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Antimicrobial Effects of *Peperomia Pellucida* (Ketumpang Air) Against Multidrug Resistant and Foodborne Organisms Including Toxicity Study in Sprague Dawley Rats

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Abstract

The objective of this study was to evaluate the *in vitro* antibacterial activities of methanol and aqueous extracts of *P. pellucida* aerial part (PPAP) against four multi-drug resistant organisms; methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococcus, extended-spectrum beta-lactamase and carbapenem-resistant Enterobacteriaceae and four foodborne pathogens; *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium*. The antibacterial potentialities of the plant extracts were evaluated at 250 mg/ml and 500 mg/ml. Only susceptible bacteria were further determined for minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations. The best extract of a single dose of 5000 mg/kg PPAP methanol extract was acutely tested on female rats by adapting the OECD guidelines No 425. Findings obtained indicated that only PPAP methanol extract was found to be a potent inhibitor towards *Bacillus cereus* with the MIC and MBC values at 3.91 mg/ml and 7.81 mg/ml respectively. Toxicity study revealed that there was neither mortality nor morbidity and absent of abnormalities on all rats examined.

Keywords: Peperomia pellucida, antibacterial, multi-drug resistant, foodborne pathogens, acute toxicity

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Introduction

The current problem of emerging multidrug resistant organisms (MDROs) is posing a global medical threat and is continually challenging the scientific community [1]. This is because, the conditions where these bacteria could resist multiple antibiotics started becoming increasingly common, leading to an increase in morbidity, mortality and cost of health care. It is estimated that 25,000 patients died each year in European Union countries from the infections caused by MDROs and had caused 1.5 billion euro spent for treatment purposes [2]. In other parts of the world, the United States recorded 26,000 deaths whereas approximately 96,000 deaths were reported in the Southern Asia region [3]. In the meantime, the outbreak of foodborne disease has become a primary concern to the developing countries all over the globe including Malaysia [4]. Over millions of years, plants have co-evolved with pathogens which warrant them to develop defense mechanisms against pathogenic bacteria. Therefore, it is rational to foresee the potential of antibacterial activities exists within a local herb.

Peperomia pellucida is locally known as a shiny bush, slate pencil plant or rat's ear in English or 'ketumpang air' among Malaysian. This plant is commonly ingested raw as a salad because of its crispiness like carrot stick and celery [5]. *P. pellucida* has also become herb of choice among old folks due to its pivotal role in providing cure for various types of illnesses such as fever, headache, eczema, abdominal pain while the aerial parts can be utilized for wound dressing. In addition, this plant also has been broadly tested for its antibacterial properties against various clinical strains of bacteria and fungal [6-9].

The use of herbal medicines products is generally safe and effective with less reported toxicity [10]. However, it is not surprised that many plants contain potentially poisonous compounds that are highly toxic to human and animals when either acutely or sub-chronically administered [11]. In this regard, *P. pellucida* is not an exception and should not be left ignored. This is because the methanol, hexane and ethyl acetate fractions of this plant were reported to be toxic in the *in vitro* experiment with its median lethal concentration (LC_{50}) was less than 1000 mg/ml [6].

Although there are numerous researches concerning the vast pharmacological activities of *P. pellucida*, none of the studies has ever been performed specifically on antibacterial activities of this plant against MDROs and foodborne pathogens as well as *in vivo* acute toxicity profile. The present study was aimed to evaluate both aqueous and methanol extracts of PPAP for its antibacterial properties specifically against four multidrug-resistant organisms and four common foodborne pathogens. Further, the experiment was proceeded to investigate the effects of a single dose administration of the best extract on female Sprague Dawley rats via acute toxicity study.

MATERIALS AND METHODS

Plant materials

Approximately five kilograms of whole fresh *P. pellucida* were collected from shady areas around the School of Health Sciences, Universiti Sains Malaysia (USM), Health Campus, Kubang Kerian, Kelantan, Malaysia. The whole plant was authenticated by Dr. Rahmad Zakaria with voucher specimen no: 11737 and deposited at the herbarium in the Department of Botany, School of Biological Sciences, USM, George Town, Penang, Malaysia.

Plant extraction

The preparation of both aqueous and methanol extracts of PPAP were conducted in the Extraction Room, Pharmacology Laboratory, Department of Pharmacology, School of Medical Sciences, USM, Health Campus, Kelantan. The fresh aerial parts were obtained by separating the roots from the whole plant. The samples were cleaned and washed thoroughly with running tap water to remove soils and impurities. The plants were subsequently divided into two portions for aqueous and methanol extractions as described below.

