International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2021; 9(2): 34-37
Received: 29-11-2020
Accepted: 08-01-2021

Syifa Nurisma Putri
Faculty of Medicine, Diponegoro University, Prof. H. Soedarto, S.H. Street, Tembalang, Semarang, Central Java, Indonesia

Helmia Farida
Department of Microbiology, Faculty of Medicine, Diponegoro University, Prof. H. Soedarto, S.H. Street, Tembalang, Semarang,Central Java, Indonesia

Winarto
Department of Microbiology, Faculty of Medicine, Diponegoro University, Prof. H. Soedarto, S.H. Street, Tembalang, Semarang,Central Java, Indonesia

Tri Nur Kristina
Department of Microbiology, Faculty of Medicine, Diponegoro University, Prof. H. Soedarto, S.H. Street, Tembalang, Semarang,Central Java, Indonesia

Corresponding Author:
Syifa Nurisma Putri
Faculty of Medicine, Diponegoro University, Prof. H. Soedarto, S.H. Street, Tembalang, Semarang, Central Java, Indonesia

Antibacterial effect of bitter melon extract (*Momordica charantia*) against *Escherichia coli* and *Pseudomonas aeruginosa*

Syifa Nurisma Putri, Helmia Farida, Winarto and Tri Nur Kristina

Abstract
Escherichia coli and *Pseudomonas aeruginosa* are gram-negative bacteria that often cause infectious diseases in humans. Pharmacological therapy to overcome infection by these two species needs to be developed by utilizing natural ingredients, such as bitter melon fruit. This study aims to examine the antibacterial effects of bitter melon extract (BME) on *E.coli* and *P. aeruginosa* by *in vitro* method. *In vitro* experimental research with post test only control *group*design was carried out using 5 groups, namely BME with gradual concentrations (100%, 50%, 25%, 12.5%, 0%) inoculated with bacteria and 1 positive control (K+) of gentamicin 16 µg/mL. Minimum Inhibitory Concentration (MIC) was assessed by comparing the clarity of the treatment solution with K+ and measuring the inhibitor zone diameter (IZD). Minimum bacterial concentration (MBC) was tested by culturing the treatment solution on solid media to see the resulted sterility. The inhibition of *E.coli* growth by BME 50% and 100% was not significantly different from K+ (p=1,000) and the IZD of BME50% for *E.coli* was 11 mm. The MBC of BME for *E.coli*was not achieved by BME 12.5%-100%. The inhibitory growth of *P. aeruginosa* by BME 25% to 100% was not significantly different from K+ (p=1,000) and the IZD of BME 25% for *P. aeruginosa* was 13 mm. The MBC of *P. aeruginosa* was not achieved by BMEin all concentrations. BMEwas bacteriostatic against *P. aeruginosa* with MIC 25% and probably bacteriostatic for*E.coli*with MIC 50%. The higher BME concentration gives gretaer IZD results.

Keywords: Bitter melon extract, antibacterial, *Escherichia coli*, *Pseudomonas aeruginosa*, *in vitro.*

