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IN VITRO ANTIMICROBIAL ACTIVITY OF KATURAY (SESBANIA GRANDIFLORA) FRUIT POD EXTRACT

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ABSTRACT

Antimicrobial resistance (AMR) is one of the current health issues that is on the rise all over the world, and it is in this respect that it has become a matter of interest to look at plant-based compounds that can be used as alternative therapeutic agents. This research assessed the in vitro antimicrobial properties of Katuray (Sesbania grandiflora) fruit pod extract (SGFPE), a culturally relevant and is a widespread Philippine plant traditionally used in food and folk medicine. The extract was tested on its phytochemical profile and antimicrobial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans using an experimental research design carried out in the College of Public Health, University of the Philippines Manila. The phytochemical screening has indicated the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, reducing sugars and traces of anthraquinone glycoside (1120 µg/mL). Antimicrobial assays showed that all the four organisms were weak to moderately susceptible to the extract with the zone of inhibition corresponding to the concentration of the extract. The 100 percent extract was the most inhibitory in all of the test organisms; but there were still large variations compared to standard positive controls, and thus it can be seen that traditional antimicrobials are more effective. On the whole, the results substantiate the ethnomedicinal applicability of Sesbania grandiflora and promote its role in the antimicrobial research based on natural products, the preservation of cultural knowledge, and the field of scientific discussions on the sustainable approaches to the issue of AMR.

KEYWORDS: Medicinal Plants, Microbes, Phytochemicals, Antimicrobial, Susceptibility Tests.

1. INTRODUCTION

Antimicrobial resistance (AMR) remains among the most pressing health-related challenges in the 21 st century and is still identified as a global threat by the World Health Organization (WHO, 2021). AMR happens when bacteria, viruses, fungi and parasites develop and become unresponsive to standard antimicrobial treatment, leading to infections that are persistent, causing illness to last longer, incurring higher medical expenses and leading to increased mortality. WHO (2021) believes that when antimicrobial drugs become less effective, the medical achievements of decades will be threatened because the common infections will not be treated. Philippines Antimicrobial resistance is on the rise, and after the realization of the Antimicrobial Stewardship Program of the Department of Health, the rates of antimicrobial resistance increased by 14 percent in 2017-2018 (Department of Health, 2018). The ever-evolving development of resistance patterns underscores the need across the globe to find alternative and plant-derived antimicrobial agents (Cowan, 1999).

The issue of developing resistant microorganisms has reawakened the world to seek alternative therapeutic resources. The human societies have long lived in an extensive microbial world, which can be helpful, or sometimes harmful. Medicinal plants have traditionally been the main source of preventive and curative administration of infectious diseases. The rising use of herbal therapy is justified by the fact that studies indicate that the use of botanical medicines in the treatment of infection as well as inflammation is growing worldwide (Ekor, 2014). Such dependence is not only biological but also a cultural identity because the traditional plant-based medicine is a part of the intangible culture of a great number of societies, including the Philippines.

The practice by the indigenous people in the ancient times is still being supported by the contemporary science. Plants have been known to have a rich repertoire of secondary metabolites including tannin, flavonoid, terpenoid, and alkaloid that are natural defenses against microbe's attack. They can be antimicrobial, antifungal, or antioxidant compounds, so these compounds are significant as new therapeutic agents. Phenolic compounds and tannins, e.g. have proven to be very active as antimicrobials against a range of pathogens (Scalbert, 1991). It has also been discovered that flavonoids possess therapeutic properties, such as antimicrobial, antioxidant, and anti-inflammatory effects (Panache et al., 2016). The Sesbania species of terpenoids and other related compounds have demonstrated potential biological activity in drug development (Afrin et al., 2019).

Katuray (Sesbania grandiflora), is of cultural as well as medicinal value in the Philippines. Katuray, which is commonly used in traditional Filipino medicine and cuisine, has been traditionally regarded to be beneficial in treating inflammation, infection, and digestive disorders. The research done on various components of Sesbania grandiflora validates its antimicrobial properties, with the plant showing the ability to act against various Gram-positive and Gram-negative microbes (Packiyalakshmi et al., 2016). Antibacterial and anti-biofilm properties of S. grandiflora extracts are also found in other studies against organisms of clinical importance (Gandhi et al., 2017). The flower extracts were found to inhibit pathogenic bacteria but favoring the growth of desirable species (China et al., 2012). The study of its antimicrobial effects does not only help in combating drug resistance in the world but also helps in the documentation and conservation of ethnomedicinal information, which is becoming an essential element in a rapidly modernizing world where traditional practices are being eroded.

Moreover, the development of digital laboratory instruments, phytochemical analysis and global databases allow researchers to record, analyze as well as share indigenous medicinal practices in a more efficient way. Optimal methods of extraction have been demonstrated to enhance production of bioactive plant compounds including optimized ethanol extraction (Chew et al., 2011). Such an element of technology is consistent with the present tendency to the digital scientific culture, the incorporation of natural science research with the aims of the cultural, societal and educational goals.