Preparation of aqueous extract

The preparation of the aqueous extract of PPAP was conducted according to Bazylko *et al*., [12] with slight modifications. In this current study, the fresh juice of PPAP was obtained by filtrating the blended aerial parts with distilled water in the ratio of 6:1 using an electronic blender. The juice was then subjected to pre-frozen at -20ºC for 48 hours before being lyophilised on the next day using a laboratory freeze-dryer for 72 hours. The crude aqueous extract was weighed using digital analytical balance and stored in an air tight container placed in a desiccator until further use.

Preparation of methanol extract

The preparation of methanol extract of PPAP was performed according to Mutee *et al*.,[13]. The PPAP was dried in an incubator at 45ºC for 72 hours to remove all water content. The dried plant materials were subsequently ground into a fine powder using a laboratory dry grinder and filtered using a stainless-steel sieve. The plant powder of 150g was weighed and dissolved in 1.5 litres of absolute methanol. Sequentially, the solution was macerated with continuous agitation using an orbital shaker for 72 hours. After that, the solution was filtered using Whatmann filter paper No 1. The filtered solution was then placed into an aluminium wrapped Schott bottle to minimize the evaporation of phytochemicals. The solution was subsequently going through solvent removal via a rotary evaporator at 42- 45ºC until a percentage yield of extracts ranged from 9-11% w/w was obtained. The dried extract was weighed using analytical balance and stored in a 4ºC chiller until further use.

***In vitro* antibacterial activities study**

Tested microorganisms

Antibacterial activities of both aqueous and methanol extracts of PPAP were investigated against MDROs which consist of two gram positive; methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) and two gram negative; extended-spectrum beta-lactamase (ESBL) and carbapanem-resistant *Enterobactericeae* (CRE). The four selected common foodborne strains comprising of two-gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two-gram negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) were further tested following the first experiment on MDROs.

All bacteria were acquired from the ATCC bacterial strains in the Culture Room, Microbiology Laboratory, School of Medical Sciences, USM, Health Campus, Kelantan, Malaysia. Stock cultures of all MDROs were grown on the blood agar whereby stock cultures of foodborne pathogens were grown on the nutrient agar by incubating them overnight for 16-18 hours at 37ºC. All bacteria strains were maintained only for three days at 4ºC before sub-cultured again on a new agar media afterwards. Only pure bacterial colonies were selected and cultured in broth/normal saline with a standard concentration of 0.5 Mc Farland. All bacteria were identified and confirmed by conventional microbiology procedures i.e bacterial colony morphology, gram staining procedures and biochemical tests for gram positive/negative organisms.

Antimicrobial Susceptibility Testing

PPAP extracts discs with concentrations of 250 mg/ml (5 mg/disc) and 500 mg/ml (10 mg/disc) and negative control discs were used for antimicrobial susceptibility testing. For positive controls, standard antibiotics mainly consisted of vancomycin (30 µg/disc), teicoplanin (30 µg/disc), meropenem (10 µg/disc) and polymyxin B (300 µg/disc) were dispensed for MRSA, VRE, ESBL and CRE respectively. Standard antibiotic discs consisted of tetracycline (30 µg/disc) was dispensed for *S. aureus* whereas gentamycin (10 µg/disc) was allocated for other remaining three bacteria strains (*B. cereus, E. coli and S. typhimurium*). All discs were stored at 4ºC prior to Kirby-bauer disk diffusion susceptibility protocols. Initially, 6 mm sterile blank antimicrobial susceptibility discs were placed individually on the complete labelled sterile plastic petri dish with date and types of discs (PPAP extracts or solvents). Further, 20 µl of sterilised PPAP extracts and solvents were impregnated to individual disk according to the designated petri dish. All petri dishes were placed at room temperature for maximum of one hour to allow absorption before being put in the 4ºC chiller prior to use.

