BG, spray dried and extrudates extracts, respectively. Inhibitory effects of bacterial growth by the extracts from different powders were in the range from 0.625 to 22.5 mg/ml expressed as MIC values and in the range from 1.25 to 45 mg/ml expressed as MBC values.

MIC and MBC values of hot air dried extract of bitter gourd are shown in Table 1. The extract of concentrations of 10 mg/ml and 90 mg/ml showed least MIC values 1.25 and 5.625 mg/ml, respectively, and MBC were 1.25 and 11.25 mg/ml against *S. epidermidis* and S. aureus, respectively.

MIC and MBC values of spray dried extracts of bitter gourd are shown in Table 3. The spray dried at 90°C, 200°C, and 175°C extracts showed least MIC values among spray dried extracts with 1.40, 2.81, and 5.625 mg/ml, respectively, and MBC were 1.40, 2.81, and 5.625 mg/ml, respectively, against *K. pneumoniae*, *B. cereus*, and *S. epidermidis*, respectively.

Table 3. The MIC (mg/ml) and MBC (mg/ml) of BG ethanol extract spray dried

Microorganisms	spray dried at 90°C	spray dried at 120°C	spray dried at 147°C	spray dried at 175°C	spray dried at 200°C

	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. cereus	22.5	45	22.5	22.5	22.5	45	22.5	45	2.81	2.81
E. coli	22.5	45	22.5	45	22.5	45	22.5	45	11.25	22.5
K. pneumoniae	1.40	1.40	22.5	22.5	22.5	22.5	11.25	11.25	22.5	45
P. aeruginosa	-	-	-	-	-	-	-	-	-	-
S. aureus	22.5	22.5	-	-	-	-	-	-	22.5	22.5
S. epidermidis	22.5	22.5	11.25	11.25	11.25	11.25	5.62	5.62	11.25	11.25
S. mutans	22.5	45	11.25	22.5	22.5	45	22.5	22.5	11.25	22.5

MIC and MBC units are mg/ml.

Table 4. Antimicrobial assays results of BG ethanol extract extrudates samples

Microorganisms	**Extrudate at 80°C**			**Extrudate at 100°C**			**Extrudate at 120°C**		
	Inhibition zone	**MIC**	**MBC**	**Inhibition zone**	**MIC**	**MBC**	**Inhibition zone**	**MIC**	**MBC**
B. cereus	10	0.625	1.25	10	2.5	2.5	10	1.25	1.25
C. albicans	R	-	-	R	-	-	R	-	-
E. coli	10	5	5	10	5	5	10	11.25	22.5
K. pneumoniae	10	0.625	1.25	11	2.81	5.62	12	1.40	2.81
P. aeruginosa	11	5.62	5.62	11	22.5	22.5	11	22.5	22.5
S. aureus	11	11.25	11.25	11	22.5	22.5	11	22.5	22.5
S. epidermidis	10	0.625	1.25	R	-	-	10	2.5	2.5
S. mutans	10	0.625	1.25	12	5.62	11.25	11	2.81	5.62

R, resistance (no inhibition zone was observed); MIC and MBC units are mg/ml; inhibition zone unit (mm).

MIC and MBC values of extrudates extracts of bitter gourd are shown in Table 4. The extrudate at 80°C extract showed least MIC value among the extrudates extracts with 0.625 mg/ml, and MBC was 1.25 mg/ml, against *K. pneumoniae* and *B. cereus*, *S. epidermidis* and *S. mutans*. The extrudates at 100°C and 120°C extract showed MIC values of 2.5 and 1.25 mg/ml, respectively, and MBC were 2.5 and 1.25 mg/ml against *B. cereus*.