Against this background that the current research fills the gaps in microbiology, pharmacognosy, and cultural heritage research by scientifically assessing the presence of phytochemical constituents and antimicrobial action of the Katuray fruit extract. This publication is a contribution to the field of biomedical research as well as the ethnobotanical knowledge of the Philippines, which supports close connection between science and culture.

1.1. Statement Of the Problem

The purpose of the study was to identify the in vitro antimicrobial activity of Katuray (Sesbania grandiflor) fruit extract on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans.

Specifically, this study aimed to:

1) Identify the phytochemical constituents of

Katuray (Sesbania grandiflora) fruit extract;

2) Determine the total mean zone of inhibition of the test organisms subjected to the fruit extracts at different concentrations:
- 100%
- 75%
- 50%, and
- 25%

3) Determine if there is a significant difference between and among the mean zones of growth inhibition of the Katuray fruit pod extract to the test organisms at the following concentrations:
- 100%
- 75%
- 50%, and
- 25%

1.2. Scope And Delimitation

The study was aimed at conducting phytochemical and antimicrobial assessment of the fruit pods of Katuray (Sesbania grandiflora) which is one of the plants in Filipino traditional medicine. The experiment was constrained to find out the activity of the extract on four common laboratory organisms, namely Staphylococcus aureus (Gram-positive), Escherichia coli and Pseudomonas aeruginosa (Gram-negative), and Candida albicans (fungi).

The plants used to gather the Katuray fruit were found in Pantay Daya and Vigan City and certified by the Bureau of Plant Industry of the Department of Agriculture to guarantee botanical correctness. The fruit pods were left to dry in the sun over a period of five days and extraction was performed on the USP-grade 95% ethyl alcohol at the College of Public Health, University of the Philippines Manila. Phytochemical was confined to qualitative analysis.

Preparation of the culture, standardization and disc diffusion method of all microbial assays were done using laboratory strains acquired at the College of Public Health. Mean, One-way Analysis of Variance (ANOVA), and Games-Howell Post Hoc Test were used in the analysis of data. The paper neither tries to measure the phytochemical content or extract any particular compound, nor does it determine toxicity, pharmacokinetics or clinical applicability. Rather, it offers the essential scientific evidence regarding the cultural and ethnomedicinal significance of Katuray, which centers on interdisciplinary additions to the natural science and the preservation of the culture.

2. LITERATURE REVIEW

Medical plants have also been the focus of traditional medicine in many cultures, with various potential medicinal effects that are receiving the scientific focus of contemporary pharmacological studies. In the process, numerous studies have been conducted on Sesbania grandiflora (Katuray) where the phytochemical composition and biological activities of the plant are studied extensively particularly in Southeast Asia. The scientific studies have continued to establish the existence of alkaloids, flavonoids, tannin, saponin, glycoside, steroid, and phenolic compounds in this plant, indicating a possible high medicinal potential. Initial studies discovered all these metabolites in various parts of plants, which verified their possible roles in antimicrobial and anti-inflammatory processes (Arun et al., 2014). Subsequent literature revealed other compounds, including lupeol, sterols, and terpenoid derivatives that have been linked with strong pharmacological effects to include antimicrobial and anti-inflammatory effects (Siddique and Saleem, 2011). A study by Reji and Alphonse (2013) indicated that S. grandiflora has a high number of phenolics and flavonoids in leaves and flowers, which contributes to the high antioxidant power in the plant. In the meantime, research on the active constituents showed derivatives that were active against Mycobacterium tuberculosis and this widened the scope of the medicinal potential of various parts of the plant (Hasan et al., 2012).

The antimicrobial, antioxidant, hepatoprotective and wound-healing activities recorded in numerous pharmacological studies have led to the therapeutic potential of S. grandifloras. These various effects were highlighted in a fundamental overview by Wagh et al. (2009) that helped in the appreciation of the plant as a multi-purpose medicinal resource. It was also reported to have anti-inflammatory effects, and ethanol extracts showed good bioactivity during in vitro tests (Abinaya & Anuradha, 2018). The plant has also been tested to have anticancer activity in addition to antimicrobial and anti-inflammatory properties. In an interesting study, it has been shown that S. grandiflora extracts have cytotoxic capability against Ehrlich ascites carcinoma in mice indicating the potential of its use in cancer therapies (Sreelatha et al., 2011). Reviews that suggest S. grandiflora as a source of valuable bioactive compounds have also shown the potential of the natural product to be used in medicine in a broad sense (Henry Ivanz Boi, 2018). In addition to these results, Ouattara et al. (2011) noted that polyphenols extracted by the plant exhibited significant antioxidant and antibacterial properties indicating the dual purpose of its

phytochemical constituents.