Minimum Inhibitory Concentration (MIC)

Only bacteria that showed susceptibility which indicated by the presence of zone of inhibition around the discs was further used in MIC test. A sterile 96-well microtitre plate was contained with 100 µl of two-fold serially diluted PPAP extracts in a Mueller Hinton Broth (MHB). Bacterial suspension of 20µl was mixed into each well. In order to ensure the accuracy of the method performed, positive controls and negative control were applied on the same plate. The 96 well plates were sealed with parafilm and wrapped with aluminium foil to avoid dehydration prior to incubation at 37ºC for 18-24 hours. Each test was done in triplicates. The determination of MIC value was conducted by selecting the lowest concentration of the PPAP extract that results in an optically clear microtitre plate well.

Minimum Bactericidal Concentration (MBC)

MBC was determined following MIC test as it was defined as the lowest concentration of extract that did not permit any bacterial growth. MBC was determined from broth microdilution assays by sub-culturing 10 µl from each clear well of the previous MIC and eventually spotted and streaked onto the MHA. The MHA plates were then incubated overnight at 37ºC for 18-24 hours.

Statistical analysis

The numerical data were analyzed using SPSS version 24.0. In *in vitro* antibacterial study of PPAP extracts, all zones of inhibition from triplicate samples were measured at least three times. The diameters of inhibition were analyzed using one-way analysis of variance (ANOVA) and followed by Bonferroni post-hoc analysis. Values were considered statistically significant when $p<0.05$.

***In vivo* acute toxicity study of PPAP methanol extract in Sprague Dawley rats**

Dosage preparation

Based on the findings of *in vitro* antibacterial activities, the animal study was conducted using methanol extract of PPAP. The oral dosage form of this extract was prepared by dissolving in 2% of carboxymethyl cellulose (CMC) sodium salt. The PPAP methanol extract was weighed based on the average body weight of rats and reconstituted with 2% of CMC sodium salt solution to a fixed volume of 1.5 ml. It was further homogenized with a homogenizer at 24,000 rotations per minute until the mixture became uniform. The prepared dose was stored at -20ºC prior to use.

Selection of animals

The procedures and protocols involved in animal study were approved by the USM Animal Ethics Committee (USM/ IACUC/ 2017/(107)(861). Five female Sprague Dawley rats, 10-12 weeks old, weighing 180-200g were acquired from the Animal Research and Service Center (ARASC), USM, Health Campus, Kelantan, Malaysia.

All animals were placed individually in polypropylene cages and kept in the ARASC holding room for a week prior to the experiment to allow acclimatization. They were maintained in a standard environmental condition at 20±3ºC with humidity of 60-70% and 12h:12h light-dark cycle (lights on from 0700 to 1900 hours). The animals were provided with standard rat pellet (Atromin, Germany) and *ad-libitum* access to tap water. Only rats within acceptable range of age and weight were included, while those presented with any abnormality were excluded from this study. The handling and use of the animals were in accordance with the institutional guidelines.

Experimental protocol

The study was performed according to the Organization for Economic and Co-operation and Development (OECD) guidelines No. 425 [14]. A maximum of five female rats were used in order to test the selected limit dose of 5000 mg/kg body weight of PPAP methanol extract. Prior to dose administration, food was withheld overnight, but with continuous supply of water and followed by measuring the body weight of rats. At 9.00 am, the extract at the single dose of 5000 mg/kg with a volume of 1.5 ml was orally administered via oral gavage to one rat at the beginning. The rat was left with careful monitoring for 24 hours. In the following day, when the first rat had survived, four additional animals were subsequently treated with the same dose of PPAP methanol extract. Following each dosing, all female rats were closely observed for mortality, morbidity or moribundity during the first 30 to 40 minutes, periodically during the first 24 hours and daily thereafter, for a total of 14 days. General observation and behavioral changes of animals were also monitored at the same time every morning. Particularly, all rats were examined for any sign of toxicity such as changes in skin, fur, eyes, mucous membranes, abnormal respiratory or locomotor progression, presence of tremor, convulsion, salivation, diarrhea, lethargy and vaginal bleeding. All observations, including the time of onset at which signs of toxicity appeared and length of recovery period were noted throughout the study period. Additionally, the changes in body weight and food consumptions were recorded for all animals.

Termination of animals

Prior to the termination of animals, food was withheld overnight but with *ad libitum* supplies of water. On the next day (day 15), the body weights of all survived rats were measured. Started from 9.00am, the rats were humanely anaesthetized via the intraperitoneal route with 100 mg/kg of sodium pentobarbital. The injection procedure was performed at the lower right quadrant of the rat's abdomen. Precaution steps were taken to avoid all the interval organs such as liver, bladder or caecum. The rat was confirmed unconscious once it did not respond to any stimuli; no jerking movement when the sole of its feet was pinched. Further, they were closely observed for the presence of abnormalities on their coat condition and excretory production. The external organs such as eyes, nose, mouth, ears and orifices were also checked and noted for the natural color, any presence of blood or abnormal mucous secretion prior to laparotomy.