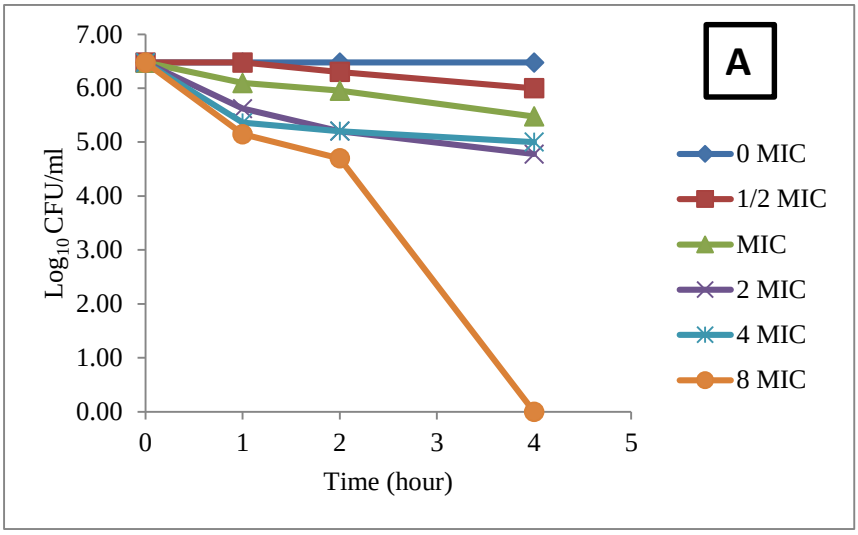
Kill-kinetic determinations are shown graphically by plotting $\log_{10}$ CFU at specified time (0, 1, 2, and 4 hour) in Figure 1. The hot air dried BG extract with 90 mg/ml concentration exhibited a significant bactericidal effect on *S. aureus* with (> 99.9 %

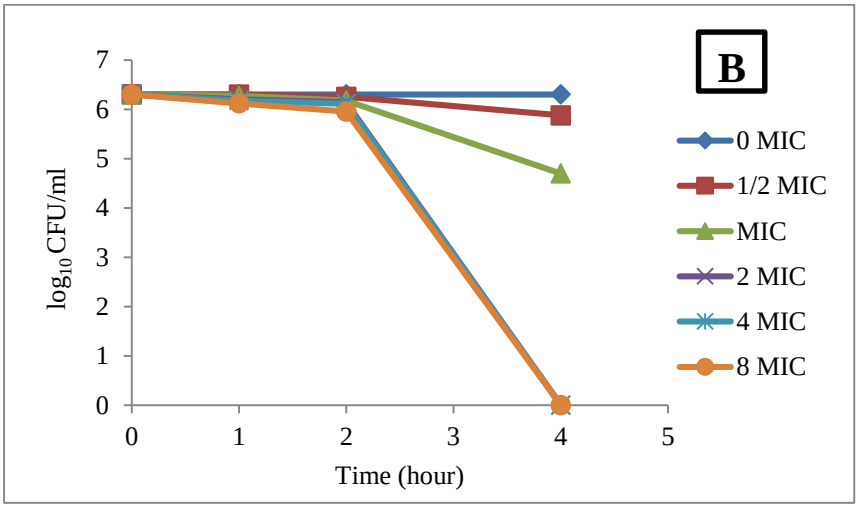
killing, 6.4-log) decrease in CFU after an hour of incubation for 8× MIC, and with decrease in CFU after 4 hour of incubation for 4× MIC, however, the rest of (MICs); 1/2× MIC, MIC, and 2× MIC demonstrated bacteriostatic effect with a ≤ 1.7-log reduction in growth after 2 h. *S. epidermidis* had bactericidal results with 8 × MIC with extract of extrudate at 80°C after one hour. Time-kill finding of *K. pneumonia*e ATCC 13733 in this study was evident and confirmed that *K. pneumoniae* ATCC 13733 is the most susceptible among bacterial tested towards the extract at smallest MIC concentration (1.25 mg/ml) after 4 h of incubation time with the treatment. That indicated that less than 2 mg of extrudate at 80°C powder could be used to inhibit *K. pneumoniae* growth completely. The rest of bacterial tested had bactericidal activity at 4 h, except *P. aeruginosa* which had bacteriostatic activity at 4 h. However, in order to achieve the bactericidal activity for bitter gourd hot air dried extract against *P.* aeruginosa, 24 h was needed. Overall, time-kill assay demonstrated that the extracts managed successfully to destroy an aliquot of 10 µl of bacterial tested.