Antimicrobial characteristics of S. grandiflora have undergone serious investigation in several studies. The studies regarding leaf extracts showed an antibacterial property towards clinically important bacterial strains, where the findings indicated that the solvent type plays a crucial role in the antimicrobial power (Padmalochana & Dhana Rajan, 2014). Lakshmi et al. (2011) gave consistent results in their study which indicated that ethanol and aqueous extracts were very effective against Gram-positive and Gram-negative pathogens. Zarkani (2016) also showed that S. grandiflorachloroform extracts exert an inhibitory effect against most microbial species and this confirms the use of this extract in the treatment of infectious diseases. Research against biofilm-forming bacteria concluded that extracts are effective in preventing biofilm growth in Staphylococcus aureus, which showed that the plant has the potential to prevent biofilm-related persistent infections (Anantaworsakul et al., 2017). Parallel studies using silkworm infection models have found major therapeutic in vivo effects of S. grandifloras extracts and have suggested its possible expanded usage in antimicrobial therapy (Anantaworasakul et al., 2017).

The methods used in extracting the bioactive compounds and the analytical techniques are very instrumental in enhancing the ability of bioactive compounds to be isolated in medicinal plants. It has been identified that ethanol extraction is one of the most effective methods to extract alkaloids, phenolics, flavonoids, and other metabolites necessary to have biological activity. The experiments on the extraction parameters proved that the concentration of the solvent used, temperature, and extraction time have a significant effect on the yield and potency of extracted compounds (Chew et al., 2011). Similar to the conventional extraction procedures, the contemporary approaches have highlighted the value of standardization of extractive techniques to enhance the recovery of bioactive constituents reiterating the significance of standard procedures in study of herbs (Gupta et al., 2012). HPLE techniques like HPLC have been successfully applied in the characterization of active constituents in the S. grandiflorum, which have further helped in the scientific confirmation of its therapeutic potential.

The issue of plant-derived compounds in solving the problem of antimicrobial resistance in general, and S. grandiflora in particular can be emphasized further by the broader literature of phytochemicals. It has also been identified that tannin can be used to prevent the growth of bacteria by damaging their cell walls and precipitating proteins (Chung et al., 2010). Surveys of the plant-based antibacterial agents have highlighted the possibility of phytochemicals to be used as alternative or complementary to antibiotics, especially in the face of increasing resistance (Barbieri et al., 2017). And in the same favor, tannic acid and its derivatives have been demonstrated to have high antiviral and antibacterial potentials, which can promote the creation of new antimicrobial substances (Kaczmarek, 2020). Taken together, these results highlight the importance of the traditional medicinal plants and the phytochemicals to fight drug-resistant pathogens.

3. METHODOLOGY

This section provides the design of the study, materials that were used, and the procedures that were followed in the study to the latter. The flow of methodology was designed in such a manner that it is easy to replicate or follow every step of the experiment in a logical manner.

3.1. Research Design

This research was based on the experimental research type using a real laboratory set-up. This type of study used randomization and comparison of the both control and experimental groups. Each group, which was selected and randomly placed in it, was put to either treatment or some form of control. Each of the subjects was administered post-tests to tell whether there was a difference between the two groups. This was close to the best approach although it was wanting in not having a pretest measure. The difference was hard to establish at the onset of the study. Randomization can serve as a way of mixing the subjects together, although it does not fully guarantee that it was actually an equivalent that was being done to each of the two groups.

It consisted of 12 Petri dishes that were disposable and had equal contents of Mueller-Hinton Agar (MHA) and could hold six antibiotic discs. Therefore, 72 samples (6 test solutions with two categories tested in four organisms: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans) were adopted.

The set-ups included:

a. Experimental group -4 concentrations of the Katuray (Sesbania grandiflora) extract:

25%, 50%, 75%, and 100%.

b. Control Group-Amoxicillin (positive control of S. aureus), gentamicin (positive control of E. coli and P. aeruginosa), fluconazole (positive control of C. albicans), and distilled water (negative control).

The experimental design was in accordance with established procedures on assessment of antimicrobial activity under concentration gradient and conventional controls.

3.2. Variables

The independent variables include the source of plant extracts and the concentration of the plant extract.

The zone of inhibition that is seen in the culture media is the dependent variable. This variable structure is traditional dose response evaluation in antimicrobial evaluation. Other experimental factors like incubation temperature, medium to be used and inoculum density were held constant during the procedures.

3.3. Data Analysis

Data analysis involved use of mean, One-way Analysis of Variance (ANOVA) and Games -Howell Multiple Comparison. The average diameter of zone of growth inhibition was tested using mean, ANOVA used to test whether there was a significant difference between and among the mean zones of inhibition and Games -Howell Multiple Comparison was used to test whether the different combinations of groups of the various treatments were significantly different. These were statistical tools that enabled proper comparison of two or more concentration groups and controls.

