International Journal of Research Studies in Education
2025 Volume 14 Number 5, 37-55

ISSN: 2243-7703
Online ISSN: 2243-7711

OPEN ACCESS

Comparative phytochemical analysis and antimicrobial activity of Abaca (Musa textilis [Neé]) processing wastes

Panonce, Marian Grace C. ✉
Visayas State University, Baybay City, Leyte, Philippines (mgpanonce13@gmail.com)
Piamonte, Robelyn T.
Visayas State University, Baybay City, Leyte, Philippines (rtpiamonte@vsu.edu.ph)
Albarico, Genesis C.
Visayas State University, Baybay City, Leyte, Philippines (genesis.albarico@vsu.edu.ph)
Panonce, Celia Natividad C.
Department of Education, Baybay City Senior High School, Leyte, Philippines (celianatividad.panpnce@deped.edu.ph)

Received: 30 December 2024 **Revised**: 27 January 2025 **Accepted**: 15 February 2025
Available Online: 20 February 2025 **DOI**: 10.5861/ijrse.2025.25845

Abstract

The Philippines is the world's best manufacturer of Musa textiles commonly known as abaca, supplying 87.5% of the world's leading demands. However, in an abaca stalk, the farmer could only recover 0.3-0.5 kg of fresh fiber, and the rest would go to waste. Baybay City, Leyte, and other abaca-producing areas also struggle with abaca production and seek additional applications for its waste. Hence, looking for ways to utilize abaca is empirical. The knowledge generated from this research may give us an insight into how we can use the abaca plants processing wastes as resources, such as its antimicrobial properties. This study was conducted to determine the antimicrobial properties of the different parts of abaca (corm, flower, leaves, pseudostem, and roots) against Escherichia coli, compare the antimicrobial properties of the extract by testing it against the bacterial wilt of tomato, Ralstonia solonacearum, using preventive and curative methods, quantify the total phytochemical content of M. textilis extracts in terms of total phenolic and flavonoid contents and evaluate the toxicity of the extracts using yeast cell model. The Results showed that the different parts of abaca can inhibit the growth of E. coli at concentrations of 5,000ppm, 10,000ppm, and 15,000ppm. Minimal differences in preventive and curative data across various extracts and concentrations prevent us from confirming a significant impact. The different parts of the abaca plant were analyzed for phytochemical content, revealing high levels of flavonoids and phenols. Additionally, toxicity tests confirmed all the abaca extracts were non-toxic.

Keywords: Musa textiles (Nee), antimicrobial properties, bacterial wilt, abaca extract, abaca phytochemicals

Comparative phytochemical analysis and antimicrobial activity of Abaca (Musa textilis [Neé]) processing wastes

1. Introduction

Abaca, commonly known as Manila hemp (Britannica, 2014), is mostly grown for its natural fiber in tropical areas such as - Costa Rica, Ecuador, and the Philippines (Kadolph, 2017). Abaca is an important asset to the Philippines which supplies around 87.5 % of the world's requirement for abaca fiber and as such is the world's leading abaca producer. The key abaca-producing areas nationwide are the Bicol Region and Mindoro in Luzon; Leyte, Samar, Negros Oriental, Iloilo, and Aklan in the Visayas; and all the provinces of Mindanao (Vancouver Philippine Consulate General, 2020). However, these key areas including Baybay City, Leyte face an incredible hurdle in terms of abaca production and utilization of abaca's waste. The challenge in abaca production is how to increase farm productivity and look for value, as in a stalk of abaca the farmer could only recover 0.3 - 0.5 kg of fresh fiber. And all the rest will go to waste. The abaca plant % is also composed of 90% water and sap. This sap will go to waste come harvesting of the plant. Hence, looking for ways how to utilize abaca is empirical. Adding technologies so that abaca farmers can increase their income from abaca farming and the development of novel products from abaca and its production wastes is one way to increase the utilization of abaca.

