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Sugarcane (*Saccharum officinarum*) Bagasse with Coconut (*Cocos nucifera L.*) Water as an Alternative to Sabouraud Dextrose Agar for the Cultivation of *Candida albicans*

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Abstract*— Saccharum officinarum, commonly known as sugarcane is an agricultural crop that is mainly used for sugar production, while Cocos nucifera L. or known as coconut water is a natural beverage that contains carbohydrates and protein, which are all needed for the growth of fungi. The aim of the present work was to evaluate the potential of sugarcane bagasse with coconut water in supporting the growth of Candida albicans to produce a cheaper alternative to Sabouraud's dextrose agar (SDA) using an experimental design. The experiment was performed at Department of Science and Technology 02 – Regional Standards and Testing Laboratory. This study used different ratio concentrations of sugarcane bagasse and coconut water mixture (75:25, 50:50, and 25:75), each concentration contains 4g of glucose from powdered sugarcane bagasse and coconut water 76.5mL and, 63mL, and 29.5mL of distilled water, respectively and are compared to the control SDA. The *Candida albicans* was inoculated in every mixture and evaluation of the colony on the first to seventh day to macroscopically observe the colony diameter of *Candida albicans*. Data was analyzed statistically using One Way ANOVA continued by Post hoc test. Since the p-value is .000 and is less than the alpha value of 0.01, the results showed that the mean colony diameter of the treatment is significantly different for at least one treatment. The Post hoc analysis test result revealed that 50:50 with a p-value of 0.0001* and 25:75 sugarcane bagasse and coconut water mixtures with a p-value of 0.0001 have a significant difference with negative control, while gave no significant value with SDA. This result proves that the mean fungal growth of *C. albicans* is influenced by the various concentrations of the sugarcane bagasse and coconut water. The upcoming studies may also include the colony factor unit and microscopic morphology of the *C. albicans.

Keywords*— *Sugarcane bagasse, Coconut water, Sabouraud's dextrose agar, Fungi, Candida albicans, Growth medium

I. INTRODUCTION

Fungi are a common type of microorganisms that significantly impacts the environment. It benefits both humans and the environment, yet on many surfaces, they may also spread diseases and contaminants (Hasanah et al., 2017). Eukaryotic fungi can be found in soil, animals, water, plant waste products, or other surfaces. Fungi can be simple single-celled microorganisms like yeasts or creatures with multiple cells like filamentous (surface molds), mushrooms, and toadstools (Hernandez & Martinez, 2018). Fungi are typically cultured on highly costly dextrose agars like potato dextrose agar (PDA), Sabouraud Dextrose Agar (SDA), or cornmeal agar (CMA). For their survival and growth, all fungi need carbon, nitrogen, and resources for energy (Sidana & Farooq, 2014). One of these organisms is *C. albicans*, a pathogenic fungal infection that resides in the digestive and genitourinary systems of around 70% of people and 75% of women as a harmless commensal. However, in immunocompromised patients, specific immunologically compromised individuals, and occasionally healthy people, it develops into a chance of infection. (Kabir et al., 2012). Thus, various media for fungal culture was successfully developed to diagnose fungal infections and produce a rapid and accurate treatments to a certain disease (Hong et al., 2017).

Coconut water is widely consumed worldwide because of its health benefits which is also a nourishing and appetizing beverage. The field of plant industry, biomedical fields and technology depends on the various chemical components that contribute to its bioactivity (Rethinam & Krishnakumar, 2022). Coconut water constitutes of carbohydrates and protein which can serves a whole nutrient content required to the cultivation of fungi (Elfarisna & Saskiawan, 2019). Since the high level of

carbohydrate can promote growth of microorganism like fungi (Basalamah et al., 2018). Another factor that influences the growth of fungi is the pH of the culture media (Anindita et al., 2024). As the pH affects the rate of growth of fungi however it can still grow at the range from 2.1-11.2. Also, temperature is another factor which considers the optimal growth at approximately 33°C but can grow in lower temperatures from 10–12°C and higher temperatures from 50–55°C (Oyedeji et al, 2023).