3.4. Ethical Considerations

The microorganisms employed in this study are very infectious and they pose a danger to humans in case they are handled, transported and disposed carelessly.

manner. This demonstrates the direction of the researchers who will adhere to avoid such harmful circumstances and the methods of safeguarding the environment. Handling. The experimental conduct was taken care of strictly. Wearing of personal protective equipment; long-sleeved laboratory gowns, mask, gloves were used with high stringentness. Since the bacteria used were pathogenic, the researchers were exposed to the minimal number of hours that was at least 3 hours. No other place was used to open the tube that contained the bacteria to be inoculated, subculture, and streaked. Washing of hands was being followed strictly. Disposal. The disinfection was performed on the workplace after the experiment with 70 percent alcohol spray and diluted Lysol solution. The decontamination of all the materials was done in the diluted solution of Lysol treated one hour followed by the washing of the materials with soap and water. Lysol solution was added to the culture media and broth holding the bacteria and discarded. All wastes were buried in the ground. Glassware (such as flasks and petri dishes) was put in an autoclave at 15 psi at 121 degrees Celsius 1 hour then disposed. The following procedures guaranteed safe procedures during handling and disposal of biological materials during the study.

3.5. Data Gathering

3.5.1. Preparation Of Lab Materials

The researchers had ready the following equipment items required in the experiment: four 50mL beakers, forceps, stirring rods, Bunsen burners, laboratory scales, disposable inoculating loops, 1 mL pipettes, 12 disposable Petri dishes, sterile, blank paper disks, and Vernier calipers. All the glass materials used in this study were sterilized at 121 degrees Celsius in 15 minutes using autoclaving. The experiment required the use of autoclave. The plate utilized during culture/isolation and in the susceptibility, testing was prepared by the laboratory staff at the University of the Philippines-College of Public Health laboratory. The testing of the antimicrobial effect of Katuray (Sesbania grandiflora) and all the mentioned types of equipment were facilitated by using and assistance of the UP laboratory at the college of public health, University of Philippines 625 Pedro Gil Street, Ermita Manila. Aseptic conditions and the presence of properly sterilized materials were prepared.

3.5.2. Plant Collection

The researchers gathered the fruit of Katuray (*Sesbania grandiflora*), specifically the ripe ones at Pantay Daya, Vigan City, Ilocos Sur.

3.5.3. Plant Verification and Authentication

Proper identification and authentication of the Katuray fruit were done with sample specimens sent to the Bureau of Plant Industry in Manila. Extraction and further analyses were done using authenticated specimens only.

3.5.4. Ethanol Extraction

The Katuray fruit pods dried in the sun of 500 grams was placed in 500 mL of 95% ethanol and allowed to mix at least 72 hours in constant motion. The solid material was then eliminated and filtration of the mixture was done with the help of Whatman filter paper. Evaporation of the resulting solution was done via rotavap in order to remove the alcohol. The 100% Katuray fruit extract remained as the

solution following the evaporation of alcohol. An extraction of plants was done at UP-College of Public Health. Ethanol was used as solvent, which helped in the extraction of a wide range of polar and semi-polar phytoconstituents.

3.5.5. Preparation For Stock Solution

The plant extract in stock solution was made by dissolving 1 g. of the dried plant extract in 100 mL of water to the final concentration of 10 mg/mL. In our study, 100 Per cent plant extract concentration was equal to 10 mg/mL. The researchers were working with the 10 mL of each extract concentration (100, 75, 50, 25) of the extract. A 10 mL concentration of the extract that was 75 percent was obtained by diluting 7.5 ml of the 10 mg/mL stock solution with 2.5 mL of water. The same technique was repeated with 50% of which 5.0 mL of stock solution was added subsequently to 5.0 mL of water. Likewise, it was likewise done with the 25 percent extract concentration, where 2.5 mL of stock solution was diluted to 7.5 mL of water. To prevent interference and contamination of antimicrobial activity, all the dilutions were prepared in sterile distilled water.

3.5.6. Phytochemical Screening

The dried fruit sample of another two hundred and fifty grams was sent to the UP- Manila College of Public Health where the phytochemical screening test was to be conducted. It is in the UP-Manila College of Public Health where only the qualitative phytochemical test was conducted. The quantitative test was not done because of the financial constraints of the researchers.

Phytochemical constituents identified included, Alkaloids, Carbohydrates, Glycosides, Saponins, Phytosterol, Phenolic compounds, Flavonoids and Proteins.