1. Introduction
Escherichia coli (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are common microorganisms causing infection. Various clinical manifestations can result from the infection of the two bacteria. *E. coli* may cause gastrointestinal infection, diarrhea, urinary tract infection, neonatal meningitis and sepsis. *P. aeruginosa* is the bacterium that commonly causes nosocomial infection. Clinical manifestations of the nosocomial infection may vary, as blue-green pus around wound infection or burn wound, urinary tract infection after catheter administration, ventilator-associated pneumonia, post-lumbar puncture meningitis, and sepsis[1-2]. Therapy to overcome the infection of the two bacteria needs to be considered. Until nowadays beta-lactam antibiotics are the common drugs to treat various infections. Today there is resistance to these antibiotics that result from the activity of beta lactamase enzymes that causes the emergence of *extended beta lactamase* (ESBL) bacteria. Previous studies have shown that *E. coli* are resistant toamoxicillin (96%), ceftriaxone (70.8%), ciprofloxacin (52%), and ampicillin (100%).The resistance *of P. aeruginosa* to various antibiotics also increases. Studies show that there is increasing level of resistance of *P. aeruginosa* to cefepime, ceftriaxone, and cefoperazone. Hence, the development of drugs to overcome the resistance problem to these bacteria is necessary [1–3]. The stewardship to develop antibiotics can be done by exploiting the potential local plants. This is considered because the materials are easily obtained with affordable price. The community has also known the use of local plants as traditional medicine. One of the local plants that are believed to provide antibacterial effect is bitter melon fruit. Many studies have tried to prove the antimicrobial effect of bitter melon fruit extract against various species of bacteria including *E.coli* and *P.aeruginosa* [4–6]. However, they have not provided information on how antimicrobial effects are shown, because these studies only show inhibitory zones on bacterial culture media, without information on how effective is the antimicrobial effect of the extract.Due to the absence of standard of minimal inhibitory zone for bitter melon extract to be said to have an antimicrobial effect, research about MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) of bitter melon extract (*Momordica charantia*) against *E. coli* and *P.*

aeruginosa and its relationship with the inhibitory zones needs to be carried out to prove the presence of antimicrobial effects of bitter melon extract on the two bacteria.

2. Material and Method

2.1. Research Design and Variable

This study is a true experimental study with a posttest only control group design on the test of antibacterial effectiveness. The treatment is by giving bitter melon fruit extract with multilevel concentrations (100%, 50%, 25%. 12.5% and 0%) with gentamicin 16µg /ml as a positive control. The outcome is an antibacterial effect that can be seen from the minimum inhibitory concentration, the minimum bactericidal concentration and the diameter of the inhibitory zone. The study was conducted by observing the results of the posttest then compared to controls.

2.2 Sample

The sample of this study was uncontaminated bitter melon extract from bitter melon fruit which was obtained from namely Merapi Plantations. The fruit is extracted in the Integrated Laboratory, Diponegoro University, which is then subjected to phytochemical test. The number of research sample is calculated using Federer formula so it results with five times repetition. Extract samples were divided into two groups, extract inoculated with *E. coli* and extract inoculated with *P. aeruginosa*.

2.3 Tools and materials

Extraction: Basin, oven, ethanol 96%, container, water bath, analytical balance, volumetric flask, aquadest

MIC, MBC, and Inhibition Zone Diameter Measurement: Reaction tubes and shelves, BHI, NaCl, Bunsen, inoculating loop, LAF, autoclave, petri dish, nutrient agar, erlenmeyer, measuring cylinder, baker glass, cork borer, sterile swab.

Procedure

Extraction: The extract is made by weighing 2 kg of washed bitter melon and cut into pieces. Bitter melon is then dried in the oven for a few days. Dry bitter melon is immersed in 96% ethanol for a full day, and then the ethanol bath is replaced the next day until there are no phytochemically active substances in the ethanol bath. It is then heated using a water bath to form a thickening solution similar to cotton candy. Multilevel concentrations of the extract are made by dissolving the extract with distilled water serially [9]. The MIC of the extract of bitter melon fruit against both bacteria is known by conducting dilution and diffusion tests. MBC is obtained by conducting a sterilization test of the pour plate technique from a sample which gives a clear picture of the MIC dilution test.

Data analysis included correlation test and comparison test of the treated group to the control group. The data distribution normality test was carried out by the Saphiro Wilk test because the sample was less than 50. The correlation test of MIC and MBC of bitter melon extract with positive control of the dilution method used the Chi-square test. The inhibition zone difference test of the treatment group compared to the control group used the One-Way ANOVA test for normal data distribution and the Kruskal Wallis test for abnormal data distribution.