The coconut water content which allows production of sclerotia, a soil-type of fungi is unknown however the growth of morel can be done using the endosperm of coconut. This demonstrates that coconut water can be used as a cheaper alternative for the cultivating morel and producing a high-quality formation of sclerotia. The effect of coconut water was characterized through subjective observational analysis and different parameters conforming to variations of nonlinear models. With the robustness property of the model, characterization of the growth of morel is presented demonstrating the effects in the culture broth of the coconut water (Evangelista et al., 2021).

The coconut extract's nutrient content comprises of water, protein, lipid, sucrose, glucose, fructose, and many others. This amount of nutrients allows the growth of the pathogen like fungi. The glucose content of *C. nucifera* varies in characterization of young versus mature coconut water. With the coconut's 7 to 8 months of maturity, there is an adequate number of sugars and nutrients and continuously decreasing as it matures more (Shubhashree et al., 2014). Water from young coconuts contains 2.18 grams if glucose per 100 mL, while mature contains 1.62 g (Burns et al., 2020). Wherein, according to Wan et. Al, the water content of young coconut contains 94 grams per 100 grams which is supported by the study of Obu (2020), coconut water contains 94% water. Moreover, the Mucor specie is cultivated by the coconut thrice more than the SDA which means that coconut has more necessary components for the growth of Mucor specie, a fungus found in soil and digestive system as a culture media compared to SDA. Hence, for the cultivation of Mucor, low-cost coconut-based media can be utilized as an effective substitute for commercially available media (Sathiyavimal, 2014). In similar study, coconut water shows to be an alternative media for the growth of *Aspergillus flavus*. With the maximum growth of *A. flavus* in a highest concentrations of coconut water which is 100% while the optimum concentration is 70%. This demonstrates that coconut water can be used as a substitute media for SDA in the growth of *A. flavus* (Ferreira et al., 2021).

Sugarcane is one of the primary agricultural crops in the Philippines with a reported number of 23 million tons of average yearly production from the year of 1990-2017 (Gudia et al.,2023). The cane juice from the sugarcane stem is extracted to make ethanol or sugar which produces a byproduct called bagasse (Canilha et al. 2012). It can be used as a potential substrate for the organism to acquires nourishment and allow synthesis of microbes of value-added goods such enzymes, amino acids, organic acids, pharmaceutically significant chemicals. As this is product usually utilized for the purpose producing invertase which is needed for sweetener development (Veana et al., 2014). It also has a carbon necessary for the growth of fungi (Linda et al., 2019). Moreover, due to its high-availability and satisfaction to the requirements for the fungal growth, it can be as a potential source of material for developing a new fungi medium which makes a cheaper media compared to various expensive media such as potato dextrose agar (PDA), SDA, or cornmeal agar (CMA)

Globally, sugarcane bagasse is produced as agricultural waste biomass during sugarcane processing. Due to its natural availability, researchers have utilized this biomass for several purposes, including energy and environmental sustainability. In addition, Philippines is reported to be one of the significant producers of sugarcane globally (Khoo et al.,2018). The sugarcane bagasse composed of 60-80% carbohydrates which is a necessary requirement for the growth of fungi in a media like SDA. As these major constituents provide an energy, carbon, and phosphorus for the cultivation of fungi. It could also serve as a carbon source for filamentous fungi to proliferate (Ferreira et al., 2021). Furthermore, sugarcane bagasse could satisfy all these needs, serve as a fungal growth medium, and replacement for expensive market media (Sidana and Farooq, 2014). One of the most widely grown crops in the Philippines is coconut fruit or scientifically known as *Cocos nucifera L*. The nation is regarded as one of the top fruit growers in the world and produces 347 million metric tons of coconuts yearly (Burns et L., 2020). Coconut water, the liquid element of the fruit known as the coconut, is one of its components. It is a natural, hydrating beverage rich in minerals, vitamins, and phytohormones while low in calories and fat (Prades et al., 2012). As one of the main contents of coconut water is sugar specifically glucose, sucrose, and fructose which all acts as a source of carbon influencing the growth of microorganism (Sekar et al., 2013). As the amount of sugar can influence the growth of the fungi making the coconut water a suitable media for the cultivation of fungi. Thus, to cultivate the growth of *C. albicans*, the study intends to create an alternative selective medium to replace SDA, utilizing locally accessible resources like sugarcane bagasse and coconut water.

