Pharmacology and Clinical Pharmacy Research
Volume 3 No 2 August 2018
ISSN:2527-7332 | e-ISSN: 2614-0020
doi: 10.15416/pcpr.v3i2.16700

Antibacterial Activity of *Cucumis melo* L. Extract and Its Nanoparticles Formulation against *Escherichia coli*

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Abstract

Over the past decade, the use of biological agents such as plants, cyanobacteria, bacteria, and fungi for synthesis of metal nanoparticles has been developed. The aim of this study was to investigate antibacterial activity of *Cucumis melo* L. peel extract and its nanoparticles formulation against *Eschericia coli*. The nanoparticles were made using silver nitrate with the ratio between *C. melo* L. extract and silver nitrate aqueous solution (1 mM) were 1:10 and 1:15. The formation of silver nanoparticles was observed after microwaved for 30, 60, 90, 120, 150, and 180 seconds by visible spectrophotometry analysis. Phytochemical screening revealed the presence of flavonoid and terpenoid within the extract. However, the characteristic of surface plasmon resonance band, which occurs in the range of 410-500 nm were not found in the nanoparticle extract, even though the reaction time was extended to 330s. Antibacterial activity against *E. coli* of the extract and its nanoparticle formulations was determined using Resazurin microtiter assay and compared to Amoxsan® as positive control. The highest *E. coli* inhibition was exhibited by the nanoparticles (79.8739±0.3859), followed by the extract (65.2821±0.9949). The nanoparticles and the extract have potent antibacterial activity compared to positive control (84.5519 ± 0.2544). In conclusion, the antibacterial activity of the *C. melo* L. nanoparticles formulation was better than its extract.

Keywords: *Cucumis melo*, silver nanoparticles, UV-Vis spectra

Introduction

Nanoparticles are the particles with the size between 1 and 100 nm. The definition varies depending on the field and its application.[1] On this size scale, the physical, chemical, and biological properties of nanoparticles differ from their properties as single atoms/molecules. Nanoparticles can be made of chemical and natural materials. Most often used materials are metals, metal oxides, silicates, non-oxide ceramics, polymers, organic, carbon, and biomolecules. Nanoparticles have several different morphologies such as spheres, platelets, cylinders, tubes, and others.[2]

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Received: 2 June 2018. **Revised:** 25 June 2018. **Published:** 1 August 2018.

Formulation of nanoparticle material is an early stage for the development of nanoscale technology. During this time, the preparation of nanoparticle materials is carried out through a bottom-up synthesis process by chemically synthesized or physically top down to obtain the desired type, size, shape, and nanoparticle composition.[1]

Over the past decade, the use of biological agents such as plants, cyanobacteria, bacteria, and fungi for synthesis of metal nanoparticles has been developed. The synthesis of nanoparticles by utilizing living things as biological agents in the synthesis process is known as nanoparticle biosynthesis.[3]

The effectiveness of inhibition is one of the criteria for selecting an antimicrobial compound. The stronger the inhibition, the more effective the compound. Damage caused by antimicrobial components may be microsidal (permanent damage) or microstatic (temporary damage/can be recovered). It depends on the concentration and culture used.[4]

Thus, the aim of this study was to evaluate the antibacterial activity of the *C. melo* L. extract and its nanoparticle formulations using resazurin reduction microplate (MRA) assay.

Methods

Materials

The tools used in this research were visible spectrophotometer (Thermoscientific Genesys 10 UV-Vis V4.002 2L9N 175013), microwave (Samsung Model ME109F), autoclave (All American Mode 25X-2, United States), incubator (Memmert Typ INE 400, Germany), micro pipette (BIO-RAD 10, 100, 1000 µL), analytical balance (Mettler AE 200), laminar air flow (ESCO MICRO PTE LTD 1750, Singapore), vacuum buchner (Sibata Scientific Technology LTD No), vacuum rotary evaporation (B-480, Switzerland), vortex (Scientific Industries Model G-560E, USA), microtiter plate 96 well (Thermo Scientific Cat No. 167008) and other standard laboratory glassware according to the procedure.