The suggested tests are the ones that were applied to identify phytochemical constituents of the plant extract.

i. Test For Alkaloids

1. Mayer's test. 2 ml of ethanolic extract was added to a few drops of reagent used by Mayer. The presence of alkaloids is shown by the formation of white or pale-yellow precipitate.
2. Dragendroff's test. Ethanolic extract (5 mL) was added to 2 mL of ethanolic extract followed by the addition of 5 mL of distilled water and 2 mL of hydrochloric acid until an acidic reaction took place. 1mL of reagents that were used by Dragendroff was added to this. A precipitate is formed that is orange or orange red, which means that there are alkaloids.
3. Hager's test. A few drops of Hagers reagent were added to 2 mL of ethanolic extract that was taken in a test tube. The presence of alkaloids is proved by formation of yellow precipitates.
4. Wagner's test. A volume of 2 ml of ethanolic extract was acidified with 15% v/v of hydrochloric acid and a few drops of Wagner reagent added to it. The precipitate is yellow or brown; the presence of alkaloids.

ii. Test For Carbohydrates

1. Molisch's test. Ethanolic extract of 2 mL was mixed with 10 mL of water and then filtered and the filtrate was concentrated. This was combined with 2 drops of freshly prepared 20 percent alcoholic solution of a-naphthol such that a layer was formed at the bottom of the mixture with the concentrated solution of sulfuric acid of 2 mL. The color changed to red violet ring meaning that carbohydrates were present and disappeared when too much alkali was added.
2. Fehling's test. Ethanolic extract of 2 mL was mixed with 10 mL of water and then filtered and the filtrate was concentrated. 1 mL of Fehling solution A and B were then added to this and boiled few minutes. Red or brick red colored precipitate is formed, which is evidence of the presence of reducing sugar.

iii.Test For Glycosides

1. Modified Borntrager's test. Boiled 200 mg of the test material and 2mL of diluted sulfuric acid. Take 2 mL of the solution and combine with ferric chloride solution during 5 minutes. Since some of the plants have anthraceneaglycone in the reduced form, when ferric chloride is involved in the extraction procedure, oxidation to anthraquinons occurs and this reacts with Bornhagers test.
2. Kedde's test. Chloroform mixed extract dried at full volume, then 1 drop 90 per cent alcohol and 2 drops 2 per cent 3,5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid keddes reagent). Brought to alkalis using 20% sodium hydroxide solution. A purple color was produced. The color development with 3.5 dinitrobenzoic acids is due to the existence of B-unsaturated-o lactones in the aglycone.

iv. Test For Saponions

1. Froth's test. A drop of sodium bicarbonate solution was put in a test tube of approximately 5 mL with ethanolic extract. The test was aggressively shaken and left at 3 minutes. The presence of saponins is reflected by formation of honeycomb-like froth.
2. Foam test. Dry 6.0 ±0.1 g of foaming agent powder, 3.6 ±0.1 g of sugar and 2.4±0.1 g of instant coffee in a 250mL beaker. We added 80 ± 1 °C 150mL water in the beaker. Instantaneously swirled the beaker contents with a teaspoon during 10 seconds with 2 cycles of 5 full rotary turns clockwise, 6 full rotary turns anticlockwise. Measure the mm liquid on the top of the foam in the liquid after 3 and 15 min.

v. Test For Phytosterol

1. Salkowski's test. Two mLs of extract were shaken using chloroform. One added slowly sulfuric acid on either side of the test tube to the chloroform layer. Development of red signaled an existence of the steroids.
2. Libermann Burchard test. 2mL of the extract was dissolved in acetic acid anhydride that was heated up until it became boiling, afterwards 1 mL of concentrated sulfuric acid was added to all sides of the test tubes. The appearance of green color means that there are steroids.

vi. Test For Phenolic Compounds

1. Ferric chloride test. When 2 mL extract is combined with ferric chloride solution of 1% it appears blue green or brownish green.
2. Gelatin test. Two mL extracts are added to several drops of 1% solution of gelatin that has 10% sodium chloride that produces white precipitate.

vii. Test For Flavonoids

1. Alkaline reagents test. With drops of sodium hydroxide solution extracts were treated. Intense yellow color formed and turns colorless on addition of dilute acid is the evidence of flavonoids.
2. Lead acetate test. A few drops of 10 percent lead acetates which produce yellow precipitates are added to alcoholic solutions of the extract.

viii. Test For Proteins

1. Xanthoproteic test. Take the 2 mL of the tested matter and put it to the test tube. Add 1 mL of strong HNO3 to the tube.

These qualitative assays collectively provided a phytochemical profile of the fruit extract.

3.6. Laboratory Procedures

3.6.1. Preparation Of Mueller–Hinton Plates

Mueller-Hinton Agar (MHA) (powder) was made by suspending 336.0 g of powder in 1 liter of purified water. The agar was well blended. The heating was on constant agitation and the agar was boiled after 1 minute to ensure that no remains were left behind. Subsequently, agar was dispensed in the agar plates and allowed to solidify at room temperature followed by storage at 4 to 8 °C.