Gross necropsy

Upon abdominal dissection, all animals were euthanized immediately by aortic exsanguinations prior to detailed macroscopical observation of the visceral organs. The organs including the heart, lung, liver, spleen, stomach, adrenal glands, kidneys, ovaries, fallopian tubes, uterus and intestines were removed, trimmed from any adherent tissues and fats prior to measurement of absolute organ weight.

RESULTS AND DISCUSSION

In recent years, there are escalations in the number of published articles reporting on the successful antimicrobial activities on variety of medicinal plants against MDROs such as MRSA, VRE, ESBL and CRE [15,16]. Despite that, our present study is the first to show the antibacterial potential of PPAP extracts against these four MDROs (Table 1 and 2). Even though our findings differ as compared to other tested plants, the weak antibacterial activities of PPAP in this study might be influenced by numerous factors including a strong defense

mechanism within MDROs, methods of extraction and types of solvents used [17].

Pertinent to the current work, we postulated that the weak antibacterial activities posed by PPAP extracts might be caused by the over-expression of MDR efflux pumps [18]. As reflected in this group of bacteria, resistant occurred when these pumps double the bacterial cell wall protection by eliminating any foreign substances (phytochemicals in our study) [18]. Coupled to the extreme defense mechanism, the presence of double membrane barrier especially in gram negative bacteria might elevate the resistance towards the PPAP plant extracts [19].

Findings of the current investigation also showed that there were no antibacterial activities of the aqueous extract of PPAP against all the four tested MDROs and foodborne pathogens (Table 1 and 3).

We found that *B. cereus* has exhibited strong antibacterial activities in a concentration-dependent manner as compared to other three bacterial strains against PPAP methanol extract (Table 4). This result was elucidated by the presence of the zone of inhibitions i.e. 6.93±0.29 mm and 7.90±0.37 mm around 250 mg/ml and 500 mg/ml methanol extract discs respectively (Fig. 1). In contrast, the weak antibacterial activities of PPAP extracts against *S. aureus*, *E. coli* and *S. typhimurium* however were in agreement with the data reported [20]. Our findings also showed absent of zone of inhibition was reported for *S. aureus*, *E. coli* and *S. typhimurium* of methanol extract of *P. pellucida* up to 100 mg/ml.

The determination of MIC was based on the potential of PPAP extract in exhibiting antimicrobial activities towards the tested bacteria. In our study, only methanol extract of PPAP was effective against *B. cereus* and thus it was employed to determine the MIC value. Based on the result obtained, the minimum concentration of PPAP methanol extract that successfully inhibited the growth of *B. cereus* was at 3.91 mg/ml. In contrast to the current finding, Wei *et al.* [17] have reported different MIC values for methanol extract of PPAP ranged from 31.25 to 125 mg/l. These ranges of values have shown to inhibit the growth of *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae*. The distinct of the MIC values achieved can be explained by the different types of solvent used during the extraction process of *P. pellucida*. This evidence has shown that 70% of methanol extract of this plant has ability to inhibit the growth of varieties of bacteria in much lower concentration instead of absolute methanol as applied in this current study [21].

Following MIC, the MBC value was determined. MBC can be defined as the ability of the plant extract to kill more than 99.9% of the inoculum as compared to an initial determination of viability in MIC [22]. The MBC value was indicated by the last well that showed an absence of growth on the Mueller Hinton agar plate. Since the growth of the bacteria was visible from the inoculation taken from well 8, thus the minimum concentration of methanol extract of PPAP required to kill *B. cereus* is at 7.81 mg/ml (Fig. 2). Therefore, this study has shown strong antibacterial activities of PPAP methanol extract was detected against *B. cereus*.

Kotiranta *et al*. [23] had reported that the foodborne disease outbreak attributed to *B. cereus* colonization occurs approximately 68% in inadequate heat and short cooking duration of rice dishes. This mainly due to the unique characteristics of the spores composed of these bacteria that is resistant to harsh conditions; heating, freezing, drying and radiation [23]. Meanwhile, the risk estimation of *B. cereus* was predicted to be high particularly to Malaysia people as rice is the main staple food in this country. Therefore, our findings may serve as an alternative strategy to combat *B. cereus* within foods.