3. Result

The MIC of bitter melon extract against E. coli showed clear samples at concentrations of 50% and 100%. Extract of concentrations 25% and 12.5% yielded fully turbid samples. It can be concluded that the MIC of bitter melon extract against E. coli is the concentration of 50%. The of bitter melon extract against *P. aeruginosa* is the concentration of 25%. This is showed by the results of a clear sample in the concentrations of 25% to 100%. The sample with the extract of concentration 12.5% was fully turbid.

The samples that showed clear results were then tested for sterility to determine the MBC of bitter melon extract against the two bacteria. It results that all samples show bacterial growth. Hence, all level of concentrations of bitter melon extract has not shown MBC on the two bacteria.

The MIC of bitter melon extract against E.coli and P.aeruginosa is also investigated by the diffusion method to determine the standard diameter of the inhibitory zone of bitter melon extract against the two bacteria. Diameters of inhibition zone to E. coli are formed at the concentrations of 50% and 100%. The diameter of the smallest inhibition zone with a concentration of 50% is 8.83 mm. The inhibition of *P.aeruginosa* is shown at the concentrations of 12.5% to 100%. The diameter of the smallest inhibition zone with a concentration of 12.5% is 8.81 mm. Data analysis of the measurement of the inhibition zone diameters of the two bacteria showed significant differences compared to positive control. It is continued with a correlation test of the extract concentration group with inhibition zone diameters. Based on the test it can be concluded that there is a strong correlation between the level of concentrations of the bitter melon extracts with inhibition zone diameters.

Table 1: The Result of the Measurement of the IZD of BME against *E.coli*

Variabel	Inhibition Zone Diameters *E.coli* (mm)					Mean ± SD	$p^{\ddagger}$
	I	II	III	IV	V		
Control (+)	22,18	21,35	21,52	21,23	21,22	21,50 ± 0,40	<0,001*
Control(-)	0,00	0,00	0,00	0,00	0,00	0,00 ± 0,00	
BME 100%	12,12	11,85	12,10	12,00	12,22	12,06 ± 0,14	
BME 50%	10,55	8,83	10,75	10,21	9,87	10,04 ± 0,76	
BME 25%	0,00	0,00	0,00	0,00	0,00	0,00 ± 0,00	
BME 12,5%	0,00	0,00	0,00	0,00	0,00	0,00 ± 0,00	

Table 2: The Result of the Measurement of the IZD of BME against *P.aeruginosa*

Variabel	Inhibition Zone Diameters *P.aeruginosa* (mm)					Mean ± SD	Median (min – max)	*p*
	I	II	III	IV	V			
Control (+)	22,15	22,23	21,52	21,71	21,81	21,88 ± 0,30	21,81 (21,52 – 22,23)	<0,001*
Control (-) (EBP 0%)	0,00	0,00	0,00	0,00	0,00	0,00 ± 0,00	0 (0 – 0)	
BME 100%	19,52	15,22	18,82	18,52	18,81	18,18 ± 1,69	18,81 (15,22 – 19,52)	
BME 50%	16,15	13,82	17,15	15,87	16,23	15,84 ± 1,23	16,15 (13,82 – 17,15)	
BME 25%	12,17	11,27	12,84	12,56	12,45	12,26 ± 0,60	12,45 (11,27 – 12,84)	
BME 12,5%	8,81	10,97	10,58	10,82	10,97	10,43 ± 0,92	10,82 (8,81 – 10,97)	

4. Discussion

The aim of this study was to measure the antibacterial effect of bitter melon extract (*Momordica charantia)* on *E.coli* and*P.aeruginosa* from laboratory experimental test. The given treatment were giving 4 stages of bitter melon extract concentration and the outcome was antibacterial effect measured from MIC and MBC. In this study, MIC was measured with dilution an diffusion method.

Bitter melon extract (BME) was gained from maceration extraction method and phytochemical content test which then showed a postivite result of flavonid and tanin content.

Extraction process and phytochemical test was done in Central of Biomedical Research, Diponegoro University.

