ORIGINAL ARTICLE

Qualitative Phytochemical Screening and Antibacterial Properties of *Momordica charantia* Methanolic Extract Against Selected Bacterial Strains

Nur Dayana Hassan Cheong, Latifah Amalina Zakaria, Hartini Yusof

Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Introduction: The persistent development of bacterial resistance against currently available antibacterial drugs necessitates the search for new antimicrobial agents. The major part of this research is to overcome drug resistance in infectious agents by utilizing medicinal plants as the main natural source in the production of new pharmaceuticals. As 25-50% of contemporary medications are derived from plants, this sparked renewed interest in therapeutic plants. Due to the largely diverse phytochemical compounds found in crude extracts of medicinal plants, they could be used as an alternative source of antimicrobial agents. This study aims to screen the phytochemical compounds and evaluate the antibacterial effect of *Momordica charantia* fruit extract, obtained by maceration in 100% absolute methanol. **Method:** Disc diffusion and broth microdilution methods were performed to evaluate the inhibitory effect of *M. charantia* fruit extract on *Staphylococcus aureus, Escherichia coli*, and *Salmonella typhimurium*. **Results:** The phytochemical tests conducted revealed that the fruit extracts tested positive for alkaloids, phenols, tannins, flavonoids, and saponins. The methanolic extract of *M. charantia* fruit demonstrated antibacterial activity against *S. aureus* with a mean inhibition zone of (17mm±0.82), but not against *E. coli* or *S. typhimurium*. For *S. aureus*, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were 125mg/ml and 500mg/ml, respectively. **Conclusion:** Based on the findings, the current study offers insight into the therapeutic potential of *M. charantia* where the methanolic fruit extract of the fruit has been shown to have antimicrobial activity against *S. aureus* and has the potential to be exploited as an antimicrobial agent.
Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP15) 154-161. doi:10.47836/mjmhs18.s15.21

Keywords: Antimicrobial activity, *Momordica charantia*, Fruit extract, Methanolic, Phytochemical compounds

Corresponding Author:
Hartini Yusof, MSc
Email: tini01@uitm.edu.my
Tel: +603-32584431

INTRODUCTION

Natural products and unique medicinal plants play a crucial role in human existence. Today the world's interest in edible plants, in particular, has risen due to the increasing need for chemical diversity and therapeutic medications from natural products. Plants are utilized in many nations medically and are a source of many potent and powerful medicines. A wide range of medicinal plant parts is used for extract as raw drugs, and they possess various medicinal properties (1). Each section of the plant includes fruits, leaves, flowers, seeds, bark, and roots contains active basic compounds that may be an essential treatment source for various adverse effects and diseases. Fruit is an example of a component of a plant that has positive effects on curing human diseases. These beneficial properties are linked to the presence of bioactive compounds with antioxidant activity that may delay or impede biomolecular oxidation, such as phenolic compounds, carotenoids, and vitamins (1).

Drug resistance to infections was relatively low in the 1980s. However, the statistics are worrisome since various antibiotics, and medication resistance to human pathogens have been documented globally (2). Foodborne pathogens such as *S. aureus*, *E. coli*, and *S. typhimurium*, among others, are the most common agents causing diseases, especially in youngsters within the communities. These species are known to develop strains that are resilient to readily available remedies. Regardless of the breakthrough of pharmaceutical businesses in the production of new antibiotics, bacteria are still resistant to these medicines (3). Therefore, the constant development of bacterial resistance against currently available antibacterial drugs necessitates the search for new antimicrobial agents. The central part of this research is to overcome drug resistance in infectious

agents by utilizing medicinal plants as the primary natural source in the production of new pharmaceuticals. It is known that concentrated fruit or seed extracts are available in different herbal formulations such as capsules and tablets, and can be found marketed everywhere these days (4). The medicinal value of these plants rests in the bioactive phytochemical components that produce specific physiological actions on the human body (5).

Momordica charantia (*M. charantia*) of the *Cucurbitaceae* family is commonly known as bitter gourd. The unripe fruit, typically characterized by a bitter taste, is expected to maintain optimum health. The plant, especially the fruits, have exhibited countless proofs of therapeutic properties (6). The *M. charantia* plants thrive in tropical and subtropical regions, including India, Asia, South America, and Nigeria, and are cultivated throughout South America as food and medicine (7). The plant has been recognized for some time, and it has been utilized in many traditional cuisine and medicines (8) such as hypoglycaemic (9) anti-HIV, antitumor, antileukemic, antidiabetic, anticancer (4), anti-inflammatory (10), antioxidant (11) and antimalarial (12).