The methanol extract of PPAP was proceeded for *in vivo* acute toxicity study since it had exhibited significant antibacterial activities against *B. cereus*. As being commonly used solvent system for extraction of bioactive phytochemicals, methanol was effective in this study because it could extract various polar compounds and certain groups of non-polar compounds too [24]. Alkaloids, flavonoids, cardiac glycosides, saponins, tannins [25] and terpenes [26] which were extracted using alcoholic solvents, are the examples of phytochemicals presence in *P. pellucida*, that are responsible for its therapeutic values. Thus, the antibacterial properties possessed by PPAP methanol extract but not in its aqueous extract in this present study might be due to the presence of terpenes (sesquiterpene oxide) [26].

In accordance with OECD Guidelines No. 425, the toxicity and safety of the PPAP methanol extract was evaluated via 14 days single oral limit test at 5000 mg/kg body weight. The limit test at the highest attainable dose can only be conducted when a substance likely to have lower toxicity [14]. To date, none of the *in vivo* acute toxicity study has been reported utilizing this high limit dose of methanol extract of PPAP in female Sprague Dawley rats. The utilization of single-sex of the experimental animal may help in reducing variability to the test population [27]. Additionally, female is more vulnerable and sensitive to the effects of chemicals [14]. The Sprague Dawley rat was chosen as animal surrogate as this strain is one of the most common, utilized laboratory animals worldwide due to relative easiness in breeding, handling and consistency in the reactions produced towards tested substances [28]. This strain has genetic stability, resistance to common laboratory diseases and less susceptible to stress [29].

Several parameters involved in the assessment of possible toxicity in the females comprising of mortality and morbidity counts, general health and behavioral status, body weight, food consumption, gross examination, absolute organ weight and LD_{50}. These parameters were relevant in acute toxicological experiment as to evaluate the interaction between the highest dose of PPAP methanol extract with only five female rats studied. Even though only a small quantity of animals was used in this study, the sequential procedures in this test permit separate decision for each animal.

Since the major endpoint for this test is death, no control group was acquired throughout this study. All five female SD rats survived until the end of the observation period. No treatment-related morbidity and mortality was noted in rats at the limit dose of 5000 mg/ml of PPAP methanol extract.

Within 24 hours post administration of PPAP extract, only one rat out of total five females showed lethargy and sleepy behavior. This condition may be explained by excessive struggling of the rat due to refusal to cooperate during oral gavage. However, the respective rat exhibited better improvement and becoming more active started the next day onwards.
Throughout the 14 days of observation phase, there were no abnormalities with regards to the skin, fur, eye color, nasal discharge, locomotor progression and respiration. Even so, at the second week of observation, 60% of rats displayed slightly abnormal fecal excretion with little mucoid in texture but still retained their normal stool shape. Tannin presence in this plant might be accountable for the presence of minor loose stools as it has potential to interrupt the protein digestibility among rats [30]. Additionally, their body weight increased normally which indicated a single high dose of PPAP methanol extract did not perturb the progression of normal health of these rats.
Damage to the target organ serves as one of the main indicators of toxicity effects after the rat has been exposed to the toxic substances. Consequently, this would result in an abnormal increase (swelling) or decrease in the organ weight [31]. As related to the present study, however, all organs appeared to be in normal shapes, sizes, positions and colors during the gross necropsy. In fact, all visceral organs showed uniformity of weight among each other. Taken all together, the PPAP methanol extract at 5000 mg/kg did not cause any obvious adverse effects on the female rats. Based on our findings, the LD_{50} of methanol extract of PPAP is estimated more than 5000 mg/kg body weight.

CONCLUSIONS

The present series of antibacterial studies suggest that methanol extract of PPAP has demonstrated zone of inhibition in a concentration-dependent manner against *B. cereus* and was able to inhibit and kill these bacteria at a minimum value of 3.91 mg/ml and 7.81 mg/ml respectively. Therefore, the PPAP methanol extract would be a favorable source to inhibit *B. cereus* growth in foods. However, this plant extract only displayed weak antibacterial activities against other tested multi-drug resistant organisms and foodborne pathogens. Our toxicity findings proposed that the LD_{50} of the methanol extract of PPAP is estimated more than 5000 mg/kg body weight. Therefore, this plant extract can be classified into category 5 or as a 'low toxic substance' according to Globally Harmonised System (GHS) [14].