Abaca farming also acts as a source of livelihood for an estimated amount of 200,000 farming families from 56 provinces within the Philippines. And as of recent years, the Bicol Region and Mindoro in Luzon; Leyte, Samar, Negros Oriental, Iloilo, and Aklan in the Visayas; and all the provinces of Mindanao have been the main key abaca-producing areas found in the Philippines. (Vancouver Philippine Consulate General, 2020).

Abaca is a large suckering plant with succulent stalks rising from a perennial rootstock, the true stem or corm. Each stalk comprises tightly wound leaf sheaths and is topped by leaf blades more than one meter long in mature plants. Its stalk contains fiber equivalent to 3-4% of its weight depending on the variety, maturity, and source (Halos, 2008). The Philippine Department of Science and Technology (PhilDOST) and the Philippine Fiber Industry Development Authority (PhilFIDA) regard abaca as the "strongest natural fiber in the world" (Vancouver Philippine Consulate General, 2020). Due to its versatile strength and resistance against salt water, abaca was widely used as ropes, twines, fishing lines and nets, rope cordages, and more. As the abaca industry expanded, so did the products. In the present time, most of the abaca fibers are pulped and processed into specialty papers, which is why the Philippines has such an abundant amount of quality grade papers compared to other countries. Abaca fiber is also used in currency notes, with the Philippine Peso bills made up of 20% abaca and Japanese yen containing up to 60% abaca. Other specialty paper products made from abaca include but are not limited to, tea/coffee bags, cigarette filter papers, sausage casings, adhesive materials, and even surgical masks. With the recent events of COVID-19, abaca was also manufactured into masks to prevent the further spread of the disease (Vancouver Philippine Consulate General, 2020). Abaca is also used to make furniture and has been incorporated into fashion. With the Republic Act No. 9242, otherwise known as "An Act Prescribing the Use of the Philippine Tropical Fabrics for Uniform of Public Officials and Employees and for Other Purposes," having been enacted on February 10, 2004, to strengthen the local demand for tropical fabrics and natural fibers, including Abaca. (Arellano Law Foundation, 2004).

The knowledge generated from this research may give us an insight as to how we can use its antimicrobial properties as a treatment for plants infected by bacterial pathogens such as the bacterial wilt of *Solanum lycopersicum* Linn commonly known as tomato wilt. Furthermore, the antimicrobial compounds in the corm, heart/flower, leaf, pseudostem, root, and seeds will be compared and analyzed to maximize their potential utilization. The general objective of this study is to determine the antimicrobial potential of abaca extracts, whilst using it against Escherichia *coli* the bacterial wilt pathogen of tomato, *Ralstonia solanacearum*. This study specifically aims to determine and compare the antimicrobial properties of the different parts of abaca (roots, leaves, pseudostem, corm, and flower) against *E. coli*, to examine the antimicrobial properties of the extract by testing it against the bacterial wilt of tomato, *R. solanacearum*, using a preventive and curative method, to quantify the total phytochemical content of *M. textilis* extracts in terms of total phenolic and total flavonoid contents, to evaluate the toxicity of the extracts using a yeast cell model.

2. Related literature

2.1 Botanical Description of Abaca

Abaca is a monocot, indigenous to the Philippines, that belongs to the Banana Family Musaceae of the Order Zingiberales, Genus Musa, Section Australimusa. The banana and abaca trees look similar to each other which, at times, causes confusion. However, the banana tree's leaves have a lighter shade of green and a much broader and round shape compared to abaca leaves. The abaca plant is usually smaller than the banana plant, which can grow up to 20-40 ft tall. And in regards to the fruit they bear, the banana fruit is edible, unlike the abaca fruit. (Cindy, 2012). The abaca plant produces up to 25 fleshy, fibreless stalks. Each stalk is about 5 cm in diameter and produces about 12 to 25 leaves with overlapping leaf stalks, or petioles, forming a herbaceous (nonwoody) false trunk that is 30 to 40 cm in diameter. The leaf blade, described to be oblong and bright green on the surface while yellow-green below, can grow up to 1 to 2.5 m in length and 20 to 30 cm in width at its widest portion. The oldest leaves are on the outside and the youngest on the inside, extending to the top, which eventually reaches a height of 8 m (Britannica, 2014).

