Original article:

The result of modified hydrothermal nanotitania extract to the Escherichia coli growth.

Ahmad Mukifza Harun[1], Nor Farid Mohd Noor[2], Mohamad EzanyYusoff[2], Razif Abas[3], Mohammad Khursheed Alam[4]

Abstract:

Background: This research was planned to search for a potential of modified hydrothermal nanotitania extract in inhibiting the growth of bacteria commonly known in medical field. It is also aims to test this substance against common medical bacteria,*Escherichia coli.* ***Materials and methods:*** In this test, suspension of modified hydrothermal nanotitania extract (together with 0.01%, 0.03% and 0.05% silver) and undoping (positive control contains TiO_2 and no silver) were prepared by mixing of TiO_2 in Mueller Hinton Broth (MH) agar. The platecontaining the bacteria and TiO_2 were observed after 24 hour, 48 hours and 72 hours incubation at 37°C for any growth of bacteria. ***Results:*** There was no growth of *Escherichia coli*in the plates containing the bacteria and modified hydrothermal nanotitania extract except in the control media. ***Conclusions:*** The finding suggested the modified hydrothermal nanotitania extractioninterfered the growth of *Escherichia coli*.

Keywords: *nano titanium dioxide, bacterial growth, silver, nanoparticles*

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Introduction

Escherichia coli (*E.coli*) is one the main bacteria causing bacterial infection in the hospital and community. It is frequently found in internal organ of human which at a time pathogenic to the human. The bacteria can be transferred from mother to neonate transmission[1]. The *E. coli*outbreak usually occurs and spreading insidethe water stream, where the infected patient present withsymptoms such as bloody diarrhea[2]. Interestingly the outbreak of *E. coli*is not only involve the human but it also infects other animal such as pig[3]. In addition, the mode of transmission of *E. coli* may occur from animal to human[4]. One of its common source of infection activity is within thehuman blood itself[5]. Despite of*E. coli*frequenttreatment with antibiotics, the resistant to the treatment is increasinglyin trend, for example third generation cephalosporin resistance[6].

In addition to antibiotics, silver ion was reported to have bactericidal action toward *E. coli*[7]. The mechanism of how the silver ion is speculated by thelost of DNA replication and protein deactivation[8]. Furthermore, zero-valent iron nanoparticles was shown deactivated the bacteria[9]. In another study, gold nanoparticles was shown to have antibacterial activity toward *E. coli*[10]. In human, defensins which is cysteine-rich peptides is proposedto be natural antibody for *E. coli* bactericidal activity referring to defensins activities against *E. coli* outer membrane[11]. Titanium dioxide (TiO_2) is a special substancethat is currentlybeing usedagainst multiple bacterias. The increasing concentration of TiO_2 nanoparticles has been found to resistthe*E. coli* growth[12]. In addition, Martinez-Gutierrez et al. investigated the potential combination of TiO_2 with silver which enhance itsantimicrobial activity[13].

Naturally produced synthesized TiO_2 nanoparticles from the extraction of *Psidium guajava* aqueous

1. Engineering Faculty, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
2. School of Dental Sciences, Universiti Sains Malaysia, Health Campus,16150 Kubang Kerian, Kelantan, Malaysia.
3. Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
4. Orthodontic Unit, College of Dentistry, Jouf University, Sakaka, Kingdom of Saudi Arabia.

Correspondence to: Ahmad Mukifza Engineering Faculty, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia. (mukifza@ums.edu.my) These authors contributed equally to this work.

leaf were shown inhibited various organism growth such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and P. mirabilis*[14].

To strengthening theTiO_2 study, *E. coli and S. aureus* were shown reduced within 48 hours of applied TiO_2combination with the silver nitrate in the research using nanoparticle-coated surgical facemask[15].

The mechanism of how the titanium dioxideprevents the bacterial growth is possibly byan observationin which TiO_2 reduced of 24-hour lactate production of oral *Streptococcus mutans*[16]. In contrast, the TiO_2-based nanocomposite photocatalyzed the action on *P. aeruginosa* PAO1 cells that reduced the expression of specific genes and proteins in regulatory, signaling and growth function of the PAO1 cells[17].

Here in this study, the modified hydrothermal nanotitania extract in combination with the multiple percentage of silver is shown inhibited the *Escherichia coli* growth. This study will enhance our modified hydrothermal nanotitania extraction of the inhibition of *Staphylococcus aureus* growth in the previous experiment[18]. This study was done in UniversitiSains Malaysia on 1st – 30th September 2018.