The purpose of this study is to investigate *M. charantia* fruit's antibacterial efficacy via assessment of the bioactive compounds present in the methanolic extract of the plant material and testing against selected pathogens. A previous study by (13) has reported that it has been observed that methanolic extract of *M. charantia* fruit has anticancer activity and the impact of cytotoxicity on several cell lines. (14) showed that antihyperglycemic effects were also discovered in *M. charantia* fruits. Another study (16) showed that *M. charantia* possesses strong antifungal and antibacterial efficacies against pathogens that infect humans. In this study, the therapeutic potentials of methanolic extract of *M. charantia* fruit as an antimicrobial agent were assessed and reported.

MATERIALS AND METHODS

Preparation of *M. charantia* Fruit Sample

M. charantia fruit was purchased in Alor Setar, Kedah, from Delima Jelita Enterprise in Simpang Empat, in powder form instead of the fresh fruit. The sample was weighed and kept at 4°C in a Schott bottle until further use.

Extraction Process of *M. charantia* Fruit

The extraction process for *M. charantia* fruit was done using the methods described by (7), with slight modifications. A ratio of 1:10 for *M. charantia* fruit to methanol solvent was used for this process. In a conical flask, 100g of powdered *M. charantia* fruit was soaked in 1000ml of absolute methanol. The flask was covered with aluminum foil, and the mixture was swirled for four days with 110 revolutions per minute (rpm) using an orbital shaker and occasional shaking. The mixture was filtered using Whatman No. 1 filter paper and evaporated at 40°C with decreased pressure using a rotary evaporator after 96 hours with intervals of stirring. 600ml of crude extract was obtained after four hours, transferred into a universal bottle, and covered using parafilm to avoid contamination. By using a rotary evaporator, 600ml of crude extract was reduced to 3.5g and was stored at 4°C until further use.

Preparation of Concentrated *M. charantia* Fruit Extract

1000mg of *M. charantia* fruit crude methanolic extract was mixed with 1ml dimethyl sulfoxide (DMSO) to produce the extract with a concentration of 1000mg/ml.

Preparation of *M. charantia* Extract Disc

Whatman No.1 filter papers were punched out into round shapes of 5mm in diameter and then autoclaved. 20µL of *M. charantia* methanolic extract was pipetted onto the prepared sterile filter paper discs and left to air dry for 1 hour. Next, 20 µl of 10% dimethyl sulfoxide (DMSO) was impregnated onto sterile filter paper discs for the negative control. The antibiotics used for positive controls against selected organisms were ampicillin (10µg) for *S. aureus*, gentamycin (30µg) for *S. typhimurium*, gentamycin (10µg) for *E. coli*.

Screening of Phytochemical Properties of *M. charantia* Extract

An aliquot of methanolic extract obtained from *M. charantia* fruits was subjected to qualitative phytochemical analysis to determine the presence of secondary metabolites. 1ml of methanolic extract was used for each analysis, except for the flavonoids and saponin tests in which 2ml of the extract was used. The presence of compounds was visualized via color changes and foam production. The results are observed and recorded.

Test for phenols

1 ml extract, distilled water, and a few drops of 10% Ferric chloride were mixed in a test tube. The formation of blue or black coloration shows the presence of the phenolic group (15).

Test for alkaloids

In a 5ml tube, 1 ml of extract, 2 ml of chloroform, and a few drops of Wagner's reagent were mixed. The development of a reddish-brown precipitate shows the presence of alkaloids (15).

Test for flavonoids

2ml of the extract was mixed with a few drops of 1% ammonia (NH3) solution in a test tube. A yellow coloration as an indication of flavonoids was observed (16).

Test for tannins

In a 5 ml test tube, 1 ml of extract and three drops of 5% iron III chloride were mixed together. The greenish-black precipitated produced indicates the presence of tannins (17).

Test for saponins

2 ml of extract was added to 5 ml of distilled water and shaken vigorously to obtain stable, persistent foam. The formation of foam indicates the presence of saponins (15).

Bacteria identification test

One entire loop of the stock culture of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *S. typhimurium* (ATCC 14028) were streaked on both blood agar and nutrient agar plates and incubated for 18-24 hours at 37°C. Colonies morphology were recorded, and single colonies were taken for basic confirmation tests such as Gram stain, cultured onto differential agar (MacConkey (MAC) agar, xylose lysine deoxycholate (XLD) agar, Salmonella Shigella (SS) agar, mannitol salt and eosin methylene blue (EMB) agar) and biochemical identification tests (indole test, oxidase test, coagulase test, catalase test, motility test, Simmon citrate test, MR-VP, and TSI).