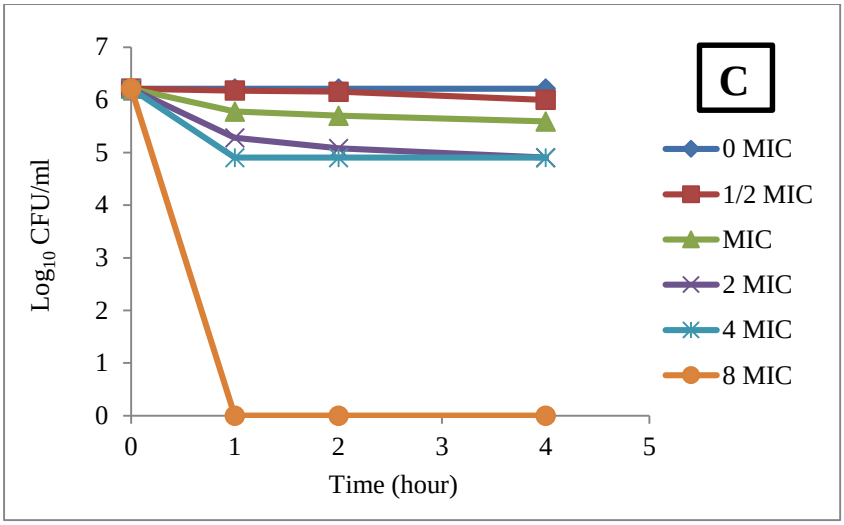
Minimum bactericidal concentration (MBC) assay results confirmed the MIC points and showed the potential action of antibacterial agent (Youssouf *et al.,* 2015). In this study, bacteriostatic effects are shown by spray dried at 120°C and 200°C extracts against *S. mutans* and *K. pneumoniae*, respectively, while remaining extracts showed bactericidal effects. *B. cereus* was the most susceptible Gram-positive bacteria and *K. pneumoniae* was the most susceptible Gram-negative bacteria among all microbes tested against all BG powders extracts.

(Ozusaglam and Karakoca, 2013). Therefore, results in this study revealed the importance of ethanolic extract from bitter gourd fruit, which were consistent with reported results by Ozusaglam and Karakoca (2013) and in which the effective dose for minimum bactericidal concentration *S. aureus* was similar with those obtained in this study with extrudate at 80°C and hot air dried treatments. Moreover, lethal points of *B. cereus* were similar to the lethal point with extrudates at 80°C and 120°C treatments, and *E. coli* was similar to its lethal point with extrudates at 80°C and 100°C and hot air dried treatments. However, the lethal point of *P. aeruginosa* in this study was higher than that reported by Ozusaglam and Karakoca (2013), although it was treated with lower concentration of hot air dried and extrudates extracts.

Yoe *et al.* (2014) found that ethanolic extract of deseeded fruit of bitter gourd (Chinese phenotype) had an antimicrobial agent, in which it could act against *B. cereus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*, through antimicrobial susceptibility disk diffusion test.