Methodology

The modified hydrothermal nanotitania extractis combined with multiple concentrations of silver, (0.01%, 0.03% and 0.05%). In addition, a control the modified hydrothermal nanotitania extractwas also used without any silver mixing (undope). One of the limitation aspects of thissubstance is its inability tobe diluted in the water, dimethyl sulfoxide (DMSO) and sulphuric acid.

The source of bacteria, *Escherichia coli* was reserved from hospitalized patient of Hospital UniversitiSains Malaysia which then developed in the microbiology lab of School of Medical Sciences UniversitiSains Malaysia.

The test was done to check whethermodified hydrothermal nanotitania extract prevent the growth of the bacteria by adding and mixing the substances to the agar media during processedof making agar media before addition of bacteria-which is similar to the method used by Ahmad et al., 2013[12]. Some media is used as control where the nanotitania extract was not added to the media preparation but only the organisms were allowed to growth.

Preparation of the modified hydrothermal nanotitania extract

Suspensions of 1g/ml TiO_2(together with 0.01%, 0.03% and 0.05% silver) and undoping (positive control contains TiO_2 and no silver) were prepared by mixing 1g of TiO_2 in 1ml of Mueller Hinton Broth (MH) before additional of 1 ml of equivalent 0.5 McFarland. For control, the agar containing only bacteria without TiO_2 was used as a negative control. Incubation of these suspensions was donein incubator with shaker for 24 hour in temperature of 37°C. Amount of 100 µl of suspension were inoculated on MH agar, spreading the bacterial inoculation was done using hockey stick. The plated were observed after 24 hour, 48 hours and 72 hours incubation at 37°C for any growth of bacteria on agar plate.

Results

1. The modified hydrothermal nanotitania extractcontaining Titanium dioxide (TiO_2) combination with 0.01%, 0.03% and 0.05% silver nanoparticle and 'titanium dioxide only' are able to inhibit the growth Escherichia coli compared to the control media.

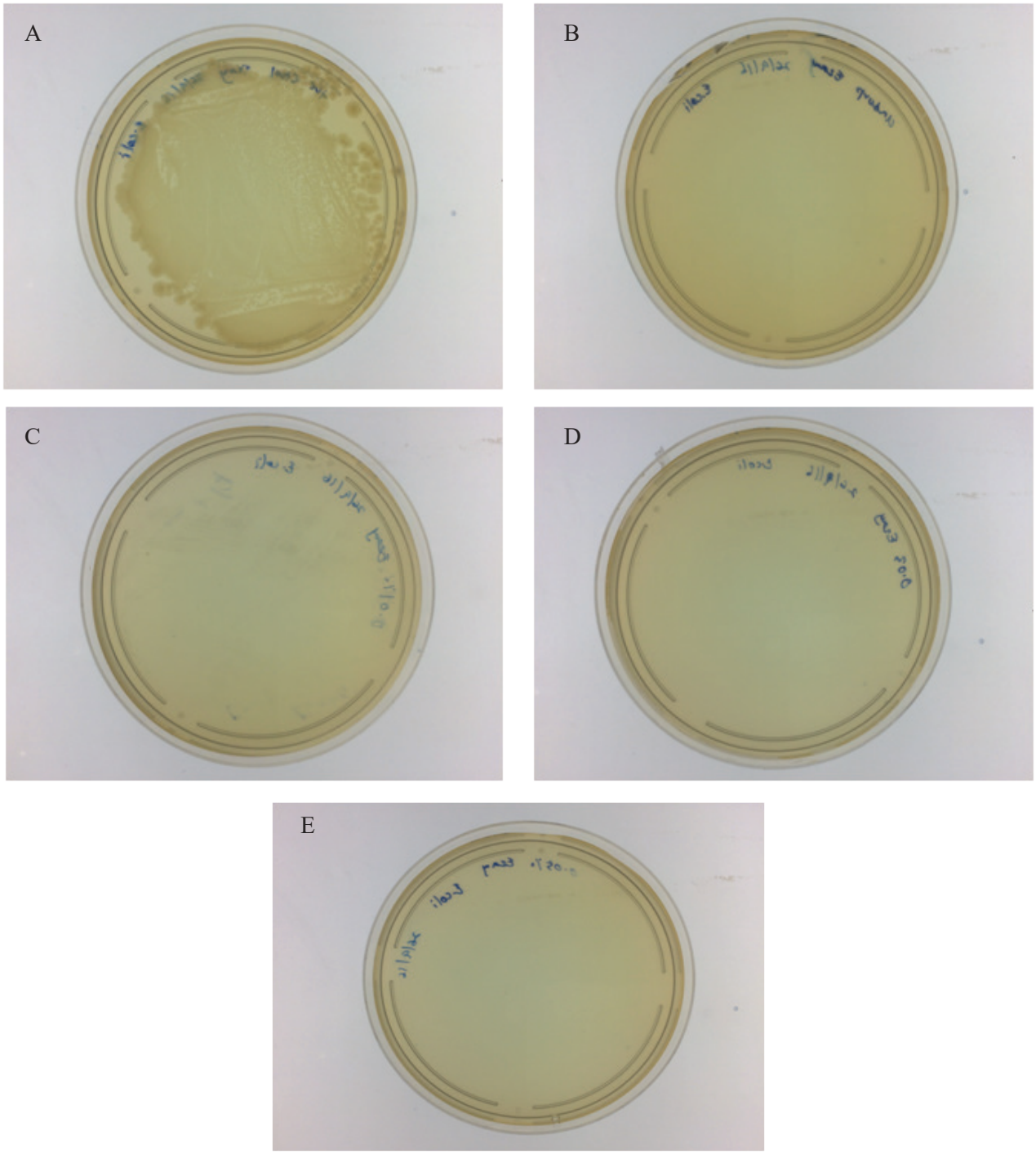
The modified hydrothermal nanotitania extractcontaining silver with multiple percentage(0.01%, 003% and 0.05%)and TiO_2 only are able to inhibit growth of *Escherichia coli* on the agar plate compared to control (no nanoparticles). In various agars containing the nanotitania extract consists of TiO_2 and mixing with silver (0.01%, 003% and 0.05%) no *Escherichia coli* was noted growth for a period of 24 hours, 48 hours and 72 hours. In the negative control agar where the nanotitania extract was not added,the organisms were seen growth successfully. The result is summarized in Table 1.