Antimicrobial Sensitivity Testing (AST)

The disc diffusion method was employed in AST and was conducted in triplicate on Mueller Hinton agar (MHA) with specified antimicrobial discs. From the nutrient plate agar, about three to five colonies of each microorganism were obtained and cultured in 5ml Mueller Hinton broth (MHB). The suspensions were then incubated at 37°C for one hour. Using the Kirby Bauer method, a sterile cotton swab was used to uniformly lawn the inoculums of each microorganism onto MH agar plates. It is critical to use a sterilized cotton swab to ensure the even distribution of bacterial inoculum suspension over MH agar. Three sterile paper discs (methanolic *M. charantia* fruit extract, positive control, and negative control) were carefully arranged with even spacings onto the bacterial lawn. The plates were incubated at 37°C for 24h, and the zones of inhibition were measured and recorded the next day.

Minimum Inhibitory Concentration (MIC)

The MIC was conducted in triplicate using a microtiter plate of 96 wells. Three to five pure colonies of each organism were inoculated in 5ml MHB to prepare for the bacterial suspensions. After incubation at 37°C for two to three hours, the turbidity of the suspensions was compared to 0.5 McFarland standard. On the microtiter plate, the wells were then marked with numbers from one to 12. Well 11 was designated as positive control and well 12 was assigned as the negative control. 100µl of MHB was pipetted into well 2 until 11, while 120µl was pipetted into well 12. 100µl of *M. charantia* extract with the concentration of 1000 mg/ml was added into well 1 and 2. The two-fold serial dilution was carried out by transferring 100µl of mixtures in well 2 through well 9. After well 9, 100µl of the suspension was discarded to standardize the final volume in each well. Then, 20 µl inoculums suspension of the tested bacterial strain were pipetted into well 1 until 11. To avoid drying, aluminum foil was used to seal the microtiter plate. The microtiter plate was incubated at 37°C for 18 to 24 hours. Turbidity and pellet development at the bottom of the well was used to monitor bacterial growth, with the first pellet spotted serving as the MIC endpoint.

Minimum Bactericidal Concentration (MBC)

The MBC value was determined by subculturing one loopful of broth from each well in MIC onto MH agar and incubated at 37°C for 18 to 24 hours. It was done by streaking an aliquot of each well, including the positive and negative control onto MH agar in triplicate. The extract concentration with the MBC value was determined by the mean lowest concentration which resulted in no bacterial growth on the medium.

RESULTS

Table I showed the summary of qualitative phytochemical screening of chemical compounds present in the methanolic extract of *M. charantia* fruit. Figure 1 shows the zone of inhibition obtained when *M. charantia* fruit extract were tested against the selected bacteria.

Table I: Phytochemical screening of methanolic extract of *M. charantia fruit*

Bioactive compounds	Methanolic extract
Phenols	Positive
Alkaloids	Positive
Flavonoids	Positive
Saponins	Positive
Tannins	Positive

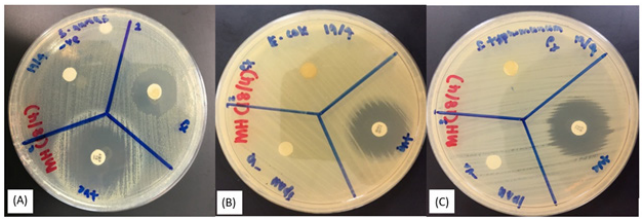

Figure 1: AST of *M. charantia* fruit extract against (A) *S. aureus*, (B) *E. coli*, (C) *S. typhimurium*

The mean inhibition zone diameter of the methanolic extract of *M. charantia* fruit against the selected bacterial strains in triplicate is shown in Table II.

The mean MIC result of *M. charantia* fruit methanolic extract against *S. aureus* in triplicate is shown in Table III. The results in Figure 2 and Table III showed no visible growth in wells number 1 to 4. Therefore, the MIC value of *M. charantia* fruit methanolic extract against *S. aureus* was 125mg/ml. The presence and absence of bacteria

Table II: The inhibition zones (mm) of *M. charantia* furit extract against selected bacterial strains.

Organism	Zone of inhibition in mm (Mean±SD)		
	Positive control (Standard antibiotics)	Negative control (10% DMSO)	Methanolic extract (1000mg/ml)
S. aureus (ATCC 25923)	23.66 ± 0.47 Ampicillin (10µg)	0.00 ± 0.00	17.00 ± 0.82
E. coli (ATCC 25922)	22.67 ± 0. 47 Gentamicin (10µg)	0.00 ± 0.00	0.00 ± 0.00
S. typhimurium (ATCC 14028)	24.00 ± 0.00 Gentamicin (30µg)	0.00 ± 0.00	0.00 ± 0.00

Table III: MIC result of *M. charantia* fruit extract against *S.aureus*.

