Antimicrobial activity of *Hylocereus undatus* (White dragon fruit) peel extract using ethanol against Methicillin-resistant *Staphylococcus aureus*

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Abstract: The worldwide development of multi-drug resistant microbial strain is progressively restricting the efficacy of the latest drugs and essentially leads to failure in the treatment of infection. *Hylocereus undatus* (White dragon fruit) peel extract possesses phytochemicals and antimicrobial properties, namely flavonoid, alkaloids, phenols, and carbohydrate, etc. In this study the antimicrobial activity of ethanolic extract of *Hylocereus undatus* peel against Methicillin-resistant *Staphylococcus aureus* was evaluated using disc diffusion and simple dilution. The result showed that the *Hylocereus undatus* (White Dragon Fruit) extract inhibits the growth of bacteria in the range of 6mm. The results in the disc diffusion method of *Hylocereus undatus* (White dragon fruit) peel extract showed that there's no significant difference in the effectiveness of antimicrobial components of extracted *Hylocereus undatus* (White Dragon fruit) peel against Methicillin-Resistant *Staphylococcus aureus* by the zone of inhibition using ethanol. Thus, these findings could be used further to understand the antimicrobial property of *Hylocereus undatus* (White dragon fruit) peel.

Keywords: Hylocereus undatus; Methicillin Resistant *Staphylococcus aureus;* antibacterial, disc diffusion.

1. Introduction

The increasing problem of multidrug-resistant (MDR) bacterial pathogens has considerably undermined the present antibacterial treatment. Indeed, even though pharmacological businesses have delivered the number of modern antimicrobials over the last decades; resistance to drugs by microbes has increased. In common, microbes show hereditary capacity to transfer and procure drug resistance that is employed as restorative agents.

Approximately between 60-90 percent of developing countries use botanical and traditional treatment and ponder it to be their main health care (Agarwal, et al., 2016) The rapidness of the adaptation and building up of resistance of microorganisms have been one of the things that affected the mortality and morbidity rate around the globe (Wangai, et al., 2019). Among all microorganisms, the *Staphylococcus spp.* is one of the most microorganisms which develops resistance quickly (Wierzchowska, 2020).

Penicillin has been a great drug, diagnosing and treating infections that have been fatal. Nonetheless, even by the mid-1940s, just a few years after the creation into clinical settings, penicillin resistance had already been identified in health facilities, within just a decade it has now become a major societal problem. S. aureus is remarkable in its opportunity to develop resistance to antibiotics (Chambers and DeLeo, 2010).

Methicillin-resistant *Staphylococcus aureus* is a gram-positive Staphylococcus bacteria that is resistant to certain antibiotics and is hard to treat unlike other kinds of Staphylococcus bacteria (Green, 2012). Since *Staphylococcus spp.* have been resistant to certain drugs, infections caused by *Staphylococcus spp*. have been on the run for years and have been considered a great threat (Turner, et al., 2019). Though the development of new antimicrobial drugs is growing, microorganisms still do not cease to fight back and continue to adapt and gain resistance to antibiotics (Boswihi, 2018).

The worldwide development of multi-drug resistant microbial strain is progressively restricting the efficacy of the latest drugs and essentially leads to failure in the treatment of infection. Antimicrobial Drug Resistance is additionally of financial concern with effect on the specialists, patients, Medical directors, drug companies, and the citizens. The unavailability and taking toll of new generation antibiotics with constrained successful spans have brought about an increment in morbidity and mortality. It requires substances from other sources to foresee demonstrated antimicrobial movement. Thus, it manages to look for more viable antimicrobial operators of materials of plant origin, in the point of finding possibly valuable present material or ingredients that will serve as a source for the formulation of modern antimicrobial drugs. The tremendous amount of therapeutic plants are acknowledged as valuable assets of organic antimicrobial compounds (Agarwal, et al., 2016).

Nowadays plants that can be used as medicine have been a fragment in development for human culture. *Hylocereus undatus* (White dragon fruit) is mostly known for having polyphenolic compounds and antioxidant

activity against various microorganisms (Hitendraprasad, 2019). *Hylocereus undatus* (White Dragon fruit), well known beneath the title "White Dragon fruit" other than its alluring color and appearance. The natural products of *Hylocereus Undatus* (White Dragon Fruit) are known for being a high source of polyphenolic components together with the antioxidant movement. Phytochemical components such as betalains, polyphenolic compounds, and carotenoids are known to have chemoprotective properties that fight against an oxidant stretch inside the body and to keep the balance between cancer prevention agents and the oxidants in the improvement of well-being. This discovery shows the application of phytochemical components of *Hylocereus undatus (* White Dragon fruit) and its connection to well-being (Liang, 2016).

