Assessment of the Antibacterial Activity of *Crescentia cujete* (Calabash) Ethanolic Fruit Extract Against *Pseudomonas aeruginosa* and *Bacillus subtilis*

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Abstract
Bacillus subtilis and *Pseudomonas aeruginosa* are bacterial species that are known to rapidly develop antibacterial resistance against antibiotics used to treat the infections that they cause. *Crescentia cujete* is a tropical tree that is considered as a herbal plant due to the secondary metabolites that it contains. This study seeks to determine if *C. cujete* ethanolic fruit extract is effective against *B. subtilis* and *P. aeruginosa.* This study also aims to compare if there is a difference in the effectivity of *Crescentia cujete* ethanolic fruit extract in four concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25 mg-mL, as well as its pure extract, against *B. subtilis and P. aeruginosa*. The study follows an experimental research design and utilizes the use of Kirby-Bauer (Disc Diffusion) method in gathering the data. Notably, results show that the *C. cujete* ethanolic fruit extract of different concentrations yield no zone of inhibition against the bacteria except for an insignificant zone of inhibition of pure extract against *P. aeruginosa*. The study concludes that *C. cujete* ethanolic fruit extract is ineffective against *B. subtilis* and *P. aeruginosa* due to lack of inhibitory activity. This study recommends the utilization of a different bacteria and another part of the plant. Additionally, the use of another extraction method may reveal different results.

Keywords: *Crescentia cujete*; *Bacillus subtilis*; *Pseudomona aeruginosa*; antibacterial activity; ethanolic extract

1. INTRODUCTION

Antibacterial Resistance (ABR) is the ability of a bacteria-causing disease to resist the therapeutic effects of antibacterial drugs. It represents a significant threat to global public health, potentially increasing the pandemic expectations in the coming years and resulting in human and economic losses (Allam & Jones, 2020). Hence, researchers are constantly developing new antibacterial treatments effective against multi-drug-resistant pathogens. For instance, *Bacillus subtilis,* although associated with food poisoning and abdominal pain, is primarily used to improve digestive health and immune system function. Similarly, *Pseudomonas* is considered a pressing therapeutic challenge for the treatment of both nosocomial and community-acquired infections as it has shown resistance to antibiotics used to treat bacterial infections (Ibrahim & Sabbar, 2018). Due to the rapid development of bacteria and their resistance to antibiotics, researchers have been looking for alternative medicine due to their nutrients that enhance the immune system, and their ability to fight foreign pathogens that enter the body (Egamberdieva et al., 2017). Because of this, herbal medicines play an essential role in the development of human culture and serve the health needs of millions of people, especially those living in the vast rural areas of developing countries (Hosseinzadeh et al., 2015).

An example of such herbal plant is *Crescentia cujete,* known locally as the Calabash tree, a tropical tree mainly growing in Mindanao and some parts of Luzon and Visayas. It is occasionally cultivated for ornamental purposes in the past, but farms such as Venida Farms in Iligan City started to grow it for other purposes (Merill, 1923, p.447 and Tan, 2019). *C. cujete* is propagated by seed and is well-suited for tropical climates (Gilman et al., 2019). It is also proven that the plant can maximize undersupply of water to grow (Aderounmu & Ogidan, 2019). It was discovered that the tree contains secondary metabolites such as flavonoids, alkaloids, glycosides, saponins, tannins, and terpenoids (Billacura & Laciapag, 2017). Secondary metabolites may aid in treating infectious illnesses which have been resistant to existing antibiotics. They have the potential to give alternative medical therapy to many individuals, particularly in poor nations where people may lack access to healthcare (Ynalvez & Compean, 2014). Moreover, several studies have supported the traditional use of the plant's parts and demonstrated that various parts of the *C. cujete* tree have numerous health benefits. It is attributed to various chemical classes and validated the plants' indigenous usage for various communicable and noncommunicable diseases (Balogun & Sabiu, 2021). Parvin et al. (2015) found that the *C. cujete* plant extract has therapeutic benefits against pathogenic bacteria and may influence disease processes by generating cellular membrane instability.