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Log10 CFU/ml
Time (hour)
0 MIC
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MIC
2 MIC
4 MIC
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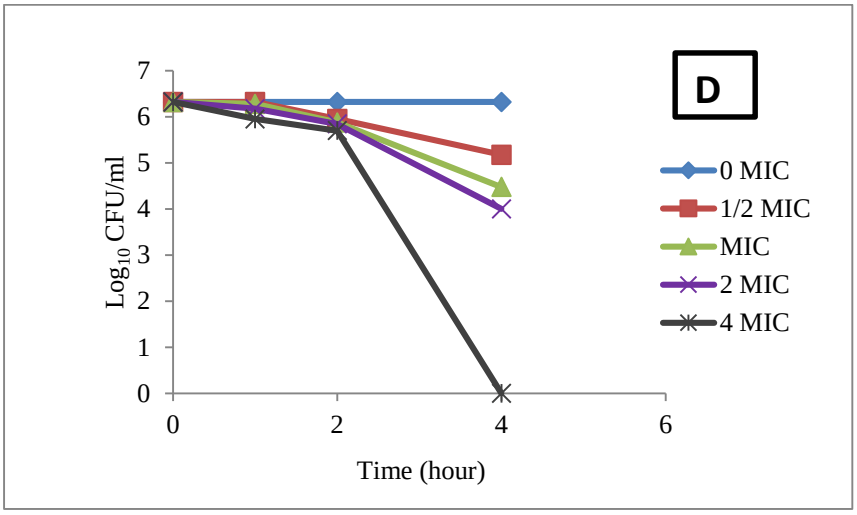
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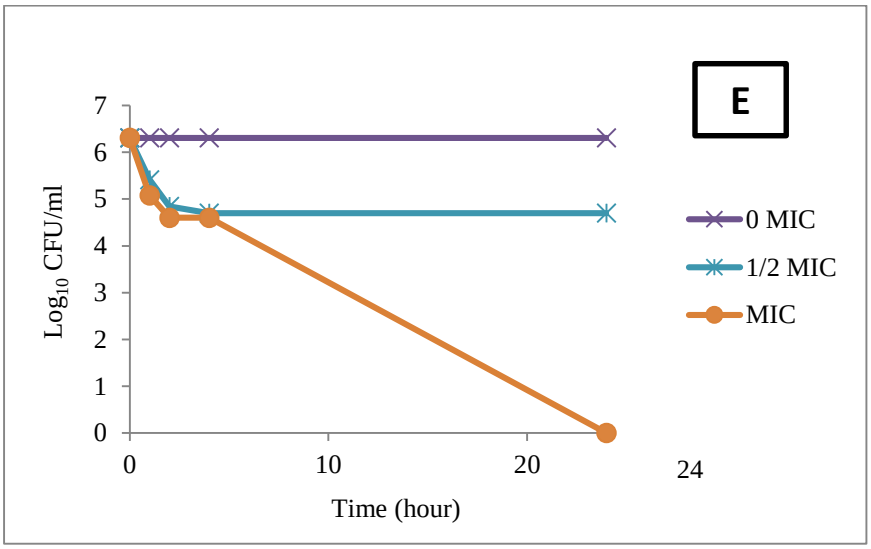
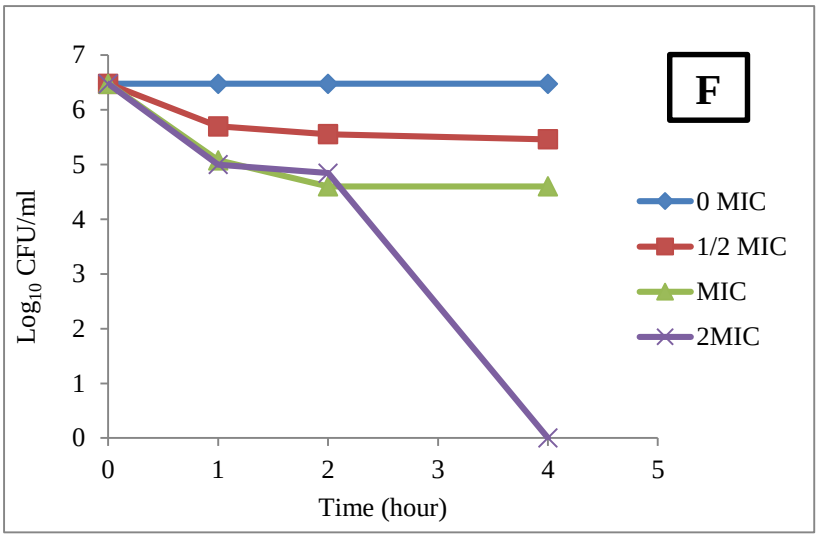