Wells	Dilution factor	Concentration of methanolic extract (mg/ml)	Result
1	1	1000.00	C
2	2	500.00	C
3	4	250.00	C
4	8	125.00	C
5	16	62.50	T
6	32	31.25	T
7	64	15.63	T
8	128	7.82	T
9	256	3.90	T
10	Positive control	-	T
11	Negative control	-	C

C: Clear (no visible growth) T: Turbid (growth)

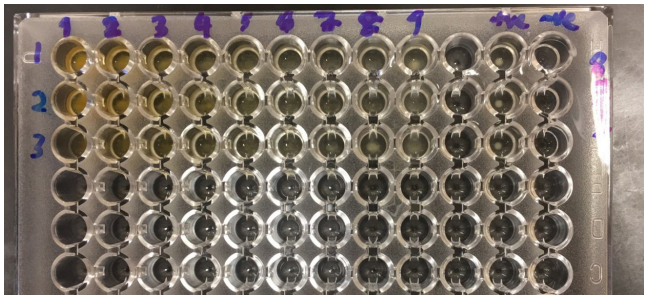

Figure 2: Microdilution broth susceptibility test of *S. aureus* by using microtiter plate (in triplicate). Clear contents of Well 1-4 observed.

growth in positive and negative control respectively validated all results.

The mean MBC result of *M. charantia* fruits methanolic extract against *S. aureus* in triplicate is shown in Table IV. The result in Table IV showed no growth in wells number 1 and 2. Thus the MBC value of *M. charantia* fruits methanolic extract against *S. aureus* was 500 mg/ml. The presence and absence of bacteria growth in positive and negative control respectively validated all results. Methanolic extract of *M. charantia* fruit inhibits *S. aureus* up to the concentration of 500mg/ml.

Table IV: MBC result of *M. charantia* fruit extract against *S. aureus*.

Well number	Concentration of methanolic extract (mg/ml)	Result
1	1000	No growth
2	500	No growth
3	250	Growth
4	125	Growth
11	Positive control	Growth
12	Negative control	No growth

DISCUSSION

Phytochemical Analysis

In this present study, absolute methanol was chosen as the solvent for the extraction procedure. This is because organic solvents can dissolve organic compounds better. The type of solvent used in an extraction procedure will largely affect the determination of biologically active compounds from plant material. In plant extractions, a suitable solvent comprises low toxicity, easiness of low heat evaporation, promotion of quick physiological absorption of the extract, conservation, and inability to cause the extract to be complex or dissociated (18). Because residual solvents can be found in the final extraction product, the solvent should be non-toxic and not interfere with or cause interruptions in the bioassay (19). As antimicrobial agents identified from plants are typically aromatic or saturated organic agents, they are most commonly acquired via first ethanol or methanol extraction (20).

Consequently, methanol, ethanol, and water are the most popular solvents employed for preliminary research of antibacterial activity in plants (21,22). According to (23), the higher polarity of solvent leads to a higher solubility of solvent compounds and may influence the capacity to extract phytochemical compounds and antimicrobial activities. Methanol, being a high polarity indexed solvent, can recover the compounds and constituents of *M. charantia* fruit better. Moreover, using methanol as an extraction solvent also prevented thermal degradation and oxidation due to its lower temperature and shorter extraction period (24).

The methanolic extract tested showed a positive result for all bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenols. Hence, this discovery of bioactive compounds can start a new drug discovery and development as the compounds are presented with significant major secondary metabolites (25). The previous study done by (26) also proves that alkaloids, steroids glycoside, saponins, and phenolic compounds that possess considerable biological importance are present in the ethanolic extract of *M. charantia*. More investigations also revealed that the phytochemical screening done on *M. charantia* seeds (27) and leaves (7) with different extraction solvents exhibited the presence of chemical constituents such as alkaloids, flavonoids, glycosides, saponins, tannins, and terpenes. The phytochemical compounds present in the extract are shown to demonstrate medicinal and physiological functions.

Firstly, according to (28), the antimicrobial action of alkaloids has been demonstrated through the inhibition of enzyme activity, among other mechanisms. Tannins have also been found to have antimicrobial properties (29,30) by attaching to proline-rich proteins, preventing the process of protein synthesis (31,32). In response to microbial infections, plants generate flavonoids, which are hydroxylated polyphenolic chemicals that have been widely researched and proven to have antibacterial action against various pathogens in vitro (33). It is summarised by (34) that the disruption of membranes, inhibition of cell envelope synthesis, nucleic acid synthesis, and bacterial toxins are among the mechanisms of antimicrobial action by flavonoids. Terpenoids have also been demonstrated to be a promising source of antimicrobial agents to possess activities against viruses, bacteria, fungi, and protozoa (35,36). A study done by (37) showed the potential of isolated saponins of *M. elliptica* to be used with other antibiotics to combat multidrug-resistant pathogens. Saponins have also been indicated to exert antimicrobial activities against various strains of bacteria (38). On the other hand, the antimicrobial assay should be broadened to include testing on other organisms as well as in vivo research. Plant extracts should not be administered directly to humans without first undergoing in vivo research. Hence, it's critical to ensure that the extract is tested for cytotoxicity, biological effects, and the method of applying extracts to humans before they're used.