The ingredients required in this study were green *C. melo* L. peel, $AgNO_3$ (Merck, Cat No. 1,01512,0250), NaCl (Merck, Cat No. 1.06404.0500), distilled water, nutrient agar (Merck, Cat. No. 1,05450,0500), nutrient broth (Merck, Cat No. 1.05443.0500), Mueller-Hinton broth (Merck, Cat No. 1.10293.0500), amoxsan® (Merck, Cat No. DKL0832401701B1), dimethyl sulfoxide (Merck, Cat No.08.02912.1000), resazurin (Merck, Cat No. 1.00138.3495), and other materials in accordance with work procedures. The test bacteria used in this study were *E. coli.*

Extract preparation

Green *C. melo* L was obtained from traditional market located in Pekanbaru. The peel was then dried under the sun. Once dried, it was made into powder and was stored in the oven. A total of 10 g of dried *C. melo* L peel was macerated with 100 ml distilled water solvent which has been heated at 60ºC. The water extract obtained was then separated by filtration. The sample residue was then extracted repeatedly for 7 days. The extract was then evaporated using a rotary evaporator until the volume was reduced by a quarter of the initial volume. This extract was then used for the biosynthesis of silver nanoparticles, phytochemical screening, and antibacterial activity analysis.

Synthesis of nanoparticles

Silver nitrate solution in water 10^{-3} M was used as a silver source. The synthesis of silver nanoparticles was carried out with

microwave to speed up the reaction of silver nanoparticles formation. This method was done by reacting water extract of *C. melo* L peel with $AgNO_3$ 10^{-3} M in microwave. The microwave was set at 600 watts then the solution was observed in 30; 60; 90; 120; 150; 180; 210; 240; 270; 300; 330, and 360 seconds. *C. melo* L peelwater extract ratio with $AgNO_3$ was 1:10 and 1:15.

Characterization of nanoparticles

The silver nanoparticles formed can be marked by the change of color. The visible spectrophotometer analysis was used to confirm silver nanoparticles formation. Silver nanoparticles have a wavelength of 405-500 nm and in this study will be measured in the wavelength range 300-600 nm.

Antibacterial activity of nanoparticles

The analysis was performed using MRA. *E. coli* bacteria from nutrient broth media was taken (one ose) and scraped into nutrient agar medium. It was then incubated at 37 ^{0}C for 18-24 hours in the incubator. Grown bacteria was taken (1 ose) and inoculated in Mueller Hinton Broth (MHB) media. It was then incubated at 37 ^{0}C for 18-24 hours to make a starter. After 24 hours of incubation, its optical density was measured at 600 nm wavelength. Media MHB and 18 ml of saline water (NaCl 0.8%) were prepared. Resazurin was prepared in a test tube. Antibacterial activity of *C. melo* L extract, its nanoparticles, and 10 µg/ml of Amoxsan® were evaluated The percentage of resistor was measured using microplate reader after incubation for 16 hours.

Data analysis

The antibacterial activity test data was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(B_0 - B_1) - ((S_0 - S_1)}{(B_0 - B_1)} x\ 100\%$$

Results and Discussion

The color changes of the solution is one indication of silver nanoparticles formation. The silver nanoparticles were characterized using visible spectrophotometry to confirm the formation of silver nanoparticles which produced an absorption peak at a certain wavelength in the range of 410-500 nm. The length of the measured wave was between 300-600 nm. However, the nanoparticle formulations did not show an absorbance in the range of 410-500 nm. visible spectroscopy measures intensity of light passing through sample. Measurements are compared at each wavelength to determine the wavelength of the sample depending on the spectrum.[5]

The visible spectrum is sensitive to the formation of silver colloids because silver nanoparticles exhibit intense peak absorption

Figure 1. The color changes in the process of colloidal biosynthesis of silver nanoparticles using *C. melo* L. peel extract. Contact time (a) 0 seconds, and (b) 330 seconds.