E
Log10 CFU/ml
Time (hour)
0 MIC
1/2 MIC
MIC
24



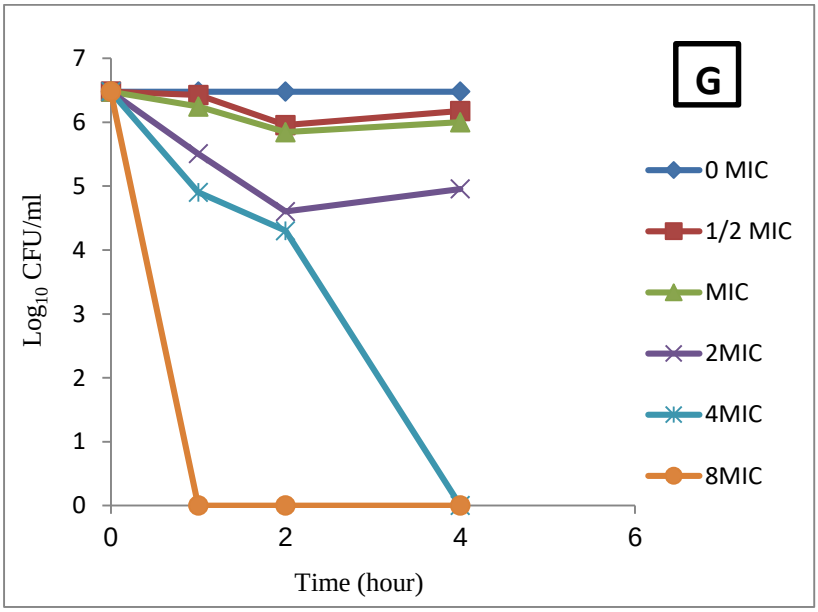


Figure 1. Representative time–kill curve plots for the susceptible bacteria following exposure to bitter gourd extracts; extrudate at 80°C extract for (A), *B. cereus* (5 mg/ml); (B), *K. pneumoniae* (1.25 mg/ml); (C), *S. epidermidis* (5 mg/ml); (D), *S. mutans* (2.5 mg/ml) and hot air dried extract for (E), *P. aeruginosa* (5 mg/ml); (F), *E. coli* (5 mg/ml); (G), *S. aureus* (22.5 mg/ml and 45 mg/ml).

Extraction of bitter gourd fruit with other organic solvents showed antimicrobial activity towards pathogenic microorganisms. Rakholiya *et al.* (2014) was recorded that *S. aureus* was susceptible to methanolic extract of powdered peel and pulp fruit of bitter gourd. Mwambete (2009) was reported that methanolic extract of fine powder of sun dried bitter gourd fruit showed antimicrobial activity against *P. aeruginosa*, *S. aureus*, *E. coli*, and *K. pneumoniae*. Those findings were in agreement with those obtained in this study, especially, to be *K. pneumoniae* the most susceptible to fruit extract among

tested microorganisms. Contrarily, in another study, fresh deseeded fruit extraction with methanol showed no activity towards selected foodborne pathogens *P. aeruginosa* and *S. aureus*; however, *E. coli* was susceptible to the extract (Lu *et al.*, 2011).

Chia and Yap (2011) found that the extracts of hexane: petroleum ether of different concentrations from powdered deseeded fruit of bitter gourd (Chinese phenotype) exhibited antimicrobial activity against *B. cereus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*, however, *S. aureus* was not susceptible to neither pure hexane or pure petroleum ether nor their mixture. In contrast, Yoe *et al*. (2014) found that *S. aureus* was susceptible to BG (Chinese phenotype) both extracts of pure hexane or pure petroleum ether in disk diffusion test.