3.6.2. Preparation Of Test Organisms

Use of bacteria was as follows; Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. The test organisms were procured at the University of the Philippines - College of Public Health laboratory. The organisms, i.e., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans were streaked in Blood Agar Plate and MacConkey agar plate, first to culture and isolate them. The culture plates were allowed to incubate at 37 °C in 24 hours. After 24 hrs, using a sterile applicator stick, 4-5 isolated colonies of the organism of the prepared culture plates were isolated and suspended in 2 mL of sterile saline solution and the saline tube was vortexed to ensure a smooth suspension was made. The suspension was then compared with vortexing to McFarland 0.5 turbidity standard after vortexing. Turbidity of the suspension was corrected by the addition of additional colonies in case the suspension is light or dilution with a sterile saline in case the suspension is heavy. This standardization played the role of having a consistent density of the inoculum in all tests of susceptibility.

3.6.3. Inoculation Of Organism to the MH Plates

Mueller-Hinton plate was streaked with the help of a sterile swab. It was streaked three times on the entire agar plate. The agar plate was then streaked followed by the plate being rimmed to clear the streaked plate of the remaining inoculum that was splashed out in the streaking process. The slide of the plate was left slightly open and left to be exposed to room temperature of 3-5 minutes. Six agar plates were used to each bacterium and fungus. Pure concentration, 75% concentration and 50% concentration were done on the first plate, the second

plate, and the third plate respectively. The 25% concentration was performed on the fourth plate and the positive control was done on the fifth plate and the negative control antibiotics on the sixth plate.

3.6.4. Preparation Of Antimicrobial Katuray (S. Grandiflora) Disc

The diluted solution in the UP-Manila College of Public Health was placed into the six millimeter (6mm) sterile blank discs to impregnate them. The solution extracted in the extract was based on the concentration of the one indicated on the sterile blank disc (e. g. 25%, 50% 75%, 100%). Prior to the application on the MH plates, all discs were completely dried. Each plate had six filter discs at the same distance. Each disc was gently pressed. The fruit extract content that is to be used will be 25, 50, 75, and 100 per cent. The discs were dried before they could be placed on the agar surface to avoid any dilution or spreading of the extract on the surface.

3.6.5. Incubation Of Inoculated Plates

The disks of MHA plates inoculated were incubated at ambient temperature (35 0 C) of approximately 16 to 18 hours. To prevent condensation of plates on the agar plate, the plates were incubated in an inverted position.

3.6.6. Antimicrobial Susceptibility Testing

The researchers used Vernier caliper to measure the diameter of disks in millimeters of the zones of inhibition. When the diameter of the zones of inhibition is not possible to measure because of overlapping of other zones of the antibiotic disks, the radius of the zone can be measured and the same multiplied with 2 to calculate the diameter of the zone of inhibition. All measurements were done at the expiry of the incubation period to allow growth inhibition to be completely expressed.

4. RESULTS

This section presents the complete results of the study, including qualitative interpretations, statistical analyses, phytochemical findings, and antimicrobial activity measurements. All data generated from the experiments are reported unchanged, with structural improvements added for clarity.

4.1. Interpretation Of Results

The qualitative interpretation of the set of samples was based on measuring the growth inhibition of bacteria. The researchers have compared the effect of the bacterial growth inhibition with the various concentrations of the fruit extract also with the positive and negative control.

To be standardized, the following interpretative range of standard zones were used (see Table 1):

Table 3: Interpretative Range of Standard Zone of Inhibition.

Zone of Inhibition (mm)	Zone Diameter Interpretative Criteria (mm)
≥ 21	+++: Strong Inhibitory Activity
14-21	++: Moderate Inhibitory Activity
7-13	+: Weak Inhibitory Activity
6	-: No Inhibitory Activity

4.2. Statistical Treatment of Data

The statistical instruments applied in the treatment of the data collected in this study were as follows:

1. Mean. The average diameter of growth inhibition of the three test organisms was determined using this, and the description of the antibacterial activity of the Katuray plant extract was done.
2. ANOVA. This was employed to ascertain whether there are any significant differences that lie between and among the mean zone of inhibition of the various concentrations of the plant extract that were exposed to the test organisms.
3. Games-Howell Post Hoc Test. This was employed to identify which pairs out of the pairs are statistically significant.

4.3. Disposal Of Utilized Culture Media and Test Solutions

During the process of carrying out the experiment, the researchers ensured that all safety measures were taken to the maximum to ensure that the bacteria would not be contaminated. The safety of the researchers was given priority, as well as the other people and the environment because the bacteria to be used were extremely infectious. Following the experiment, 70 percent alcohol spray and watered-down Lysol solution were used to disinfect the workplace. The decontainment of all the materials was done using the diluted Lysol solution in one hour after which they were washed with the soap and water. The Lysol solution was added into the culture media and the broth containing the bacteria and allowed to flood overnight and then

buried underground. All of the disposable items were buried in soil. Glass (such as flasks and petri-dishes) was autoclaved at the conditions of 15 psi and 121 degrees Celsius during 1 hour and then washed using detergent and water.