This study is pioneered and conducted to provide information about the *Hylocereus undatus* (White Dragon fruit) peel, for the public health campaign. To encourage people to use *Hylocereus undatus* (White Dragon fruit) by means of evaluation of functioning properties of *Hylocereus undatus* (White Dragon fruit) peel extract.

2. Materials and Methods:

2.1 Sample Preparation

The *Hylocereus undatus* (White dragon fruit) was obtained from a known local supermarket at Builtamart located in Tomas Morato, Quezon City, Philippines.The materials being used in the extraction were *Hylocereus undatus* (White dragon fruit) peels, 865 g, fresh and 95% ethyl alcohol, 1.5 L. The fruits gathered were rinsed with water and hand peeled.The fresh 865 g white dragon fruit peels were air-dried for 3 days and were pulverized using Wiley mill producing 95 g of dried sample which was immerse in 1.5 L of ethyl alcohol for 48 hours with occasional stirring. The mixture being filtered and obtained was concentrated using a rotary evaporator at 60˚C under vacuum for 2 hour.

2.3 Preparation of extract

The extraction of the air dried peel of *Hylocereus undatus* (White dragon fruit) was conducted according to the method of the Department of Science and Technology. The extract that is concentrated was being transferred to an evaporating dish and concentrated using a water bath at 60˚C to obtain a semi-solid extract. After that, the concentrated crude extract is being collected. Put it in an amber bottle and labelled.For the result of extraction the Crude extraction of dried 95 g dragon fruit peels produced 1.0 L ethanolic extract. 5.2 Concentration of the filtrate resulted in 3.8 g (4.0%) of semi-solid extract.

2.4 Disc diffusion

Antibiotic susceptibility disks. Antimicrobial disks are prepared in the Marikina Medical Center. Storage of the disks are important to produce good results.

Cartridges containing commercially prepared paper disks must be properly sealed and the temperature for the storage should be 8°C or frozen at -14°C in a non-self-defrosting freezer. Disks should be stored at room temperature upon removing the protective plastic packaging. Upon opening store the cartridges in a storage container containing desiccant for no more than 1 week.

2.5 McFarland standard

McFarland standards allow visual comparison of bacterial density and a suspension of either barium sulfate or latex particles.

A Wickerham card, which is a small card with parallel black lines, was among the most common. A 0.5 McFarland standard refers to a bacterial suspension having about 1 and 2 x 108 E. coli CFU/ml.

A 0.5 McFarland standard can be made throughout using the procedures below.

1. Add a 0.5-ml aliquot of a 0.048 mol/liter BaCl2 (1.175% wt/vol BaCl2 • 2H20) to 99.5 ml of 0.18 mol/liter H2SO4 (1% vol/vol) with constant stirring to maintain a suspension.
2. Make sure that the density of the turbidity standard is correct by measuring the absorbance with the use of a spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.
3. The barium sulfate suspension is transferred in 4- to 6-ml aliquots into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.
4. Store and tightly seal in the dark at room temperature.

Kirby-Bauer procedure with the use of the McFarland standard.
1. Before using a mechanical vortex mixer, vigorously agitate the barium sulfate standard and check for a uniformly turbid look. When you're using a latex-based standard, gently flip it instead of employing a vortex mixer.
2. In preparation of the inoculum the student adds bacterial colonies to the saline, then compares the results of the suspension to the McFarland standard. Then side by side and not more than 1 inch from the face of the Wickerham card hold both standard and inoculum tube and compare the appearance of the lines from both suspensions. Do not hold the tubes flush against the card. If the bacterial suspension looks lighter than the 0.5 McFarland standard, add more organisms to the tube from the culture plate. If the suspension appears thicker or denser than the 0.5 McFarland standard, add more saline to the inoculum tube to dilute the suspension to the relevant density. It can be simpler to do it again from the beginning than to go on diluting a bacterial suspension that is too thick or dense for use (Hudzicki, 2009).

2.6 Preparation of Mueller-Hinton plate

1. First, let a MH agar plate (one for each microorganism to be tested) to reach a room temperature. To avoid condensation, it's best to leave plates in the plastic sleeve while they warm up.
2. If a visible liquid appears on the surface of the agar, let the excess liquid drain and evaporate the plate by inverting the plate and then leave the lid agar. Plates can be dried in a 35°C incubator or at ambient temperature in a laminar flow cabinet
(usually 10 to 30 minutes).
3. Label each MH agar plate appropriately for each organism to be examined (Hudzicki, 2009).

2.7 Preparation of inoculum

1. First, Touch four or five isolated colonies of the organism to be examined with a sterile inoculating loop or needles.
2. Next is to suspend the microorganism in a 2 ml of sterile saline.
3. To facilitate a solid suspension, the saline tube should be vortexed.
4. Modify the turbidity of this suspension to a 0.5 McFarland standard. If the suspension is too light, add additional organism or dilute with water. If the suspension is too heavy, use sterile saline.
5. This suspension should be used within 15 minutes of being prepared. (Hudzicki, 2009).