4.1. MIC of BME against *E.coli* and*P.aeruginosa*

This study concluded that MIC of BME on *E. coli* and *P. Aureginosa* was 50% and 25% concentration respectively. The result of this study was consistent with prior studies that shown BME antibacterial effect particularly on *E. coli* and *P. Aureginosa.*[12,13]

MIC test with diffusion method was also provided information about minimal inhibitory growth zone diameter by BME was at least 11 mm and 13 mm for *E. coli* and *P. Aureginosa* respectively to be considered with significant antibacterial effect on these species. This finding could be a reference for future studies.

Saeed *et al*. study shown significant effect from flesh and skin of Momordica charantia in inhibited the growth of 11 species of gram negative bacteria including *E.coli* dan *P.aeruginosa* [10].

Prior studies shown antibacterial effect of BME from its phytochemical content such as tannin, flavonid and saponin. In this study, BME extracted with maceration method shown tannin and flavonoid as its active components. Tannin inhibited protein synthesis making up cell walls by forming covalent cross ties on several functional organic group of protein. Tannin as antibacterial component destroyed cytoplasm membrane of bacteria, thus altering protein component stability control from bacteria cell.[11]

Flavonoid, particularly catechins gained from bitter melon, has antibacterial effect from forming compound complex against extracellular protein which can alter bacterial cell membrane integrity. Thus, there was a possible antibacterial effect of BME against *E.coli* and *P.aeruginosa* from inhibition of protein synthesis making up cell walls by forming covalent cross ties on several functional organic group of protein.[12]

The advantage of this study compared to previous studies was in this study, dilution and diffusion test was done to find antibacterial effect of BME against *E.coli* dan *P.aeruginosa*.

MIC test from diffusion method was matched with MIC test from dilution method and thus supporting the statement conclued from MIC.

4.2. MBC of BME against*E.coli*and*P.aeruginosa*

MBC of BME against *E.coli*was not answered from this study. This matter was due to all tested samples in this study was still showing bacterial growth.Antimicrobial compound defined as bactericidal if MBC was equal to or 3 times greater than MIC, and defined as bacteriostatic if MBC was 3 times greater than MIC [13].

The result of MBC test of BME against *P.aeruginosa* until 100% concentration was not sterile, while MIC of BME aginst *P. aeruginosa* was 25% concentration, from this we can conclude that BME have bacteriostatic effect against *P. aeruginosa.* Whether bacteriocidal or bacteriostatic effect against *E. coli* could not be concluded from this study due to MIC was equal to 50% and author did not tested 150% concentration.

From MIC test of BME, we know that BME could be developed as slow effect antibacterial compund. The example of its usage were as ointment, or oral medicine in immunocompetent patient.[14,15] BME could not be expected with fast effect antibacterial, such as in antisepctic or disinfectant.

4.3. Correlation of BME Concentration Level with Diameter of *E. coli* and *P.aeruginosa* Inhibitory Zone

MIC of BME against *E. coli* was 50% concentration. The diameter of inhibitory zone of *E.coli* growth in this concentration was11 mm to be defined as sensitive against *E.coli.*MIC of BME against*P.aeruginosa* was 25% concentrationThe diameter of inhibitory zone of *P. aureginosa* growth in this concentration was13 mm.This result was consistent with prior studies which shown BME antibacterial effect particularly on *P.aeruginosa* growth.However prior studies about antibacterial effect of BME against *E.coli* and *P.aeruginosa* had not been using dilution and diffusion method to test MIC, thus these studies had had strong biases. Due to antibacterial effect could not be determined only from the diameter of inhibitory zone, while the correlation of inhibitory zone and MBC still unknown [5, 6, 16]. This study added up information about minimum diameter of inhibitory zone of BME to be defined as having antibacterial effect against *E.coli* and *P.aeruginosa* which were 11 mm and 13 mm respectively.

Correlation test between BME concentration and diameter of inhibitory zone shown very strong significant correlation, thus the result of BME with higher concentration tested shown greated diameter of inhibitory zone. However, this conclusion still could not be extrapolated in other concentrations with wider range, due to this conclusion was only made on certain concentration (0, 12,5%, 25%, 50% and 100%).