4.4. Overview Of Study Outcomes

The objectives of the study were to establish the antimicrobial in vitro activity of the fruit extract of Katuray (Sesbania grandiflora) on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. Particularly, the study objectives were to: (1) Find out the phytochemical components of Katuray (Sesbania grandiflora) fruit extract; (2) Find out the total mean zone of inhibition of the test organisms that are exposed to the fruit extracts at the different concentrations: (100%, 75%, 50%, and 25%); (3) Find out whether there is significant difference in selectivity and specificity between and among mean zone of growth inhibition of the Katuray fruit pod extract to the test organisms at

Pantay Daya, Vigan City was the location where the plant specimens were collected. The only fruit used was that of the Katuray. The plant sampled was dried in the sun after five days. To establish the authenticity of the plant, it was identified and authenticated by the Bureau of Plant Industry, through the Department of Agriculture in Manila, to certify the name of the plant scientifically. The extraction and screening of phytochemicals of the dried Katuray fruit and alcohol were performed at the College of Public Health, University of the Philippines, Manila. The ethyl alcohol used in plant extraction was of USP Grade, 95%. The phytochemical analysis was also limited to qualitative analysis only.

The organisms used in the experiment were obtained in College of Public Health, University of the Philippines, Manila. These were Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa to represent gram-positive, gram-negative bacteria and Candida albicans to represent fungi.

The statistical instruments that were applied to treat the data included Mean, One-way Analysis of Variance, and Games-Howell Post Hoc Test.

4.5. Phytochemical Constituents and Antimicrobial Activity

Phytochemical assay of fruit pod extract Sesbania grandiflorum indicates low levels of alkaloids, saponins, tannins and flavonoids, carbohydrates, reducing sugars, and phytosterols. Anthraquinone glycosides traces were also identified. These phytochemical findings are the basis of the antimicrobial findings presented in Table 2, showing the mean of the zone of growth inhibition of the various concentrations of the extracts (100%, 75%, 50% and 25%) with the test organisms.

Table 2: Mean Zones of Inhibition (Mm) of Katuray Extract at Different Concentrations.

	Test preparations					
	25%	**50%**	**75%**	**100%**	**Negative control**	**Positive control**
Test organisms	**Mean Zones of Inhibition (in mm)**					
Staphylococcus aureus	7.1	8.89	11.44	12.77	6	21.33
Escherichia coli	9.4	12	13.9	17	6	24.78
Pseudomonas aeruginosa	10	12.3	13.7	17.2	6	24.78
Candida albicans	8.11	10.33	12.67	16.33	6	24.78

As shown in Table 2, all organisms exhibited increasing zones of inhibition with increasing extract concentrations.

4.6. Statistical Analysis of Antimicrobial Activity

4.6.1. Anova Results

Notable Difference between the Zone of Growth Inhibition of the various Concentrations of the Katuray (Sesbania grandiflora) fruit pod extract to the Test Organisms.

The outcome of ANOVA revealed that they all have significant difference among and between the mean zone of growth inhibition of the Sesbania grandiflori fruit pod extract on the test organisms at the following concentrations: 100%, 75%, 50%, and 25%.

ANOVA analysis showed:

1. *Staphylococcus aureus*, Df (5, 12) = 2080.431, $p<0.05$. (reject H_0)
2. *Escherichia coli*, Df (5, 12) = 1136.224, $p<0.05$. (reject H_0)
3. *Pseudomonas aeruginosa*, Df (5, 12) = 2404.006, $p<0.05$. (reject H_0)
4. *Candida albicans*, Df (5, 12) = 646.068, $p<0.05$. (reject H_0)

4.6.2. Games–Howell Post Hoc Test

The GamesHowell Post HOC Test outcome revealed that there is a substantial variation amongst

diameters of the zone of growth inhibition induced by the varying concentrations of the Sesbania grandiflora fruit pod extract on all the test organisms with the exception of the findings on the diameters of the zone of growth inhibition between the Negative Control and the 25% extract, the 25% and the 50% extract, and the 75% and the 100% extract against Staphylococcus aureus where no significant variation were noted.

5. DISCUSSION

The given research was focused on the analysis of the phytochemical composition and antimicrobial activity of Sesbania grandiflorum fruit pod extract against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. The screening through the use of phytochemicals identified the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, reducing sugars, phytosterols, and anthraquinone glycosides traces. They are also well known to be involved in antimicrobial action because of the ability to disrupt microbial cell walls, enzyme inhibition or protein complexation. Their presence of these bioactive constituents is one of the reasons why the antimicrobial patterns were observed during the disc diffusion assays.

The antimicrobial activity outcome showed a definite dose dependence reaction with the highest level of extract concentration (75% and 100%) giving out the greatest inhibitory areas in all test organisms. Higher concentrations showed reduced activity but still had some measureable inhibition indicating the extract may have compounds with a broad spectrum of effects. At higher concentrations, the bacteria E. coli and P. aeruginosa were relatively more susceptible which is interesting since Gram-negative bacteria in general are more resistant to it owing to the structure of their outer membrane. In the meantime, S. aureus was moderately inhibited throughout the concentrations and C. aureus antifungal sensitivity, though observed, was less than that of fungal pathogens, which is in line with the established challenge of inhibiting fungal pathogens.