2.8 Measuring zone sizes

The zones of inhibition should be uniformly circular and there was a presence of a confluent lawn of growth if the plate was inoculated correctly, properly and all other conditions were correct.

The inoculum was too light that the test must be repeated when individual colonies are apparent across the plate.

The area that should show no obvious,visible growth that could be detected with an unaided eye must be the zone magine. The researcher must not use a magnification device when observing zone edges.

When calculating the zone of inhibition for swarm microorganisms like proteus spp., disregard the thin veil of swarm development in a zone of inhibition which is otherwise apparent.

Antagonists in the medium such as trimethoprim and sulfonamides may allow some minor growth;therefore, disregard slight growth (20% or less of the lawn of growth) and focus on measuring the more obvious margin to determine the zone diameter. (Hudzicki, 2009).

2.9 Simple dilution

1.Prepare dragon fruit then mix with saline in a clean new tube: 1st test tube (label 100%) - 1 mL dragon fruit ONLY,2nd test tube (label 75%) – 750 ul dragon fruit plus 250 ul saline, 3rd test tube (label 50%) – 500 ul dragon fruit plus 500 ul saline and 4th test tube (label 25%) – 250 ul dragon fruit plus 750 ul saline.
2.Prepare 15 pieces of filter paper 6mm in diameter, then use a puncher however, sanitize first. After that, soak the filter papers in test tubes, 3 filter papers must be used per tube.
3.Soak 1 6 mm filter paper onto a test tube with saline.
4.Soak it all for at least a day, the pat dry or oven dry. DO NOT WASH in water.
5.Prepare 14 pieces of MHA. Then, prepare an inoculum of SAU or MRSA. Using sterile cotton swabs, the turbidity was corrected to 0.5 McFarland standard (108 CFU/mL) and streaked onto Mueller-Hinton agar plates.
6.Impregnate the filter papers into the MHA. Use Rifampin for Positive control.

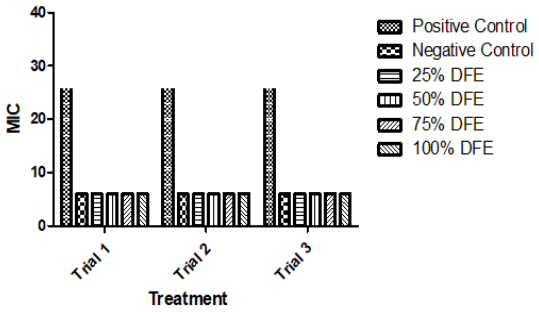
7.Incubate for 24 hrs, then use a caliper to measure the zone of inhibition.

3. Results and Discussion

With the use of a caliper to measure the zone of inhibition it was found that *Hylocereus undatus* (White dragon fruit) peel extract has a 6mm zone of inhibition in the first, second and third trials. In 100% extract it has 6mm in 1 trial, 6mm in 2 trials and 6mm in 3 trials, in 75% extract it has 6mm in 1 trial, 6mm in 2 trials and 6mm in 3 trials, in 50% extract it has 6mm in 1 trial, 6mm in 2 trials and 6mm in 3 trials, and in 25%% extract it has 6mm in 1 trial, 6mm in 2 trials and 6mm in 3 trials. *Hylocereus undatus* extract exhibited a P value of >0.05, meaning they are not significant and are not effective against Methicillin-Resistant *Staphylococcus aureus*.

Therefore, there is no significant difference in the effectiveness of antimicrobial components of extracted Hylocereus undatus (White Dragon fruit) peel against Methicillin-Resistant *Staphylococcus aureus* by the zone of inhibition using ethanol.

Table 1. Antimicrobial Activity of *Hylocereus undatus* (White dragon fruit) peel extract using disc diffusion method.



Used two-way ANOVA, the Graph of the result was formulated to present the summary of the findings. Based on the Disc Diffusion test, The positive control which is Rifampin, possessed a difference of 26 mm while the negative control which was Normal Saline Solution showed 6mm. 100%, 75%,50%, and 25% concentration also showed 6mm in the disc diffusion method.

4. Conclusion

On the basis of the results and findings in this study, this research came up with the following conclusions:

More than 0.05 confidence level of the p value means that there is no significant difference. While, if the confidence level of the P value is less than 0.05, it is significant.

The negative control along with the different concentrations of 100%, 75%, 50% and 25% Hylocereus undatus extract exhibited a P value of >0.05, meaning they are not significant and are not effective against Methicillin-Resistant *Staphylococcus aureus*.

There is no significant difference in the effectiveness of antimicrobial components of extracted *Hylocereus undatus* (White Dragon fruit) peel against Methicillin-Resistant *Staphylococcus aureus* by the zone of inhibition using ethanol.

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