The limitation of this study was the concentration of BME higher than 100% or between 12,5% - 50% with smaller interval was not tested.

The implication of this study was as reference of future studies in testing antibacterial effect of BME. The result of MIC from dilution and diffusion method could be a novel reference in antibacterial effect of BME.

5. Conclusion

The result of this study concluded that BME have bacteriostatic effect on *P.aeruginosa* with MIC 25% and minimum inhibitory zone diameter of 13 mm. BME also have probable bacteriostatic effect on *E. coli* with MIC 50% and minimal inhibitory growth zone diameter of 11 mm.There was very strong correlation between BME concentration and inhibitory zone diameter on *E. coli* and *P. aeruginosa.*

6. References

1. Nasrallah GK, Schweizer HP, El Zowalaty ME *et al*. *Pseudomonas aeruginosa* : arsenal of resistance mechanisms, decades of changing resistance profiles, and future antimicrobial therapies. Future Microbiol 2015;10(10):1683-1706. doi:10.2217/fmb.15.48
2. Parathon H, Kuntaman K, Widiastoety TH *et al*. Progress towards antimicrobial resistance containment and control in Indonesia. BMJ 2017;358:j3808-j3808. doi:10.1136/bmj.j3808

3. Mihankhah A, Khoshbakht R, Raeisi M, Raeisi V. Prevalence and antibiotic resistance pattern of bacteria isolated from urinary tract infections in Northern Iran. J Res Med Sci 2017;22:108. doi:10.4103/jrms.JRMS_889_16
4. 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 2013;7(10):579-586. doi:10.5897/JMPR012.1161
5. Lu Y, Liu Y, Liang W, Chyuan J, Cheng K. Antibacterial and cytotoxic activities of different wild bitter gourd cultivars (*Momordica charantia* L. var. abbreviata Seringe), 2011.
6. Rachmawati N, Nursyamsi. Efek Antibakteri Ekstrak Etanol Buah Pare (*Momordica)* pada Media Pembenihan Difusi 2015, 2(1).
7. Thakur M, K Sharma R. Bitter Gourd: Health Properties and Value Addition at Farm Scale A 2016, 1.
8. Jia S, Shen M, Zhang F, Xie J. Recent Advances in *Momordica charantia* : Functional Components and Biological Activities 2017. doi:10.3390/ijms18122555
9. Depkes RI. Parameter standar umum ekstrak tumbuhan obat, Jakarta: Departement Kesehatan Republik Indonesia. Ed V 1989:9(11):16. doi:615.32
10. Saeed S, Tariq P. Antibacterial Activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia* 2005;37(4):997-1001.
11. Cag Y, Caskurlu H, Fan Y, Cao B, Vahaboglu H. Resistance mechanisms 2016;4(17):2-9. doi:10.21037/atm.2016.09.14
12. Kurhekar J. Tannins-Antimicrobial Chemical Components 2016, IX.
13. Ocampo PS, Arnoldini M, Fekete G, Ackermann M, Bonhoeffer S. Antagonism between Bacteriostatic and Bactericidal Antibiotics Is 2014;58(8):4573-4582. doi:10.1128/AAC.02463-14
14. Hidayat S. Uji Aktivitas Antibakteri Infusa Daun Mangga Bacang (*Mangifera foetida* L.) terhadap Staphylococcus aureus secara *In vitro*. FK Univ Tanjungpura Pontianak 2015.
15. Li J, Xie S, Ahmed S, Wang F, Gu Y. Antimicrobial Activity and Resistance : Influencing Factors. 2017;8:1-11. doi:10.3389/fphar.2017.00364
16. Svobodova B, Barros L, Calhelha RC *et al*. Bioactive properties and phenolic profile of *Momordica charantia* L. medicinal plant growing wild in Trinidad and Tobago. Ind Crop Prod 2017;95:365-373. doi:10.1016/j.indcrop.2016.10.046.