These results support the larger data of the antimicrobial properties of S. grandiflora and phytochemical-containing extracts of plants. The medicinal value of this plant has been emphasized in previous research, which revealed antibacterial, anti-inflammatory, and healing effects that are mostly present in the leaves and flowers of this plant (Arfan et al., 2016). The necessity of using new antimicrobial agents, which has been stressed by the global health authorities, is the reason why the compounds of plant origin can be seen as a valuable alternative or a supplement to the current policies in antimicrobial resistance treatment (D'Andrea et al., 2019). The activity was also consistent with the realization that the plant phytochemicals (e.g., flavonoids, tannins, saponins, etc.) tend to inhibit bacterial growth, which is also supported by the research on phytochemical activity of other edible and medicinal plants (Hochma et al., 2021).

Moreover, the antimicrobial and antimicrobial adhesive capabilities of S. grandiflora mentioned in the earlier study by Guzman et al. (2018) support the current research study, especially in demonstrating the preventing properties of the extract against pathogenic microorganisms. The traditional ethnopharmacological approach justifies the traditional medicinal worth of this plant as reported by Patra et al. (2020) who reported the significance of phytochemical diversity of medicinal plants and their biological uses. In assessing the change patterns of antimicrobial responses, the interpretations standards that were applied in this research comply with the recognized thresholds of the method of testing susceptibility in case of Clinical and Laboratory Standards Institute (CLSI, 2015; CLSI, 2017), methodological rigor and reliability.

Along with these encouraging findings, a number of limitations should be mentioned. To begin with, phytochemical screening was carried out in a qualitative and not a quantitative manner; hence, the specific concentration of the bioactive compounds is still not known. This restricts further correlation study of individual phytochemicals and antimicrobial activity. Second, fruit pods of S. grandiflora were only tested and other body parts like leaves, barks, and flowers might harbor varying or stronger phytochemical compounds. Third, even though the disc diffusion technique is popular, it does not give minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) values, which would give more accurate information about antimicrobial potency. Also, the research did not test the possible cytotoxicity, which should be done prior to the therapeutic uses.

This research has important implications both in scientific and cultural fields. Scientifically, the proving that S. grandiflor fruit extract has antimicrobial properties towards bacterial and fungal pathogens, enhances the accumulating amount of literature that medicinal plants could be a possible source of new antimicrobial agents. The findings support the traditional Philippine ethnobotanical knowledge, which is that Katuray has

been used historically in the local health practices. This kind of discovery facilitates conservation of indigenous medicinal heritage as it encourages sustainable utilization of natural resources.

The limitations experienced in this study are to be dealt with in future research. Quantitative phytochemical analysis is also suggested to identify the levels of such principal compounds as flavonoids, saponins, tannins, and alkaloids. These compounds would be isolated and purified in order to enable researchers to determine the exact constituents of antimicrobial action. Additional research ought to be done to assess antimicrobial potency using other parts of the plants to compare the stems, leaves, flowers and the bark. Simpler antimicrobial tests like MIC, MBC, and time- Kill tests must also be carried out in order to have a more enriched and detailed pharmacological information. Furthermore, cytotoxicity and in vivo procedures are necessary to identify safety, therapeutic possibilities and potential S. grandifloras extracts use in pharmaceutical or nutraceutical practice.

6. CONCLUSIONS

Depending on the results of the experiment, the researchers were able to conclude that the fruit pod extract of Katuray (Sesbania grandiflora) comprises a variety of phytochemical compounds, such as alkaloids, saponins, tannin, anthraquinone glycosides, flavonoids, carbohydrates, reducing sugars, and phytosterols, which is likely to lead to the antimicrobial activity of this extract. The extract showed weak to moderate inhibitory activities of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans, which reveals that the following organisms are in vitro susceptible to different extract concentrations. The statistical tests proved that there is a significant difference between the inhibition zones that were generated by the four concentrations and this created a definite dose response relationship whereby the larger the concentration, the larger the antimicrobial effect. The 100% extract always had the broadest zones of inhibition, although all positive controls had a higher value than the extract at both concentrations, which underscores that even though S. grandiflora fruit pods have potential antimicrobial activity, they are currently not comparable to standard pharmaceuticals. On the whole, the research confirms the scientific validity of traditional knowledge about Katuray and its medicinal properties to the existing discussion about natural products, cultural heritage, and the discovery of alternative antimicrobial resources. These findings highlight the need to adopt more interdisciplinary studies that would facilitate the crossing of the gap among laboratory science, ethnobotanical knowledge, and sustainable health innovation strategies.

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