2.2 Center of Origin

Abaca originally came from the Philippines. Its germplasm found in other countries can be traced back to the Philippines. And different varieties of abaca still possess the same names such as Bongolanon, Libuton, Putian, Sinaba, and Putian. (Castillo, 1956). Filipinos cultivated, processed, traded, and used abaca products for tax payments and enjoyed a century-old monopoly in the world trade market (Corpuz, 1997). Abaca was spread and introduced to numerous countries with similar climates for abaca to prosper and break the Philippine monopoly on abaca. It began when in 1818 an officer from the US Navy, Lt. John White, brought 41 tons of abaca fiber from the Philippines to the United States for the purpose of making marine cordage (Dempsey, 1963). In 1822, an attempt was made to introduce abaca to other countries such as Borneo, Florida, German East Africa, India, and West Indies. But it ended up unsuccessful and commercially unviable (Copeland, 1911).

In 1923, the US Navy began to solely rely on the Philippines to supply them with abaca and use them as raw materials for their marine cordage. This sparked a reaction where the US government introduced abaca to many Latin American countries with a humid, tropical, and warm climate similar to that of the Philippines in able to have a reliable supply of abaca fiber (Spencer, 1953). The Philippine abaca was introduced in Panama, Ecuador, Costa Rica, Guatemala, Honduras, Dominican Republic, Brazil, British and French Guiana, Cuba, Jamaica, Puerto Rico, Martinique, Guadeloupe, Trinidad, Mexico, St. Vincent, Bolivia, Peru, Nicaragua and El Salvador (Dempsey, 1963). In 1925, several abaca plantations were established in British Borneo, Malaya, New Caledonia, Queensland, and Sumatra using abaca seed pieces from the Philippines (Torres & Garrido, 1939). In 1958, abaca was introduced in Vietnam using seed pieces from Costa Rica. However, Among the several countries introduced to abaca, only Ecuador was ever able to produce abaca for export. Abaca was successfully cultivated in Ecuador after World War II when a Japanese abaca farm owner from Davao conducted a field trial. And now, Ecuador supplies an estimated 10% of abaca to the world (PhilFIDA, 2015). While the Philippines remains the world's leading abaca producer, supplying 87.5% of the world's abaca (Vancouver Philippine Consulate General, 2020).

2.3 Uses of Abaca

The Philippine Department of Science and Technology (PhilDOST) and the Philippine Fiber Industry

Development Authority (PhilFIDA) regard abaca as the "strongest natural fiber in the world" (PhilFIDA, 2022). During the 19th century, due to its versatile strength and resistance against salt water, abaca was widely used as ropes, twines, fishing lines and nets, rope cordages, and more. The abaca industry was and still is flourishing to this day with the Philippines as the world's leading abaca producer. However, as the world began to change and evolve, so did the abaca industry. The industry has expanded and has found different ways to utilize abaca over the years. (FAO, 2022).

Most of the abaca fibers are pulped and processed into specialty papers, which is why the Philippines has such an abundant amount of quality grade papers compared to other countries. Abaca fiber is also used in currency notes, with the Philippine Peso bills made up of 20% abaca and Japanese yen containing up to 60% abaca. Other specialty paper products made from abaca include but are not limited to, tea/coffee bags, cigarette filter papers, sausage casings, adhesive materials, and even surgical masks (Vancouver Philippine Consulate General, 2020). During the early stages of the Covid-19 pandemic, a mask mandate was placed in an attempt to lessen the spread of the disease. Abaca was manufactured into a face mask by Salay Handmade Products Industries, Inc., in Misamis Oriental in Mindanao2. The Philippine Department of Science and Technology (DOST) approved and tested the material. The test results revealed that the product could filter 7 times better compared to ordinary cloth materials. (Vancouver Philippine Consulate General, 2020).