AST, MIC, and MBC

Only one of the bacteria species tested, *S. aureus* was sensitive to the methanolic extract of *M. charantia* fruit. At 1000mg/ml, the methanolic extract had an antibacterial activity with a mean inhibition zone of 17.00mm against *S. aureus*. Both *E. coli* and *S. typhimurium* were resistant with no zones of inhibition measured. This is backed up by prior research (39), which discovered a significant zone of inhibition for *S. aureus* when tested with methanolic extract and demonstrated that *M. charantia* fruit extracts had more significant antibacterial activity than other plant sections. *S. aureus*, the sole gram-positive bacteria tested, yielded a sensitive result in AST whereby the gram-negative bacteria, *E. coli*, and *S. typhimurium*, were resistant to methanolic extract of *M. charantia* fruit. As a result, the extract was unable to inhibit both *E. coli* and *S. typhimurium*. However, a study done by (7) reported that 100mg/ml of ethanol extract of *M. charantia* leaves exhibited high antibacterial activities against both *E. coli* and *S. aureus* with inhibition zones of 17 mm and 16 mm respectively. Previous work done by (40) also demonstrated that when tested against *S. aureus* and *E. coli*, the ethanolic extract of *M. charantia* seeds showed a strong antimicrobial potential against both species. The leaf of *M. charantia* also demonstrated antimicrobial activity against *E. coli*, *S. paratyphi, S. dysenterae* (41). On the contrary, (42) reported that the methanolic extract of *M. charantia* showed good activity against *E. coli* but inactive against *S. aureus*. In another study, the ethanol extract of unripe *M. charantia* fruit showed significant antimicrobial efficacy against *S. aureus* and *P. aeruginosa* with inhibition zones of 20.52 mm and 19.02 mm, respectively (43).

The MIC and MBC tests are antimicrobial assays that should be performed to determine the inhibitory effects of plant extract. The MIC and MBC tests were intended to assess the lowest feasible extract concentration that may inhibit the growth or even kill the organisms using two-fold serial dilution. This analysis revealed that using broth microdilution assays, at the extract concentrations below 1000 mg/ml, all the tested organisms were inhibited. The MBC result therefore shows corroboration from the preceding MIC result. A similar study done by (44) showed that the methanolic extract of fresh *M. charantia* leaves was effective against all tested organisms (*S. aureus*, *B. cereus*, and *E. coli*) and the lowest MIC value (128µg/ml) was against *S. aureus*. However, acetyl acetate extract of the similar plant section presented the lowest MIC value against *E. coli*.

The variety of results presenting different efficacies against different species might be affected by several variables, including the environmental aspect of plant production and harvest, the methods and solvents used for extraction, which influenced the production and potentials of secondary metabolites as antimicrobial agents. However, the broth microdilution method is considered the 'gold standard' to investigate the antimicrobial activity of extracts as it has a greater sensitivity than other methods (45).

Compared to gram-negative organisms, the extract prepared from *M. charantia* fruits has a higher tendency to inhibit the gram-positive organisms. Gram-positive bacteria yield highly sensitive results compared to gram-negative bacteria because they lack the outer membrane, lipopolysaccharides components as a protective barrier that causes them to be easily penetrated by other compounds compared to gram-negative bacteria (46,47). This may be due to the differing chemical makeup of both bacteria' cell walls. The chemical composition of gram-negative microorganism cell walls contains lipopolysaccharide, peptidoglycan lipoprotein, and external porin protein membrane, whereas the cell walls for gram-positive microorganisms consist of peptidoglycan, teichoic acid, and porins protein. The chemical makeup of gram-negative organisms makes them resistant to all extract particles, which can penetrate the bacteria's inner membrane and disrupt the membrane itself. Similarly, gram-negative bacteria are less permeable and more challenging to kill when compared to gram-positive bacteria because their cell walls contain more lipids (48). The result of this study suggests that methanolic extract *M. charantia* fruit might be an alternative antibacterial agent for treating infections, specifically those caused by gram-positive

organisms.

Despite all the findings, several research gaps are identified in this study. Firstly, quantitative analysis of the phytochemical compounds discovered was not done and only qualitative analyses were conducted to determine the presence of the metabolites in *M. charantia* fruit extract. In order to obtain a more specific result and to identify the secondary metabolites, a quantitative analysis should be carried out as done by (49) where 13 phenolic compounds obtained from *M. charantia* fruit extract were quantified using UPLC-MS. This allows a confident identification of bioactive compounds that is responsible for the antimicrobial properties of the *M. charantia* species. The medicinal qualities of this species should be scientifically proven to establish the potential of this fruit as an antimicrobial agent before its application and usage are widened in the healthcare sector. Other than that, considering that this study is a preliminary analysis of the antimicrobial potential of *M. charantia* fruits, cytotoxic screening of the extract was not performed. Cell viability is measured via the standard colorimetric MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide] assay. This test rely on the ability of succinate dehydrogenase, a mitochondrial enzyme in reducing yellow water soluble MTT to a purple colored formazan, and is measured spectrophotometrically (50). This test is necessary to ensure the extract is not harmful to the cells and can be used as an alternative to conventional drugs once developed further.