II. METHODS

The researchers utilized an experimental design in this study. This research was conducted at the Department of Science and Technology 02 – Regional Standards and Testing Laboratory within two weeks. The procedure was performed in the DOST–02, as well as the incubation period and observation of the growth of *C. albicans*.

A. Gathering and preparation of plant and microbial specimen

The researchers obtained the fifteen pieces stems of sugarcane from Santo Tomas, Isabela, and the coconut was brought to Tuguegarao City, Cagayan. *C. albicans* subculture was requested at Cagayan State University.

a) Coconut water. Collected coconut water was filtered to remove any contaminants using standard filter paper (Prado et al., 2015). The filtered coconut water was transferred to a sterile container to avoid contamination before use (Aysha et al., 2012).

b) Sugarcane bagasse. Sugarcane bagasse was treated with steam under pressure and heated to generate bagasse powder. The powder was placed in a cloth carry bag before being placed in a cabinet drier and dried in the cabinet drier for 20 minutes at 70 degrees Celsius. The finest material was gathered and dried for 24 hours before being used in the sedimentation process to remove the pith component of the bagasse. A blender was used to powdered the bagasse. This was adapted from the study of Sidana and Farooq (2014).

B. Agar preparation

Four concentrations of the sugarcane bagasse and coconut agar were prepared, this includes 75:25, 50:50, and 25:75. Each concentration contains 3.455 g, 2.91 g, and 2.365 g of powdered sugarcane bagasse; 25 %, 50 %, and 75 % of coconut water with water content of 23.5 mL, 47 mL, and, 70.5 mL added with 76.5 mL, 63 mL, and 29.5 mL of distilled water, respectively. To check the pH level to 5.6 ± 0.2. Combined each concentration, adding 1.5 grams of bacteriological grade agar and 1 gram of peptone into a beaker and mixed the solution thoroughly.

Afterward, the prepped agar solutions were autoclaved at 121 degrees Celsius for 15 minutes. The agars were cooled to 45 to 50 degrees Celsius before outing on the petri dishes and labelling accordingly. Each concentration was added to the Petri dishes and allowed to sit until it solidified (SDA Fungal Medium: Introduction, Principle, Composition, Preparation, 2020).

C. Dilution, Inoculation, Incubation and Observation of C. albicans

C. albicans are obtained from a subculture which is performed under aseptic conditions. The *C. albicans* suspension was prepared using a sterile 0.85% of normal saline solution (NSS). The density was also adjusted to a turbidity of a 0.5 McFarland standard which is $1X10^6$ to $5X10^6$ CFU/mL (Pootong et al., 2017). The diluted *C. albicans* was incorporated into the medium using a disposable applicator stick (Kutama et al., 2022). The medium was placed in the incubator after the inoculation and the incubator was set at room temperature. The observation of the *C. albicans* was done every 24 hours to check the growth and on the seventh day, the growth, as indicated by the colony diameter, was observed (Widhorini et al., 2021).

D. Measuring the Colony in Diameter

The researchers used the vernier caliper to measure the diameter of biggest circular colony of the fungi. After the incubation, using the vernier caliper, the colony growth diameter of *C. albicans* was measured on the seventh day (Widhorini et al., 2021). The colony of each replicate were measured in millimeters and recorded.

E. Data Analysis

In this analysis, One-Way Analysis of Variance (ANOVA) was used to determine if there is a significant difference between the growth of *C. albicans* using SDA and sugarcane with *Cocos nucifera L.* agar. For an in-depth analysis, Tukey Honestly Significant Difference (HSD) was used to compare the fungal growth of the three treatments with the negative control and the commercialized media to consequently determine which treatment can be used as an alternative to SDA in cultivating *C. albicans*. Moreover, a significance level of 0.05 was used in this study.