In general, due to a lack of studies on bitter gourd time-kill assay when obtained data, the time-kill assay could address precisely the lethal points and the needed time for the extracts of extrudate at 80°C and hot air dried of bitter gourd to completely inhibit the growth of bacterial tested for 4 h.

The least kill concentration was 1.25 mg/ml recorded against *K. pneumoniae*, however, the result of *K. pneumonia*e ATCC 13733 was not comparable with findings of other studies, which found that 8, 4, 2 × MIC of the dried rhizome of java turmeric could completely kill *K. pneumonia*e ATCC 13733 in less than 2 h (Sylvester *et al*, 2015). The highest kill concentration was 22.5 mg/ml against *S. aureus*. The fastest killing times were recorded after 1 hour of incubation time with *S. epidermidis* and *S. aureus*, respectively, at the extracts concentration of 5 and 45 mg/ml, respectively.

In general, the extract yields for hot air dried, extrudates, and spray dried of BG were 6, 3, and 1%, respectively. The extraction with absolute ethanol at solvent to solid ratio 0.02 g/ml resulted in the presence of a rich yield of saponins content in the hot air and extrudate at 80°C extracts (Figure 2.). Saponins compounds that existed in BG extracts might have a non-specific action different from other secondary metabolites in showing their antibacterial action. Saponins may interact with a cell's cholesterol. This action would destroy the membrane. If saponins content concentrations were high enough, the saponins would act as a lipophilic compound which changes bacterial cell membrane fluidity and increases permeability. This could explain the powerful antibacterial activity of BG extrudate 80°C and hot air extracts which had a high concentration of saponins content with

10.8% and 10.3%, respectively. Low concentrations of saponins content in BG spray extracts in general with an average 1.5% might be enhanced the uptake of polar of other metabolites that were exciting in the extracts, therefore, increasing their antibacterial activity in an apparently synergistic way (Wink, 2010). Therefore, antioxidant content, phenolic content, alkaloids, terpenoids, steroids, proteins and flavonoids were found in the extraction of powdered fruit with absolute ethanol (Amira *et al.*, 2013; Supraja and Usha, 2013; Ozusaglam and Karakoca, 2013) could be responsible for effectiveness of the extracts as a natural antimicrobial agent.

Bukar *et al.* (2010) found that *Moringa oleifera* seed chloroform extracts were susceptible to *Escherichia coli* and *Salmonella typhimurium* when they contained only saponins. However, the presence of saponins, alkaloids, and flavonoid in *Moringa oleifera* seed or leaf ethanol extracts enhanced its antimicrobial property against foodborne pathogens isolated from food samples. This same study also suggested testing the sanitizing effect of *Moringa oleifera* chloroform and ethanol extracts on certain foods.

Extrudate at 80°C and hot air dried extracts of BG deseeded fruit showed the lowest lethal points, which was 0.625 and 2.5 mg/ml, respectively, at 24 h. These results of effective antibacterial parameters of BG powders ethanol extract were in agreement partially with common minimal inhibitory concentrations (MIC) recorded using plant saponins-rich extracts from 5 mg/ml up to 10 mg/ml against the bacteria (Oleszek, 2000). In addition, BG extracts had MIC range from 0.625 mg/ml up to 5.625 mg/ml. Some studies reported that plant with saponins-rich extracts were more active against Gram-positive bacteria than Gram-negative bacteria with MIC of 1.25 mg/ml and 1.25 to 5 mg/ml, respectively (Oleszek, 2000). In this study, three of Gram-positive bacteria and one Gram-negative bacteria had MIC with 0.625 mg/ml and it was even low than that reported. Two Gram-negative bacteria and one Gram-positive bacteria had MIC with 2.5 – 5.625 mg/ml, and it in the same reported range of MIC.

Therefore, these extracts would be of interest for the control of food bacterial pathogens associated with safety problems in the food processing industry as well as among end consumers. These extracts can be used as a safer alternative of chemical sanitation agent such as chlorine, in which can be added to wash water to reduce microbial contamination on produce.