Despite its reputation as the "miracle fruit", *C. cujete* still lacks research in the Philippines. There has also been no research into the antibacterial properties of its fruit against *B. subtilis* and *P. aeruginosa.* Given these gaps existing in the current scenario, the study intends to determine the antibacterial activity of *C. cujete* towards the inhibiting growth of *B. subtilis* and *P. aeruginosa* to provide medical treatment for bacterial infections involving the bacteria and further research on the fruit extract of *C. cujete*.

2. METHODOLOGY

2.1 Plant Material

The fruit samples of *C. cujete* were obtained from Imus City, Cavite and correctly identified by the Bureau of Plant Industry, Malate, Manila. The *C. cujete* fruit was extracted by the Department of Science and Technology-Industrial Technology Development Institute (DOST-ITDI). Four different ethanol concentrations, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25-mg/mL were done through maceration according to DOST-ITDI Chemical and Energy Division (CED) Work Instructions Manual with modification.

2.2 Preparation of Inoculum and Inoculation of MHA Plates

A 200µL of *P. aeruginosa* and *B. subtilis* suspension was transferred to Tryptic Soy broth, which is adjusted following the 0.5 McFarland Turbidity Standard. The inoculum was incubated for 24 hours at 37°C. A 100µL of the inoculum was spread evenly across the surface of MHA plates.

2.3 Placement of Antibiotic Discs and Incubation of Plates

For treatment groups, blank antibiotic discs were immersed in four solutions with different concentrations and a pure extract. Two antibiotic discs with the positive control and negative control were set to an inoculated MHA plate for each bacteria. The MHA plates were inverted and incubated for a maximum of 24 hours at 37°C.

2.4 Measuring ZOI

The zone edge was read at the point of complete inhibition held about 30 cm from the eye. The inhibition zone diameters were measured to the nearest mL using a ruler.

3. RESULTS AND DISCUSSION

Table 3.1 presents the measured zone of inhibitions (ZOI) of the *C. cujete* ethanolic fruit extracts against *B. subitilis* to determine its antibacterial effectivity against the bacteria. All triplicates of the different concentrations of the extract showed no ZOI. This indicates that all concentrations of the extract do not exhibit an antibacterial effect against *B.*

subtilis. Moreover, there is no significant difference in the effect of *C. cujete* ethanolic fruit extracts on *B. subtilis* in the different concentrations.

Table 3.1

Antibacterial activity of *C. cujete* ethanolic fruit extracts against *B. subtilis*

Treatment	M	Sd	Min.	Max.
10 mg/mL	0.00	0.00	0.00	0.00
5 mg/mL	0.00	0.00	0.00	0.00
2.5 mg/mL	0.00	0.00	0.00	0.00
1.25 mg/mL	0.00	0.00	0.00	0.00
Pure Extract	0.00	0.00	0.00	0.00

Note. $N = 3$ discs.

Table 3.2 shows the average zone of inhibition (ZOI) of the different concentrations of *C. cujete* on the growth of *P. aeruginosa* which will determine its antibacterial property. The average ZOI derived from the triplicates of the study wherein all discs of each concentration had no ZOI present except for the pure extract which had a ZOI of 7 mm. The positive control, Ciprofloxacin, had a ZOI of 35 mm which is subsequently bigger compared to the pure extract but is not statistically significant.

Table 3.2

Antibacterial activity of *C. cujete* ethanolic fruit extracts against *P. aeruginosa*

Treatment	M	Sd	Min.	Max.
10 mg/mL	0.00	0.000	0.00	0.00
5 mg/mL	0.00	0.000	0.00	0.00
2.5 mg/mL	0.00	0.000	0.00	0.00
1.25 mg/mL	0.00	0.000	0.00	0.00
Pure Extract	7.00	0.000	7.00	7.00

Note. $N = 3$ discs.