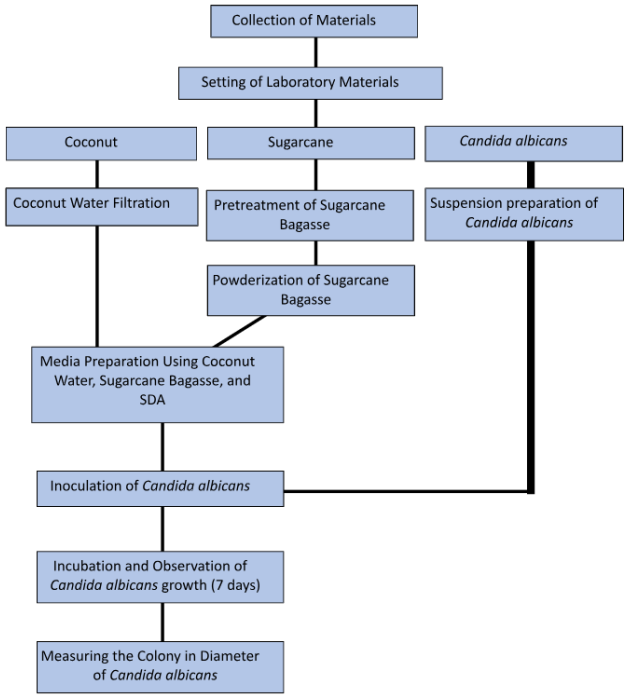

Fig. 1. Methodological Framework followed in the conduct of the study

III. RESULTS AND DISCUSSION

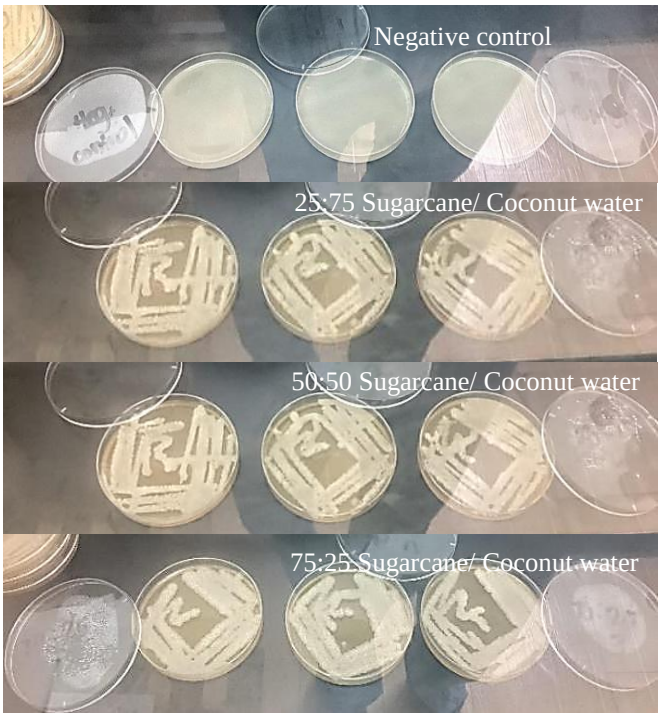

Fig. 2. Colonies of *C. albicans* formed in the different agar preparations

Macroscopically, the *C. albicans* that grew both on SDA and alternative media of sugarcane bagasse and coconut water showed a small, round, cream colored to yellowish, and 2-4 mm in diameter. No fungal or bacterial contamination was seen in any of the treatments prepared.

TABLE I. MEAN COLONY DIAMETER OF *C. ALBICANS* IN THE DIFFERENT TREATMENTS

Treatments	Mean Colony Diameter (mm)
25:75 Sugarcane bagasse and coconut water	3.80
25:75 Sugarcane bagasse and coconut water	3.30
25:75 Sugarcane bagasse and coconut water	1.90
Sabouraud's dextrose agar	3.84
Negative control	0

Based on the data presented on Table 3, there is no colony growth of the fungi in the control after seven days of observation while there are seen growth of the fungi in other treatments including the SDA. In treatment 1, there is a recorded growth of 1.90 mm diameter of fungi colony where the minimum growth is 1.30 mm and the maximum growth among the replicates is 2.24 mm. Treatment 2 posts a higher mean fungal growth of 3.30 mm as compared to the treatment 1 where the minimum growth is recorded at 2.30 mm and the maximum is 4.43 mm. Meanwhile, treatment 3 has the highest fungal growth among the three treatments with a mean value of 3.80 mm with minimum and maximum growth at 3.30 mm and 4.68 mm, respectively. Furthermore, the commercialized medium, SDA remains to have the highest mean growth as compared to the three treatments including the control with a mean growth of 3.84 mm.