CONCLUSION

This research discovered that the methanolic extract of *M. charantia* fruits has antibacterial activity and was efficient against the gram-positive bacteria, *S. aureus*. The capacity of the fruit extract to function as an antibacterial agent may be developed and utilised to treat infectious illnesses. The crude extract from *M. charantia* fruit can only inhibit gram-positive bacteria since no inhibition zones were found for the tested gram-negative species, *E. coli*, and *S. typhimurium*. *S. aureus* was the most sensitive to the extract, exhibiting a clear inhibitory zone around the methanolic extract disc. The extract was more efficient against gram-positive bacteria than gram-negative bacteria. Variations in these species' susceptibilities to the extract may be attributed to inherent characteristics of these organisms, such as the permeability of their cell wall surfaces to the particles in the extracts.

According to the results from MIC and MBC tests, the extract was shown to be effective against certain tested bacteria. Even so, it can also be concluded that the extract was inadequate to completely replace the current drugs in use, instead it could be used as a complement when combined with the existing antibiotics. Concurrently, additional studies are compulsory to be performed on the extract of *M. charantia* prior to its commercialization and public usage. The screening test of phytochemical compounds conducted in this study also showed favorable results where the saponins, tannins, alkaloids, phenol, and flavonoids were present. The screening for phytochemicals was done simultaneously in this study to determine the presence of bioactive components involved for novel drug discovery and development may be carried out thereafter.

Since this is a preliminary study on *M. charantia* extract, the phytochemical screening procedure was technically insufficient and less accurate, thus further research should be carried out with confirmations and quantifications of compounds using high performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS). When compared to just employing reagents and subjectively observing the color changes in test tubes, these analytical techniques will give more accurate and effective findings. A quantitative phytochemical analysis should also be conducted to provide a more in depth information of the extract. For future investigations on *M. charantia* fruit extracts, procedures such as killing assays, SDS-PAGE, screening, and quantitation of antioxidants, and in vivo testing by means of animal models are strongly suggested.

ACKNOWLEDGMENT

The authors thank the Faculty of Health Sciences at the UiTM Selangor for providing the laboratory space and financial support.

REFERENCES

1. Ayala-Zavala JF, Rosas-Dom´ınguez C, Vega-Vega V, Gonz´alez-Aguilar GA. Antioxidant enrichment and antimicrobial protection of fresh-cut fruits using their own byproducts: Looking for integral exploitation. J Food Sci. 2010;75(8):175–81. doi: 10.1111/j.1750-3841.2010.01792.x
2. Yeo YL, Chia YY, Lee CH, Sow HS, Yap WS. Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of *Momordica charantia* L. J Appl Pharm Sci. 2014;4(10):16–23. doi: 10.7324/JAPS.2014.40104
3. Nascimento GGF, Locatelli J, Freitas PC, Silva GL, Piracicaba UM De. Antibacterial activity of plant extracts and phytochemicals on antibiotc resistant bacteria. Brazilian Arch Biol Technol. 2000;31:247–56.
4. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: a review. J Ethnopharmacol. 2004;93:123–32. doi: 10.1016/j.jep.2004.03.035
5. Afolabi F, Afolabi OJ. Phytochemical constituents of some medical plants in south. IOSR J Appl Chem. 2013;4(1):76–8. doi: 10.9790/5736-0417678
6. Mozaniel S de O, Wanessa A da C, Fernanda