The lack of zone of inhibition in *B. subtilis* and the small inhibition in *P. aeruginosa* could be due to the phytochemical compounds lacking in the extract. Terpenoids, alkaloids, and phenolics such as flavonoids, phenolic acids, and polyphenols are said to be the main groups of compounds that inhibit the growth of microorganisms (Saxena et al., 2013).

Billacura and Laciapag (2017) noted that there is a slight presence of alkaloids in the crude ethanolic extract of the *C. cujete* fruit. Alkaloids protect the plant by guaranteeing survival against microorganisms through allopathically active chemicals (Saxena et al., 2013). Natural alkaloids can also disrupt the cell membrane of bacteria, prevent protein synthesis, and affect DNA (Yan et al. 2021). Despite the presence of alkaloids, plant extracts may nevertheless have little to no inhibitory capability against different types of bacteria in some instances. In addition to having flavonoids, Hasanah et al. (2018) identified that the fruit extract of *C. cujete* also contained alkaloids and was not as effective as an antibacterial agent. In Mahmood et al. (2019)'s study, *Eurycoma longifolia* also contained alkaloids but it still proved to have little to no inhibition capabilities against various bacteria tested.

Hasanah et al. (2018) also noted that the ethanolic extract of the fruit flesh contains quite the amount of alkaloids and in addition, some presence of flavonoids. Flavonoids are compounds said to have antibacterial properties. They interfere with cell wall permeability which makes it easy for other compounds like phenolics, alkaloids, and triterpenoids to pierce and damage the cell wall thus inhibiting bacterial growth (Anyasor et al., 2011). Other ways wherein flavonoids show antibacterial activity is through directly killing the bacteria, activating antibiotics, and reducing bacterial pathogenicity (Cuhsine & Lamb, 2011). There are instances where in despite the presence of flavonoids, plant extracts could still exhibit little to no inhibition capabilities to different types of bacteria Hasanah et al. (2018) reported that despite the presence of flavonoids in the fruit ethanolic extract of the *C. cujete* fruit, it was not able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. Mahmood et al. (2019) studied five plant extracts and even with the presence of flavonoids in the plant extracts of *Eurycoma longifolia*, *Averrhoa bilimbi*, and *Morus nigra*, they prove to have little to no inhibition against the different bacteria used in the study.

Despite the presence of some antibacterial compounds in phytochemical tests done on the *C. cujete* fruit extract, it was proven that it is less effective as an antibacterial against *B. subtilis* and *P. aeruginosa* than that of the commercially available ciprofloxacin. However, this does not rule out the fruit's potential as an antibacterial, as it has been discovered to contain a variety of antibacterial compounds in its fresh form (Billacura and Laciapag, 2017), and the ethanolic extract is effective against *Vibrio harveyi* (Rahmaningsih, 2017). Similarly, the study of Parvin et al. in 2015 successfully found antibacterial activity in the leaves and stem bark while also utilizing a different bacteria from what is used in this study.

4. CONCLUSIONS

Based on the findings of the study, it has been concluded that the ethanolic fruit extract of *C. cujete* does not exhibit an inhibitory effect on *B. subtilis* but exhibits an inhibitory effect on *P. aeruginosa*, insignificantly. On the other hand, the commercially available antibiotic ciprofloxacin is shown to be more effective for both bacteria, *P. aeruginosa* and *B. subtilis*. Future studies may utilize other pathogenic bacteria. Other plant parts of *Crescentia*, such as the stem, leaves, and roots may be utilized by future researchers to test on *P. aeruginosa* and *B.subtilis*. Moreover, future researchers may also use another extraction method called decoction for the plant part and a different solvent for extraction, particularly methanol. Due to its phytochemical composition, it is recommended to conduct further studies about other biological properties of the *C. cujete* crude extract.

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