Abaca has also been incorporated into furniture and fashion. Abaca furniture. Now in terms of fashion, abaca is used as a textile in making Baro't saya, which is the traditional Filipino wear for women and Barong Tagalog for men (Vancouver Philippine Consulate General, 2020). And on top of that, Republic Act No. 9242, otherwise known as "An Act Prescribing the Use of the Philippine Tropical Fabrics for Uniform of Public Officials and Employees and Other Purposes," was enacted on February 10, 2004, to strengthen the local demand for tropical fabrics and natural fibers, including Abaca (Arellano Law Foundation, 2004).

2.4 Economic Importance of Abaca in the Philippines

The Philippines is regarded as the largest abaca fiber producer, attributing to 87.5% of the world's abaca imports. Abaca farming also acts as a source of livelihood for an estimated amount of 200,000 farming families from 56 provinces within the Philippines. And as of recent years, the Bicol Region and Mindoro in Luzon; Leyte, Samar, Negros Oriental, Iloilo, and Aklan in the Visayas; and all the provinces of Mindanao have been the main key abaca-producing areas found in the Philippines. (Vancouverpcg 2020). During the years 2006 – 2016, for a decade, the country's production of abaca fiber was relatively stable, generating an average of 67,329 metric tons and US$100 million in foreign earnings per year from the different varieties of abaca products. (Vancouverpcg 2020). In the course of the year 2020, the estimated global abaca fiber market size was an astounding USD$420.9 million and is still expected to increase in the upcoming years. (Grand View Research, 2022). While the abaca industry in the Philippines has been steadily expanding over the past years, abaca fiber production is faced with a massive problem, stemming from major abaca growing regions being hit by viral diseases, which need to be handled and addressed accordingly if the Philippines wishes to maintain its title as the world's leading abaca supplier.

2.5 Problems and Concerns in the Abaca Industry

A few years ago, in Leyte alone, the abaca industry underwent a crisis, in which thousands of hectares of abaca were destroyed due to the bunchy-top disease which is considered the most injurious disease among the two other diseases – abaca mosaic and abaca bract mosaic- that affect the country. The loss of income due to the destruction of the crops caused by the bunchy-top disease led to flow-on impacts on households' food security, education, health, and even psychosocial well-being. It also caused the government and research efforts to become more committed to finding means to eradicate this disease (Nuñez, 2013). The challenge in abaca production is how to increase farm productivity and look for value. Adding technologies so that abaca farmers can increase their income from abaca farming. Development of novel products from abaca and its production wastes is one way to increase the utilization of abaca (Sinon et al., 2011), in a stalk of abaca the farmer could only recover 0.3 - 0.5 kg of fresh fiber. And all the rest will go to waste. Hence, looking for ways on how to utilize abaca is empirical.

2.6 Antimicrobial Compounds Found in Abaca

Traditionally, crude extracts are collected from the different parts of medicinal plants, these plant parts may include the fruit, flower, leaves, roots, stems, etc., and were used as herbal medicine to treat and combat human diseases (Khan et al., 2013). These medicinal plants contain several phytochemicals namely, alkaloids, flavonoids, tannins, terpenoids, and more, which hold both antimicrobial and antioxidant properties (Talib & Mahasneh, 2010). Tribes originating from the Philippines have used abaca as a treatment for certain conditions and injuries. The Higaonon tribe of Rogongon, Iligan City from Mindanao uses the sap from young abaca shoots to treat diarrhea, they partially roast it then squeeze out the sap from the shoot and drink the juice thrice a day (Olowa et al., 2012). In Davao, Philippines, the Matigsalug tribe uses the abaca plant for relapse (Guevara & Garcia, 2018).

