Antibacterial Effect of Kiwi Fruit Extract (*Actinidia chinensis*) to *Streptococcus pyogenes* and *Staphylococcus aureus*

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ABSTRACT

This study was conducted to test the antimicrobial activity of Kiwi fruit (Actinidia chinensis) as an alternative antibacterial agent against gram positive microorganism such as Staphylococcus aureus that causes infections that colonize the skin and mucosal surface, and Streptococcus pyogenes which causes sore throat, pharyngitis, necrotizing fasciitis and Streptococcal toxic shock syndrome. The use of fruit extract was considered due to the emergence of multiple drug resistant bacteria due to multiple exposure and improper administration of antibiotics. The researchers of the study generally aim to evaluate the effectiveness of Actinidia chinensis fruit extract against Staphylococcus aureus and Streptococcus pyogenes which specifically aims to determine its minimum inhibitory concentration by using micro broth dilution method and to also determine if there is a significant difference between the percentage inhibition of the minimum inhibitory concentration of vancomycin and kiwi fruit extract against the two microorganisms. Results showed that kiwi fruit extract has its minimum inhibitory concentration at 3.125mg/ml with a percentage inhibition of 80.27% against S. aureus and, also has its minimum inhibitory concentration at 0.731mg/ml with a percentage inhibition of 79.60% against S. pyogenes. The researchers found out that kiwi fruit extract has no significant difference to vancomycin against S. aureus but is significantly different from vancomycin against S. pyogenes. Therefore, kiwi fruit extract was concluded to be potential alternative medicine in treating S. aureus related infections but has a lower antimicrobial activity compared to vancomycin in treating S. pyogenes related infections.

Keywords: Antibacterial, Kiwi fruit, Staphylococcus aureus, Streptococcus pyogenes

INTRODUCTION

Streptococcus pyogenes is a gram-positive coccus belonging to the Lancefield Group A *streptococcus* which is commonly found in the upper respiratory tract and in the skin. This bacterium is responsible in developing certain infections like pharyngitis, necrotizing fasciitis and streptococcal toxic shock syndrome. *S. pyogenes* may also produce autoimmune diseases such as rheumatic fever and acute post streptococcal glomerulonephritis and rheumatic heart disease (Beye, El Karkouri, Labas, Raoult, & Fournier, 2017). *S. pyogenes* infections are spread by large droplets produced in the upper respiratory tract. School-aged children are the mostly infected individuals of *S. pyogenes* that carry this bacterium but are usually asymptomatic (Turgeon, 2013). Over 616 million cases of *S. pyogenes* occur each year worldwide (Sanyahumbi, Colquhoun, Wyber, & Carapeti, 2016). Infection of this bacterium in the throat is self-limiting but antibiotic treatment such as penicillin and amoxicillin are suggested to lessen the severity of the infection and prevent the progression to acute rheumatic fever (Wessels, 2016).

Staphylococcus aureus is gram positive cocci and a catalase positive microorganism and also an opportunistic pathogen which is associated with human infections that colonizes the skin and mucosal surfaces. This is considered as the most virulent species of *Staphylococci* (Tille, 2014). Patients that are colonized by *S. aureus* are more susceptible in acquiring healthcare associated *S. aureus* compared to those individuals who are not colonized with this bacterium. In 2016, there were a total of 5,474 isolates of *Staphylococcus aureus* reported and 61.5% of these are Methicillin- resistant *Staphylococcus aureus*. These reported MRSA rate did not differ significantly from the MRSA rate of the year of 2015 which is 62.5% (Antimicrobial Resistance Surveillance Program-Philippines, 2016). Recent studies have shown that vancomycin is considered as the antibiotic of choice in utilizing serious infection with Methicillin-resistant

Staphylococcus aureus and Methicillin-susceptible *Staphylococcus aureus* and other gram-positive infections despite of its continuous usage for over half of a century (Phillips, Wells, Martinello, Smith, Woodman, & Gordon, 2016)

Antibiotics such as penicillin and methicillin may have an adverse reaction when it is administered to patients including the development of allergic reaction Micromedex, Multum, & Kluwer, 2018). A reported hypersensitivity to penicillin is associated with the increase use of antibiotics such as vancomycin, clindamycin, aztreonam, fluoroquinolones and aminoglycosides. These antibiotics are more costly with broader spectrum and more toxicity which increases the risks of the patient of contracting the infection with resistant pathogen (Blumenthal, Shenoy, Varughese, Hurwitz, Hooper, & Banerji, 2015).

Most medicinal plant consists great amounts of antioxidant and antibacterial property. Any compound that exist in plants or fruits is termed phytochemicals. These phytochemicals are non- nutrient in nature but have a good preventive action in utilizing certain illnesses. *Actinidia chinensis* which is considered as one of the most used public fruits worldwide with different biological properties such as antioxidant, anti-allergic and cardiovascular protective effects and is known to possess high levels of flavonoid. (Al-Kawaz, & AL-Mashhady, 2016). Flavonoids possess anti-tumor, anti- inflammatory, anti-ischemic, anti- allergic and anti-bacterial activities (Wu, Ma, Li Deng, Yin, & Huang, 2015).