TABLE II. SIGNIFICANT DIFFERENT IN THE MEAN COLONY DIAMETER OF THE DIFFERENT TREATMENTS

F-value	p-value	Decision
16.068	0.000*	Reject Ho

***** *SIGNIFICANT AT 0.01 LEVEL*

Table II shows that the mean colony diameter of the treatment is significantly different for at least one of the treatments. Moreover, the table shows that each treatment has different significant value with the negative control and SDA. With the p-value of less than 0.01, the Treatment 2 with 50% sugarcane bagasse and 50% coconut water mixture had a significant difference with negative control with the p-value of 0.001*, while there is no significant difference with treatments 1, 3, and SDA. Treatment 3 media containing 25% sugarcane bagasse and 75% coconut water recorded significant with negative control with the p-value of less than 0.001 and did not show any significant growth with the treatments 1, 2, and SDA. Moreover, SDA has a significant difference with the negative control. To support this, One-way ANOVA with a p-value of less than 0.001 suggests that at 5% level of significance, there is a significant difference among the mean growth of the treatments, control, and the commercialized media in terms of mean fungal growth. In this analysis, treatments that have significantly different mean fungal growth with the negative control are considered to have more growth in terms of cultivating *C. albicans*.

The researchers utilized sugarcane bagasse and coconut water to determine its effectiveness in cultivating *C. albicans*. The results indicate that in terms of macroscopic morphology, SDA and all the treatments of the prepared alternative media are comparable. While in colony diameter, all treatments showed a no significant difference with SDA.

The fungi that were inoculated on the alternative media and SDA was observed macroscopically to confirm the presence of *C. albicans*. SDA is a solid medium that isolates and enhances the growth of pathogenic and non-pathogenic microorganisms, but is naturally acidic inhibiting the growth of many bacteria. Macroscopically, the colony growth of fungi on alternative media appeared to be consistent with the characteristics of *C. albicans* growth in the control medium, SDA, which produced a creamy, smooth, and pasty colonies measuring about 3-4 mm after 48 hours (Sophia et al. 2021). Also, both medium produced a yeast-smelling colony.

Another method used for determining the effectiveness of the alternative media was through measuring the colony diameter. This study showed that at the seventh day of incubation in room temperature, the growth of the fungus was visible in all three treatments and the control. However, the alternative media that showed comparable result with the control (SDA) is the treatment 3 (25:75). This contains the minimum concentration of sugarcane bagasse with maximum concentration of coconut water. In terms of the coconut water content, this result is consistent with the study of Widhorini et al. (2021), wherein, 70% of coconut water is the optimum concentration and 100% for the maximum growth of fungi, which is the *A. flavus*. Their findings suggest that coconut water can be used as an SDA alternative for *A. flavus*, as coconut water contains the nutrient, such as carbohydrate and protein, needed for the growth of the fungi (Elfarisna & Saskiawan, 2019). The fungi use carbohydrates as their source of carbon and energy. High carbohydrate content is required for fungi to grow, which is also the major component (dextrose) of SDA. Another previous study by Ofeh et al. (2019) provided the proximate composition of sugarcane bagasse, which has a 64.70% of carbohydrate, 4.81% protein, 0.20% lipid, and 0.75% ash that have been used as major constituents for the cultivation of filamentous fungi (Prescott et al., 2023). In the same study of Ofeh et al. (2019), the sugarcane bagasse supports the growth of Alternaria and Microsporum species; the

former exhibits rapid and more growth, and utilizes higher proportion of the sugarcane bagasse than the latter.