ISSN:2527-7332 | e-ISSN: 2614-0020

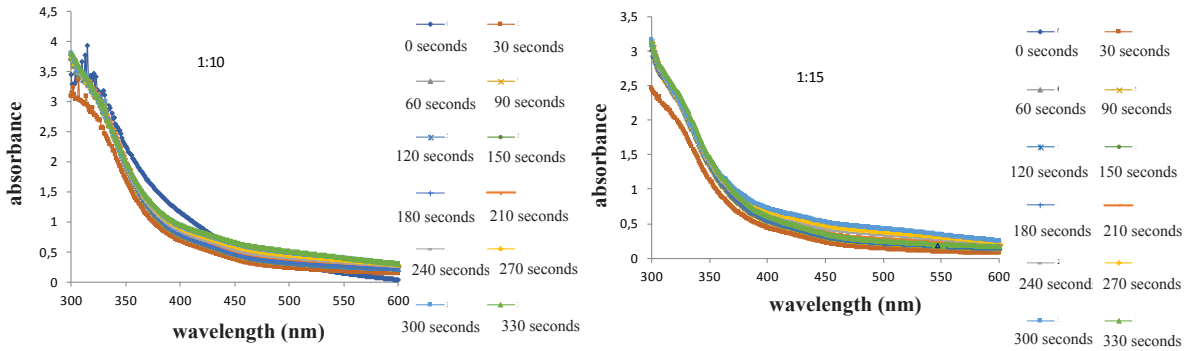

Figure 2. The UV-Vis spectrum of *C. melo* L peel extract and its nanoparticles

due to surface plasma excitation (illustrating the joint excitation of electron conduction in metals).[6] The spread of nanoparticles depends on wavelengths with short wavelengths (ultraviolet or blue light) spread intensely rather than longer wavelengths (red light). The spreading of the larger particle light is independent of the wavelength.[7]

Phytochemical screening results showed that *C. melo* L extract contained flavonoids and terpenoids. However, it is estimated that the amount of the compound is insufficient to reduce the silver ions. It could be caused by the high content of the water in the extract even though it has been concentrated.

The results of antibacterial activity test of *C. melo* L extract, silver nanoparticles, and a positive control against *E. coli* bacteria can be seen in Table 1. The highest *E. coli* inhibition was shown by the nanoparticles (79.8739±0.3859), followed by the extract (65.2821±0.9949). The nanoparticles and the extract have potent antibacterial activity compared to positive control (84.5519 ± 0.2544). Silver nanoparticles have the ability to interact and penetrate cell walls that cause structural changes in cell membranes, such as cell membrane permeability. It could inhbit bacterial growth by creating 'holes' on the cell surface and free radicals to the cells.[8]

The MRA is a method which uses resazurin as a color indicator in detecting bacterial cell activity. MRA is an effective method for testing the reactivity of natural products such as extracts from plant sources and microbes.[9] Resazurin ($C_{12}H_7NO_4$) is an active compound of Alamar blue which is known to be an indicator of oxidation reduction reactions used to assess cellular metabolic functions. Resazurine has a non-fluorescent blue color in the form of resorufin. The color changed from blue (resazurin) to pink (resorufin) as an indicator of the cells reduction. The color changes in resazurin is performed by enzymes

Table 1. Antibacterial activity of C. melo L extract, silver nanoparticles, and positive control

Sample	% Mean of Inhibition
Extract	65.2821 ± 0.9949
Silver nanoparticles	79.8739 ± 0.3859
Positive control	84.5519 ± 0.2544

in cells mitochondrial and cytoplasmic.[10]

Conclusion

In conclusion, the antibacterial activity of the *C. melo* L. nanoparticles formulation was better than the water extract

Acknowledgement

Not declared.

Funding

None.

Conflict of interest

Not declared.

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