Recent study had shown that the fruits of *Trigonosciadum brachytaenium* (Boiss) Alava which possess a flavonoid compound exhibited a great antimicrobial activity against the selected bacteria used in their experiment. Therefore, *Actinidia chinensis* which also possess flavonoids like *Trigonosciadum brachytaenium* (Boiss) Alava may also exhibit an antibacterial activity that can be used as an alternative to prevent *Streptococcus pyogenes* and *Staphylococcus aureus* infections (Akhavan, Jahangiri, & Shafaghat, 2015).

This study evaluated the effectiveness of *Actinidia chinensis* fruit extract in preventing the bacterial infection of *Staphylococcus aureus* and *Streptococcus pyogenes* using micro broth dilution method. Specifically, to determine the Minimum Inhibitory Concentration of *Actinidia chinensis* fruit extract against *Streptococcus pyogenes* and *Staphylococcus aureus* and to determine significant difference between the percentage of inhibition of the mean minimum inhibitory concentration of *Actinidia chinensis* fruit extract to the percentage of inhibition of the mean minimum inhibitory concentration of vancomycin between 50mg/ml to 0.098mg/ml for *Streptococcus pyogenes* and *Staphylococcus aureus*

MATERIALS AND METHODS

Preparation of the Extract

The kiwi fruit's mesocarp part was washed, cut in half, oven dried for 15 hours at 60^{o}C and grounded into powder using mortar and pestle within 30 minutes and stored at a room temperature until it was used. Ten grams of the fruit powder was soaked in 100 ml distilled water for 24 hours with intermittent shaking every 5 hours. Mixtures were centrifuged for 5 minutes at 3400 rpm. The supernatants collected were evaporated by freeze-drying. The extracted powder of the kiwi fruit was dissolved in distilled water to a final concentration equal to 100 mg/ml (Abdallah & Ismail, 2017).

Micro-broth Dilution Method

One hundred microliters of Muller Hinton broth were distributed in all 96-well microplates, fifty microliters of kiwi extract and antibiotics were transferred into the first row of the 96 well microplates and served as the positive control. Ten, two-folds serial dilution of the kiwi extract and antibiotics were carried out such that 50 microliters from the first wells were transferred, resulting in the samples of the first row to be serially distributed in descending order with a concentrations of 50mg/mL, 25mg/mL, 12.5mg/mL, 6.25mg/mL, 3.13mg/mL, 1.56mg/mL, 0.78mg/mL, 0.39mg/mL, 0.195mg/mL and 0.098mg/ml respectively, while distilled water was added and serve as negative control. Afterwards, 50 microliters of bacterial suspension, equal to 0.5 McFarland standard was added to each well and the plates were incubated

at 37◦C for 24 hours. After incubation, 40 microliters of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride was added to the reaction mixture. Optical densities of samples were read at 650nm. The well with the lowest concentration or with a 79.5% inhibition or higher was considered as the Minimum Inhibitory Concentration for these tested samples (Aumeeruddy-Elalfi, Gurib-Fakim, & Mahomoodally, 2015).

Data Analysis

Gathered data were analyzed using T-test with three trials to determine if there is a significance difference between the percentage of inhibition of mean minimum inhibitory concentration of the vancomycin between 50mg/ml to 0.098mg/ml and percentage of inhibition of the mean minimum inhibitory concentration of the *Actinidia chinensis* extract.

RESULTS AND DISCUSSION

The minimum inhibitory concentration of vancomycin for *S. aureus* was 0.098mg/ml that inhibits 82.86% of the growth of the microorganism and the minimimum inhibitory concentration of kiwi extract for *S. aureus* was 3.125mg/ml that inhibits 80.27% of the microorganisms**.** *Actidindia chinensis* fruit or the so called kiwi fruit possesses flavonoid (Fiorentino et al., 2015) which is responsible for its antibacterial properties. According to Xie et al. (2015), flavonoids are well-known anti-bacterial agents in many types of microorganisms. This coincides with the study conducted by Donkor et al. (2012), claiming that the fruit extract *Physalis angulata* which also possess flavonoid compound exhibited a high antimicrobial activity against *S. aureus*. This plant was concluded to be a good treatment for boils, sores and wounds. Furthermore, the study that was conducted by Ogbonna et al. (2013) states that medicinal plant such as *Xylopia aethiopica* which also possesses flavonoid has anti-tumour, anti-asthmatic, anti-inflammatory and antimicrobial property against *S. aureus*. A similar result was exhibited in the study of Radovanovic and Andelkovic (2013) which proved that wild berry fruit containing high amount of flavonoid was also determined to be an effective antibacterial agent against *S. aureus*. Fruit or medicinal plant that possess flavonoid truly exhibits a great antimicrobial activity against *S. aureus*. In relation to that, kiwi fruit which also possess flavonoid, was determined to be an effective antimicrobial agent against *S. aureus*.