WFB, Marilena EA, Gracialda CF, Raul N de CJ. Phytochemical profile and biological activities of *Momordica charantia* L. (Cucurbitaceae): A review. African J Biotechnol. 2018;17(27):829–46. doi: 10.5897/AJB2017.16374
7. Mada SB, Garba A, Mohammed HA, Muhammad A, Olagunju A. Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of *Momordica charantia* L. leaves. J Med Plants Res. 2013;7(10):579–86. doi: 10.5897/JMPR012.1161
8. Polito L, Bortolotti M, Maiello S, Battelli MG. Plants producing ribosome-inactivating proteins in traditional medicine. Molecules. 2016;21(11):1–27. doi: 10.3390/molecules21111560
9. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol. 2003;84(1):105–8. doi: 10.1016/s0378-8741(02)00144-7
10. Kobori M, Nakayama H, Fukushima K, Ohnishi-Kemayama M, Ono H, Tastunobu F, et al. Bitter gourd suppresses lipopolysaccharide-induced inflammatory responses. J Agric Food Chem. 2008;56:4004–11. doi: 10.1021/jf800052y
11. Ahmed I, Lakhani MS, Gillett M, John A, Raza H. Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. Diabetes Res Clin Pract. 2001;51:155–61. doi: 10.1016/s0168-8227(00)00224-2
12. Alam A, Uddin R, Subhan N, Rahman M, Jain P, Reza HM. Beneficial role of bitter melon supplementation in obesity and related complications in metabolic syndrome. J Lipids. 2015;2015. doi: 10.1155/2015/496169
13. Alshehri MA. Anticancer activity of methanolic extarct of *Momordica charantia* against human colon, liver and breast cancer cell lines-In vitro. J Biol. 2016;6(6):106–11. doi: 10.1155/2015/496169
14. Choudhary SK, Chhabra G, Sharma D, Vashishta A, Ohri S, Dixit A. Comprehensive evaluation of anti-hyperglycemic activity of fractionated *Momordica charantia* seed extract in alloxan-induced diabetic rats. Evidence-based Complement Altern Med. 2012;2012. doi: 10.1155/2012/293650
15. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Int Pharm Sci. 2011;1(1):98–106.
16. Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pac J Trop Biomed. 2013;3(9):705–10. doi: 10.1016/S2221-1691(13)60142-2
17. Saranraj P, Stella D, Sathiyaseelan K, Samuel S. Antibacterial potentiality of ethanol and ethyl acetate extract of Acalypha indica against human pathogenic bacteria. J Ecobiotechnology. 2010;2(7):23-27. Available from: https://updatepublishing.com/journal/index.php/jebt/article/view/81
18. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plants Res. 2010;4(2):104–11. doi: 10.5897/JMPR09.030
19. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotechnol. 2008;7(12):1797–806. doi: 10.5897/AJB07.613
20. Serkedjieva Y, Manolova N, Bankova V. Anti-influenza virus effect of some propolis constituents and their analogues (esters of substituted cinnamic acids). J Nat Prod. 1992;5(3):294–7. doi: 10.1021/np50081a003
21. Lourens ACU, Reddy D, Başer KHC, Viljoen AM, Van Vuuren SF. In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. J Ethnopharmacol. 2004;95(2–3):253–8. doi: 10.1016/j.jep.2004.07.027
22. Parekh J, Karathia N, Chanda S. Screening of some traditionally used medicinal plants for potential antibacterial activity. Indian J Pharm Sci. 2006;68(6):832–4. doi: 10.4103/0250-474x.31031
23. Tomsone L, Kruma Z. Comparison of different solvents for isolation of phenolic compounds from horseradish (Armoracia Rusticana L.) leaves. Res Rural Dev. 2013;1:104–10.
24. Savsatli Y, Seyis F. Determination of suitable solvents for extraction of different fruit parts of bitter melon (*Momordica charantia* L.). Res J Agric Sci. 2016;9(1):18–22.
25. Srinivasulu S, Pallavi Y, Gayatri Devi B, Padma Jyothi HK. Phytochemical and HPTLC studies on fruit extracts of Momordica cymbalaria Fenzl, a medicinally inportant plant. Not Sci Biol. 2017;9(3):350–60. doi: 10.15835/nsb939910
26. Ullah M, Showkat M, Ahmed NU, Islam S, Absar N. Evaluation of *Momordica charantia* L. fruit extract for analgesic and anti-inflammatory activities using in vivo assay. Res J Med Plant. 2012;6(3):236–44. doi: 10.3923/rjmp.2012.236.244
27. Oragwa LN, Efiom OO, Okwute SK. Phytochemicals, anti-microbial and free radical scavenging activities of *Momordica charantia* Linn. (Palisota Reichb) seeds. African J Pure Appl Chem. 2013;7(12):405–9. doi: 27. 10.5897/AJPAC2013.0526
28. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Front Microbiol. 2019;10(911). doi: 10.3389/fmicb.2019.00911
29. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother.