While previous studies have focused on a single medium constituent and different fungi, the result of the present study demonstrate the potential of both coconut water and sugarcane bagasse in cultivating *C. albicans* that can cause Candidiasis, which were among the top causes of fungal infections in the Philippines (Batac & Denning, 2017). All the treatments prepared contains a higher amount of sugarcane bagasse than the glucose content of coconut water; however, the growth, in terms of diameter, favors the treatment with the highest glucose content of coconut water, which might suggest that the coconut water is more capable in growing *C. albicans* than the sugarcane bagasse. However, based on the findings of similar studies, a more plausible explanation is the difference in the exact nutrient and glucose content of the two constituents used in the study, just like in the sugarcane bagasse composition provided by Ofeh et al. (2019). The biochemical and physio-chemical properties of the coconut water depend on the maturity stage and variety of the fruit. For instance, in young coconut, sugars like glucose and fructose are higher whereas, in mature coconut, sucrose is the predominant sugar (Sekar et al., 2013). In this trial, the coconut water was acquired from young coconut fruit, which is 6 months old. According to Yong et al. (2009) and Burns et al. (2020), the glucose content of coconut water from this age is 2.61g/100mL and 2.18g/100mL, respectively; and in this study, it followed the findings of Burns et al. in 2020. Furthermore, the variation of coconut was not identified.

These factors may cause deviation to the total glucose content of the prepared alternative media (4g/100mL water), resulting into a much better growth since *C. albicans* require nutrients, more importantly carbohydrates, for growth (Sophia et al., 2021); and these nutrients are found in coconut water and sugarcane bagasse.

Due to the lack of data on the approximate composition of the two main ingredient, the results cannot confirm which of the two contributes more to the growth of the fungi. Thus, further investigation on this aspect may be needed. Moreover, the colony count and colony size must be considered when determining the best media (Sophia et al. 2021), and determination of microscopic morphology may also be suggested to confirm and differentiate the growth of *C. albicans*.

IV. Conclusion

Based on the results of the study, it can be concluded that sugarcane bagasse and coconut water can be both used as a cheaper alternative and effective medium for the growth of *C. albicans*. The colony diameter and macroscopic morphology of the various concentration closely resembles the colony on SDA control media. The concentration containing the highest coconut water showed the highest fungal growth comparable to the SDA and rreported that a coconut water has the greater capability to cultivate a fungus compared to the sugarcane bagasse. As the age of the coconut water that was used are tender or young which composed of a considerable amount of glucose content necessary for the growth of the fungi. Moreover, water content could also be a contributing factor because the highest fungal growth contains the least amount of water, hence a more concentrated amount of sugar content present which further promote the growth of fungi. However, the percentage of sugar do not always result to increasing growth rate of fungi as sugars such as glucose and lactose, when mixed with other sugars may reduce the growth of fungi. In contrary, the present study revealed that despite the various number of sugar and nutrients contain by the coconut water and sugarcane, cultivation of the fungal growth was observed. Furthermore, the alternative media provide a less expensive option to expensive commercial laboratory medium for the growth of *C. albicans* due to a naturally available and inexpensive materials needed for the agar. Therefore, sugarcane bagasse and coconut water can be a material of choice for creating a new agar medium for fungi which could substitute Saboroud's Dextrose Agar because of its availability and meets the requirements for fungal growth.

V. Recommendations

The sugarcane bagasse with coconut water agar proved to be a new and cost-effective microbial media for culturing *C. albicans*, hence could be an alternative to saboroud's dextrose agar. Thus, the alternative agar can be released and sell commercially to provide laboratories a cheaper but effective option in the market. Moreover, research laboratories or companies can also apply or use the materials and procedures done in the present study to manufacture the alternative agar and provide a widely access to the agar.

However, future researchers should consider adding more concentrations or treatments for the sugarcane bagasse and coconut water. The growth of the culture should be subjected for colony count and incubation period should be checked from first day until the seventh day. Also, extracted juice from the sugarcane can be utilized for the future research as an alternative nutrient for the growth of fungi. Since we only cultured *C. albicans*, other species are suggested to substantiate the acceptability of this alternative media.

The limitation of this study is represented by the fact only *C. albicans* are exhibited to grow in the alternative media. Moreover, the microscopic morphology of the organism is not presented. The incubation period is only recorded at the seventh day which became one of the controls of the method.

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