There was no significant difference between the percentage growth inhibition of vancomycin and the kiwi extract. Therefore, kiwi fruit extract is an effective antibacterial agent against *S. aureus* and its antimicrobial activity is comparable to vancomycin. This coincides with the study conducted by Maliehe, Shandu, and Basson (2015), claiming that *Syzygium cordatum* fruit extract which contains flavonoid was also found to exhibit a comparable anti-microbial activity from ciprofloxacin against microorganism such as *S. aureus.* Moreover, a study by Okeke et al. (2015) stated that the *Citrus limonum* fruit extract which posssess flavonoid was also proved to be as good as gentamycin in treating *Staphyolococcus aureus* related infection. This fruit extract was concluded to be a good substitute for gentamycin in inhibiting *S. aureus*. Furthermore, a similar result was also evident in the recent study conducted by Mann et al. (2015) stating that fruit extract of *Baccaurea sapida* containing flavonoid was also capable of exhibiting a comparable result to that of tetracycline in treating infections caused by *Staphylococcus aureus.* In relation to the studies conducted above, kiwi fruit extract which contains flavonoid, was proven to be a potential alternative medicine to *S. aureus* due to its comparable antimicrobial activity from vancomycin in treating *S. aureus* related infection.

The minimum inhibitory concentration of vancomycin for *S. aureus* was 0.098mg/ml that inhibits 82.88% of the growth of the microorganism and the minimimum inhibitory concentration of kiwi extract for *S. aureus* was 0.731 mg/ml that inhibits 79.60% of the microorganisms. Flavonoid is one of the substances that is present on kiwi fruit extract that showed a great antimicrobial activity against gram positive microogranisms such as *S.pyogenes* and *S.aureus* (Djouossi et al., 2015)**.** This coincides with the

study conducted by Akthar et al. (2008) wherein *Pimpinella anisum* fruit extract which was found to possess flavonoid compound was determined to have an antibacterial activity against microorganisms such as *S. pyogenes*, *E. coli*, *K. Pneumoniae*, and *S. aureus* but is inferior to ciprofloxacin as the positive control. Furthermore, another study was conducted by Sekar et al. (2014), exhibiting that rambutan fruit peel extract that possesses flavonoid was also determined to be an effective antibacterial agent against gram positive bacteria such as *S.pyogenes* but has no antimicrobial activity in microorganisms that are considered gram negative bacteria. Furthermore, a similar study regarding antibacterial activity was shown in *Garcinia mangostana* plant extract, found to possess a flavonoid compound which is responsible for its antibacterial activity against *S. pyogenes* (Bhat & Al-Daihan, 2013). Moreover, Jaganathan et al. (2018) also proves that plant or fruit extracts with flavonoid content shows antibacterial activity. Grape seeds' phytochemical property was studied first and found out to possess flavonoid component that was responsible for its role as an effective antibacterial agent against *S. pyogenes.*

There was a significant difference between the percentage growth inhibition of vancomycin and the kiwi extract. Therefore, kiwi fruit is a an effective antibacterial agent against *S. pyogenes* but its antimicrobial activity is inferior to vancomycin. This corresponds to the study conducted by Cock and Mohanty (2013), proving that *Terminalia ferdinandia* fruit extract which possesses flavonoid, exhibits a good anti- microbial activity against *S. pyogenes.* But even though it can inhibit microorganism such as *S. pyogenes*, it's anti-microbial activity was determined to be inferior to chloramphenicol which was the standard drug used in the study. Another related study was conducted by Kaushik et al. (2010), claiming that fruit extracts of *Elletaria cardamonum* which contains flavonoid is also good antibacterial agent against *S. pyogenes* related infection. This fruit extract was proved to have a great anti- microbial activity against *S. pyogenes* but its capability to inhibit this microorganism is lower compared to the the standard drug used which was tetracycline. Furthermore, a recent study was conducted by Tabatabaei- Yazdi, Alizadeh-Behbani, and Zanganeh (2015), claiming that *Mespilus germanica* which contains flavonoid was capable of treating *S. pyogenes* related infection but was found out to have a lower antimicrobial activity against ciprofloxacin. Even though kiwi fruit has the capability to treat infections related to *S. pyogenes* infection, its anti-microbial activity is lower compared to vancomycin, therefore claiming it as not a good substitute for vancomycin in treating *S. pyogenes* infection.

CONCLUSION

Based on the results , the researchers concluded that *Actinidia chinensis* or the so called kiwi fruit is an effective antimicrobial agent against microogranisms such as *Staphylococcus* aureus with a minimum inhibitory concentration of 3.125mg/ml and *Streptococcus pyogenes* with a minimum inhibitory concentration 0.731mg/ml.

The researchers also found out that there is no significant difference between the kiwi fruit extract and vancomycin in terms of their anti-microbial activity against *S. aureus* which means that they have comparable antibacterial activity. However, the researchers found out that even though kiwi fruit is an effective antimicrobial agent against *S.pyogenes*, its antimicrobial activity was deemed to be inferior than vancomycin. Therefore, kiwi fruit extract was concluded to be a potential alternative medicine to vancomycin in treating *S. aureus* infection. However, in *S. pyogenes*, it has a lower antimicrobial activity compared to vancomycin.

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