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Table 1 The antibacterial activities of aqueous extract of PPAP against four tested MDRO

	Zones of inhibition (mm)* Mean±SEM						
Types of discs	**250 mg/ml**	**500 mg/ml**	**Distilled water**	**VA**	**TEC**	**MEM**	**PB**
MRSA	-	-	-	17.80±0.12	NA	NA	NA
VRE	-	-	-	NA	8.87±0.09	NA	NA
ESBL	-	-	-	NA	NA	29.80±0.12	NA
CRE	-	-	-	NA	NA	NA	8.90±0.10

Antibiotic used (µg/disc): VA=Vancomycin (30), TEC=Teicoplanin (30), MEM=Meropenem(10), PB= Polymyxin B (300).
The concentration of each plant extract was 5mg/disc for 250mg/ml and 10mg/disc for 500mg/ml.
Data are expressed as mean±SEM (n=3 for each type of disc).
*= Inhibition zones include the diameter of the disc (6mm).
- = no antibacterial activity.
NA=Not applicable.

Table 2 The antibacterial activities of methanol extract of PPAP against four tested MDROs

	Zones of inhibition (mm)* Mean±SEM						
Types of discs	**250 mg/ml**	**500 mg/ml**	**Distilled water**	**VA**	**TEC**	**MEM**	**PB**
MRSA	-	-	-	16.80±0.10	NA	NA	NA
VRE	-	-	-	NA	8.50±0.25	NA	NA
ESBL	-	-	-	NA	NA	29.80±0.12	NA
CRE	-	-	-	NA	NA	NA	10.77±0.12

Antibiotic used (µg/disc): VA=Vancomycin (30), TEC=Teicoplanin (30), MEM=Meropenem(10),
PB= Polymyxin B (300). The concentration of each plant extract was 5mg/disc for 250mg/ml and 10mg/disc for 500mg/ml.
Data are expressed as mean±SEM (n=3 for each type of disc).
*= Inhibition zones include the diameter of the disc (6mm).
- = no antibacterial activity.
NA= Not applicable.

Table 3 The antibacterial activities of the aqueous extract of PPAP against four tested foodborne pathogens

	Zones of inhibition (mm)* Mean±SEM				
Types of discs	**250mg/ml**	**500mg/ml**	**Distilled water**	**TE**	**GEN**
S. aureus	-	-	-	27.77±0.12	NA
B. cereus	-	-	-	NA	22.93±0.07
E. coli	-	-	-	NA	19.77±0.09
S. typhi	-	-	-	NA	20.87±0.09

Antibiotic used (µg/disk) : TE=Tetracycline (30) and GEN= Gentamycin (10).
The concentration of each plant extract was 5mg/disc for 250mg/ml and 10mg/disc for 500mg/ml. Data are expressed as mean±SEM (n=3 for each type of disc).
*= Inhibition zones include the diameter of the disc (6mm).
- = No antibacterial activity.
NA= Not applicable.

Table 4 The antibacterial activities of the methanol extract of PPAP against four tested foodborne pathogens

	Zones of inhibition (mm)* Mean±SEM				
Types of discs	250mg/ml	500mg/ml	10% DMSO	TE	GEN
S. aureus	-	-	-	27.77±0.12	NA
B. cereus	6.93±0.29[a]	7.9±0.37 [b]	-	NA	25.80±0.06
E. coli	-	-	-	NA	20.83±0.09
S. typhi	-	-	-	NA	22.80±0.12

Antibiotic used (µg/disk): TE=Tetracycline (30) and GEN= Gentamycin (10).
The concentration of each plant extract was 5mg/disc for 250mg/ml and 10mg/disc for 500mg/ml. Data are expressed as mean±SEM (n=3 for each type of disc).
*= Inhibition zones include the diameter of the disc (6mm).
[a]= Statistically significant ($p<0.05$) when compared 250mg/ml of PPAP extracts to the positive control group (Gentamycin).
[b]= Statistically significant ($p<0.05$) when compared 500mg/ml of PPAP extracts to the positive control group (Gentamycin).
- = No antibacterial activity.
NA= Not applicable.