In general, this study shows that BG powders, extrudates, hot air dried and spray dried, respectively, had a potential antibacterial action in low dosage. Food processing methods of extrusion-cooking, hot air and spray had no such negative impact on bitter gourd's phytochemicals (Kusat *et al.*,2021; Wang *et al.*,2021) that contributed to such effect on bacteria growth. More studies are needed to introduce processed bitter gourd in food system**.**

Conclusion

This study has demonstrated that ethanol extracts enriched-saponins content of dried and extruded bitter gourd fruit had potential bactericidal activity against food pathogens with concentration of 10 mg/ml in 24 h, except for Candida albicans. The extrudate at 80°C had high levels of saponins; therefore, minimum inhibitory concentrations assay showed that the extract succeeded in antibacterial action against S. epidermidis, B. cereus, S. mutans, E. coli and K. pneumoniae. Time-kill findings showed that K. pneumoniae was evidently the most susceptible among bacterial tested. Therefore, these results indicated that dried and extruded BG fruit extract could be used as food ingredient and preservative/sanitizer in food industry.

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دراسة مضادات الميكروبات للثمار المجففة والمعالجة بالبثق الحراري لنبات الخيار المُر ضد أنواع من الميكروبات المسببة لفساد الأغذية

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المستخلص

الخيار المُر .*Momordica charantia* L هو أحد أنواع الثمار الشائع تواجدها في المناطق الاستوائية ولأجل منافعه العديدة لصحة الانسان بدأت زراعته في المناطق الغير استوائية. وهذه الثمار من عائلة القرعيات وتعرف أيضا باسم القثاء المُر أو الكارلا (Karela) في المنطقة العربية. هذه الدراسة تهدف لتقصي وتقييم وجود مضادات للميكروبات في الخيار المر المجفف بطريقة فرن الهواء الساخن والرذاذ وعملية البثق الحراري (Extrusion) ضد أنواع من البكتيريا سالبة وموجبة غرام ونوع من الخمائر المبيضة البيضاء. تم اجراء اختبارات عديدة وهي حساسية المضادات الحيوية والتركيز الفعال للمستخلص (MIC – MBC) وكذلك تقدير الزمن المستغرق (kill time) للمستخلص للتأثير كمضاد للنمو الميكروبي. أظهرت النتائج أن الخميرة المبيضة البيضاء Candida *albicans* غير حساسة لأي مستخلص من مساحيق الخيار المُر، بينما أظهرت أنواع البكتيريا التي شملتها هذه الدراسة حساسية متفاوتة. حيث أظهرت نوعا البكتيريا *K. pneumoniae*, *S. epidermidis*) عدم القدرة علي النمو في وجود كل المستخلصات المجففة بالهواء الساخن والرذاذ وكذلك المعالجة بتكنولوجيا البثق بينما أظهرت أنواع البكتيريا *B. cereus, E. coli, S. mutans, S. aureus and P. aeruginosa*) اختلافا في درجات عدم النمو تأثرا بالمستخلصات ولقد كان جليا في هذه الدراسة أن المستخلص الناتج من عملية التجفيف بالهواء الساخن وعملية البثق الغذائي الحراري علي درجة حرارة 80 درجة مئوية كان لهما القدرة علي كبح النمو الميكروبي لأغلب أنواع البكتيريا خلال أربع ساعات فيما عدا بكتيريا *P. aeruginosa* . وفي النهاية نستخلص من هذه الدراسة أن مستخلص مسحوق الخيار المر المجهز عبر عمليات التجفيف فرن الهواء الساخن والرذاذ والطهي بالبثق الحراري يحتوي على خواص حيوية مضادة للبكتيريا المفسدة للأغذية والمتسببة بالتسمم الغذائي والأمراض.

الكلمات المفتاحية: الخيار المر، طريقة فرن الهواء الساخن، الرذاذ، عملية البثق الحراري، التأثير المضاد للنمو الميكروبي

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