Studies on the chemical composition of abaca fiber have been conducted in the past several years. In this study, conducted by José C. del Río and Ana Gutiérrez, the leaf fibers of Abaca used in manufacturing high-quality paper pulp production were studied in depth and the results revealed that the total fiber's lignin content was 13.2%. The analysis of the abaca fibers by pyrolysis coupled with chromatography-mass spectrometry (Py-GC/MS) predominantly released compounds that arise from both lignin and p-hydroxycinnamic acids, while also showing high amounts of 4-vinyl phenol. In the presence of tetramethylammonium hydroxide, the pyrolysis of the abaca fibers released high amounts of p-coumaric acid (as the methyl derivative) (Rio & Gutiérrez 2006). p-coumaric acid has dual mechanisms of bacterial activity which disrupts cell membranes and the binding of bacterial genomic DNA that effectively inhibits cellular function, replication, transcription, and expression of bacterial pathogens, which later on leads to the cell's death (Lou et al 2011). The results also revealed after Py-GC/MS of the abaca fibers, products deom p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) propanoid units, with a predominance of the later (H:G:S molar ratio of 1:5:1:4:9) were released. Sinapyl and coniferyl acetates were also found in the abaca. The most predominant compounds found in the abaca fiber were free sterols which made up 24% of the total extract and 24% of fatty acids. Furthermore, significant amounts of steroid ketones (10%), triglyceridse (6%), ω-hydroxyfatty acids (6%), monoglycerides (4%), fatty alcohols (4%), and a series of p-hydroxycinnamyl (p-coumaric and ferulic acids) esterified with long chain alcohols and ω-hydroxyfatty acids together with minor amounts of α-hydroxyfatty acids, diglycerides, steroid hydrocarbons, sterol esters, and sterol glycosides. (Rio & Gutiérrez 2006)

2.7 Importance of Tomato in the Philippines

Tomato, scientifically known as *S. lycopersicum,* is considered one of the most profitable crops and the second most important fruit vegetable, right after eggplant, in the Philippines (Altoveros & Borromeo 2007). In 2021, tomato production of 75,127.03 metric tons in Ilocos Region was 2.30 percent higher than the 73,435.34 metric tons output in 2020. (Table 1). Ilocos Region topped all the tomato producing regions, contributing 33.32 percent to the national output of 225,450.60 metric tons. Out of the total production of the Ilocos Region, the provinces of Ilocos Norte and Ilocos Sur contributed 35.82 percent and 35.21 percent respectively while Pangasinan and La Union had shares of 25.82 percent and 3.15 percent, respectively (Phil. Statistics Authority, 2021). And consumption of tomatoes amongst Filipinos continues to rise due to the vegetable's wide variety of uses and nutritional value. Tomatoes have been widely studied and found to have health properties such as; carotenoids, chlorophylls, dietary fibers, glycoalkaloids, minerals, organic acids, polyphenols, and vitamins C and E (Asensio et al 2019; Martínez-Huélamo et al 2016).

2.8 Importance of tomato diseases

Tomatoes, while being one of the most profitable crops in the Philippines and extensively cultivated all around the world, face an array of pest and disease problems which leads to lower yield and quality of the products. Tomato is susceptive to multiple diseases namely fusarium wilt, root rot, verticillium, and many other diseases. However, bacterial wilt's pathogen also scientifically known as *Ralstonia solanacearum* Smith., is one of the most important and serious soil-borne diseases not only in the tomato industry but also in many plants that are situated in tropical/subtropical warm climates (Hayward 1991). This disease has become a major biotic factor limiting the growth and development of crops throughout the world as it affects over 200 plant species in 50 families (Hayward 2000). And one of the families that the bacterial wilt affects is the solanaceous family including the eggplant, pepper, potato, tobacco, and tomato, most of which, contribute to the Philippines' economy (Anith et al 2004).

2.9 Management of Tomato Wilt

The use of soil organic amendments can be considered as a way to control bacterial wilt in a sustainable manner. The application of soil organic amendments is a traditional practice for maintaining not only soil health but also fertility. Which in turn causes an increase in yield and quality of crops. These organic amendments contain growth-promoting hormones such as IAA and GA, macro and micronutrients, vitamins, as well as beneficial microorganisms. This does not only provide nutrients to the soil but is also a viable method for controlling soil-borne diseases (Sreenivasa et al 2010). In addition, the increase of beneficial microbes improves the physiochemical of the soil and generates biological competition and antagonism which naturally suppresses soil-borne pathogens. (Janvier et al 2007). Streptomycin is an antibiotic that acts against both gram-positive and gram-negative bacteria, it kills the bacteria by disrupting the function of bacterial cell's ribosomes, which are responsible for creating the proteins linking amino acids together. This is why streptomycin is considered a good drug that can fight against Tomato wilt, a gram-positive bacteria. (One Secret Hunt 2012-2022).