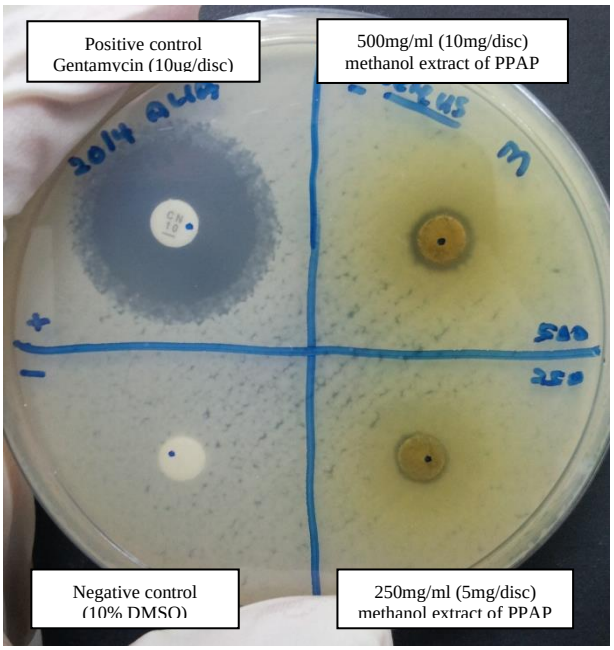

Fig. 1 Zone of inhibition of PPAP methanol extract against *B. cereus*

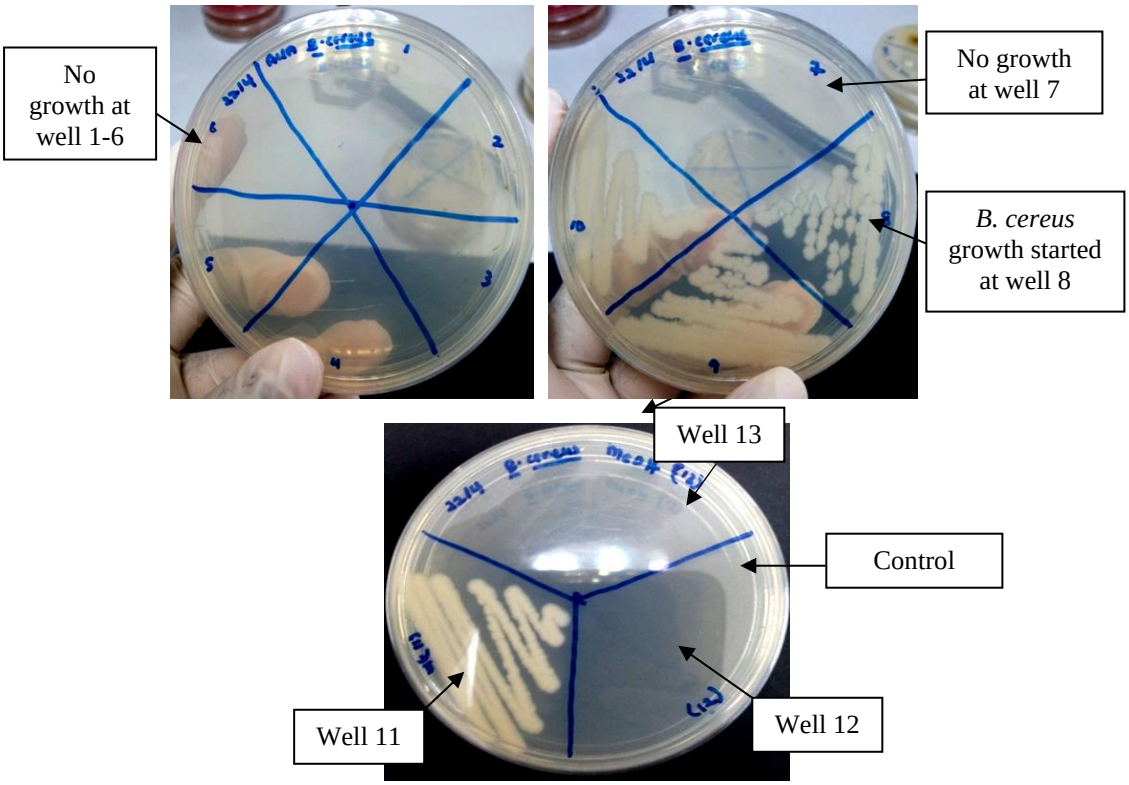

Fig. 2 The determination of MBC value of PPAP methanol extract against *B. cereus*