2001;48(4):487–91. doi: 10.1093/jac/48.4.487
30. Min BR, Pinchak WE, Merkel R, Walker S, Tomita G, Anderson RC. Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Sci Res Essays. 2008;3(2):66–73. Available from: https://academicjournals.org/article/article1380355448_Min%20et%20al.pdf
31. Sanches NR, Cortez DAG, Schiavini MS, Nakamura CV, Dias Filho BP. An evaluation of antibacterial activities of Psidium guajava (L.). Brazilian Arch Biol Technol. 2005;48(3):429–36. doi: 10.1590/S1516-89132005000300014
32. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, et al. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 1996;50(1):27–34. doi: 10.1016/0378-8741(96)85514-0
33. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82. doi: 10.1128/cmr.12.4.564.
34. Gyrniak I, Bartoszewski R, Kryliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemistry Reviews. 2019;18(1):241-72. doi: 10.1007/s11101-018-9591-z
35. Park YJ, Baskar TB, Yeo SK, Arasu MV, Al-dhabi NA, Lim SS, et al. Composition of volatile compounds and in vitro antimicrobial activity of nine. Springerplus. 2016;5(1). doi: 10.1186/s40064-016-3283-1
36. Hassan STS, Masarc R. Bioactive natural products with anti-herpes simplex. J Pharm Pharmacol. 2015;67:1325–36. doi: 10.1111/jphp.12436
37. Tagousop CN, Tamokou JDD, Kengne IC, Ngnokam D, Nazabadioko LV. Antimicrobial activities of saponins from Melanthera elliptica and their synergistic effects with antibiotics against pathogenic phenotypes. Chem Cent J [Internet]. 2018;12(97):1–9. Available from: https://doi.org/10.1186/s13065-018-0466-6
38. Khan MI, Ahhmed A, Shin JH, Baek JS, Kim MY, Kim JD. Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study in vitro and in vivo. Evidence-based Complement Altern Med. 2018. https://doi.org/10.1155/2018/3486106
39. Mwambete KD. The in vitro antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*: A Tanzania medicinal plant. Afr Health Sci. 2009;9(1):34–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2932517/
40. de Lucena Filho JHS, de Freitas Lima R, de Medeiros ACD, Pereira JV, Granville-Garcia AF, de Brito Costa EMM. Antimicrobial potential of *Momordica charantia* L. against multiresistant standard species and clinical isolates. J Contemp Dent Pract [Internet]. 2015 [cited 2021 Aug 22];16(11):854–8. doi: 10.5005/jp-journals-10024-1770
41. Omoregbe R, Ikuebe O, Ihimire I. Antimicrobial activity of some medicinal plants extracts on *Escherichia coli*, *Salmonella paratyphi* and *Shigella dysenteriae*. Afr J Med Med Sci [Internet]. 1996 [cited 2021 Aug 22];25(4):373–5. Available from: https://pubmed.ncbi.nlm.nih.gov/9532310/
42. Ahmad B, Khan AA. Antibacterial, antifungal and phytotoxic activities of Luffa cylindrica and *Momordica charantia*. J Med Plants Res. 2013;7(22):1593–9. doi: 42. 10.5897/JMPR11.1454
43. Ozusaglam MA, Karakoca K. Antimicrobial and antioxidant activities of *Momordica charantia* from Turkey. African J Biotechnol. 2013;12(13):1548–58. doi:10.5897/AJB2012.2932
44. Costa JGM, Nascimento EMM, Campos AR, Rodrigues FFG. Antibacterial activity of *Momordica charantia* (Curcubitaceae) extracts and fractions. J Basic Clin Pharm. 2011;2(1):45–51. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3979203/
45. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(1):5–16. doi: 10.1093/jac/48.suppl_1.5
46. Sibi G, Shukla A, Dhananjaya K, Ravikumar KR, Mallesha H. In vitro antibacterial activities of Broccoli (Brassica oleracea L.var italica) against food borne bacteria. J Appl Pharm Sci. 2013;3(5):100–3. doi: 10.7324/JAPS.2013.3519
47. Wan Norhana MN, A. MNA, Poole SE, Deeth HC, Dykes GA. Effects of bilimbi (Averrhoa bilimbi L.) and tamarind (Tamarindus indica L.) juice on Listeria monocytogenes Scott A and *Salmonella typhimurium* ATCC 14028 and the sensory properties of raw shrimps. Int J Food Microbiol [Internet]. 2009;136(1):88–94. doi:10.1016/j.ijfoodmicro.2009.09.011
48. Jones S. Permeability rules for antibiotic design. Nat Biotechnol [Internet]. 2017;35(7):639. doi:10.1038/nbt.3919
49. Kenny O, Smyth TJ, Hewage CM, Brunton NP. Antioxidant properties and quantitative UPLC-MS analysis of phenolic compounds from extracts of fenugreek (Trigonella foenum- graecum) seeds and bitter melon (*Momordica charantia*) fruit. Food Chem [Internet]. 2013;141(4):4295–302. doi:10.1016/j.foodchem.2013.07.016
50. Njeru SN, Obonyo MA, Nyambati SO, Ngari SM. Antimicrobial and cytotoxicity properties of the crude extracts and fractions of Premna resinosa (Hochst.) Schauer (Compositae): Kenyan traditional medicinal plant. BMC Complement Altern Med 2015;15(1):1–9. doi: 10.1186/s12906-015-0811-4