Table 1: TheEscherichia coli growth on tested agar for 24 hours up to 72 hours.

Test/hours incubation	24 hours	48 hours	72 hours
Bacteria only (negative control)	Growth	Growth	Growth
Undoping (positive control)+bacteria	No growth	No growth	No growth
TiO_2 with 0.01% silver+bacteria	No growth	No growth	No growth
TiO_2 with 0.03% silver +bacteria	No growth	No growth	No growth
TiO_2 with 0.05% silver +bacteria	No growth	No growth	No growth

Discussion

In ourexperiment, the modified hydrothermal nanotitania extractcontaining TiO_2 together with multiple percentage of silver compound (0.01%, 0.03% and 0.05%) and the undoping containing TiO_2 onlywere tested against the bacteria*Escherichia coli*. The objective of our study is to observe whether the modified hydrothermal nanotitania extracthas capacity to constrain the growth of the bacteria so that it can be used against various activity of *E. coli*



Picture: Testing the modified hydrothermal nanotitania extractwith *Escherichia coli.* Control media-contain bacteria only (A). TiO_2 only (B). TiO_2 containing 0.01% silver with bacteria (C). TiO_2 containing 0.03% silver with bacteria (D). TiO_2 containing 0.05% silver with bacteria (E).
Note there was no bacterial growth in (B),(C),(D) and (E) agar plate.

such as outbreak.

The characteristic of ourthe modified hydrothermal nanotitania extractwas,its powerlessnessto be diluted in water, dimethyl sulfoxide (DMSO) and sulphuric acid. In our experiment, the method is used to identify ability of these substances in preventing the growth *Escherichia coli* where identical method used by Ahmad et al., 2013(mentioned as above).

The results showed the modified hydrothermal nanotitania extractwas capable to be mentioned as having antibacterial propertiesup to 72 hours of post addition in the agar containing nanotitania extract. These results explain the potential of the modified hydrothermal nanotitania extractto be used for asantibacterial based on its chemical properties.

This study support other finding using nanotitania such as silver modified nanotitania and titanium dioxide- anatase nanoparticles[19-21].

The shape of a substance may influence the bactericidal activity which was shown by shape of silver nanoparticle study[22].In the future, the further experiment should be done to assess whether the shape of this modified hydrothermal nanotitania influences the bactericidal activity.

The limitation of our study is our the modified hydrothermal nanotitania extractis not dissolved in the water so it limits various other test for antibacteria.

Conclusion:

The modified hydrothermal nanotitania extractinhibits the growth of *Escherichia coli.*

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Author's contribution:

Data gathering and idea owner of this study:AMH, NFN, MEY, RA and MKA

Study design: AMH, NFN

Data gathering: AMH, NFN, MEY, RA

Writing and submitting manuscript: AMH, NFN, MEY, RA and MKA

Editing and approval of final draft: AMH, NFN, MEY, RA and MKA

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