In November 1953, a sudden outbreak in a tomato hydroponic farm of Land Authority at Vega Alta, P.R. was reported. Due to the special conditions in the hydroponic farm, quick measures for identification and control of the disease were crucial. The pure culture was obtained by extracting the ooze from pieces of the infected stems placed in water. The isolated bacterium was then later on identified to be *Pseudomonas solanacearum E.F.S*, now known as *R. solanacearum*. Since broth cultures were accessible, sensitivity tests with antibiotic discs (Difco) 2 were carried out by seeding nutrient agar plates with 24-hour-old broth cultures (Perez, 1969). The results of the sensitivity test revealed that the bacterium was very sensitive to a concentration of 1 fig. of streptomycin in vitro. Based on the test results, it was recommended to apply streptomycin sulfate directly into the main tank of the nutrient solution in the affected areas, for the final concentration of approximately 4 ng. of streptomycin could be reached. The heavily affected plants were all removed while the slightly affected plants were left. And within a few days, the slightly affected plants recovered completely, and no more cases appeared. In the following week, a second dose of streptomycin was given to ensure the affected area completely recovered (Perez 1969).

3. Materials and methods

Collection and Preparation of Abaca Plant Samples - The Abaca plant samples were collected from a mature Abaca plant at the National Abaca Research Center (NARC) Waterwheel area. The samples were then washed and cleaned thoroughly, segregating the plant parts into leaves, roots, flowers, corm, and pseudostem, labeled, and then weighed. It was air-dried for a month. After a month of air drying, the different plant parts were once again weighed to determine the moisture content within each plant part.

Abaca Plant Extraction & Drying in Vacuo - After weighing, the plant samples were cut and milled. Each plant sample was then soaked in 40% ethanol using a 1:2 ratio (sample weight: ethanol volume) and secured in sealed containers under refrigerated conditions for 3 days in the dark. After soaking, the plant samples were then filtered to collect the extract. The pseudostem was shredded and squeezed for the collection of its sap. The collected extracts, excluding the pseudostem sap, were then dried in vacuo.

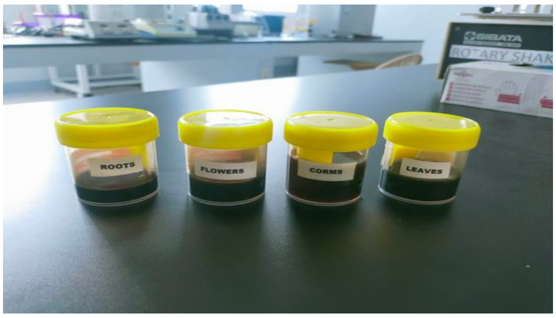

Figure 1. The collected abaca extracts after drying in vacuo

Antimicrobial Activity of Abaca Plant Extracts Against E.coli - The abaca plant extracts were subjected to the bacteria Escherichia coli at rates of 15,000, 10,000, and 5,000 ppm, with three replications per ppm. Each abaca plant extract was then individually tested in vitro. The tests were done at the Plant Disease Diagnostic Laboratory.

Preparation of Culture Medium - Forty-two (42) grams of nutrient agar powder was mixed into 1500 mL of sterilized water and heated in an oven for 10 minutes to make 1500 mL of Nutrient Agar. 15mL of the nutrient agar was poured into 63 test tubes with 51 of those test tubes transferred into the petri dishes. Three hundred microliter of the pathogen *E. coli*, provided by the Plant Disease Diagnostic Laboratory (PDDL) which provides such services, was placed into each petri dish and spread all over the plate's surface using an L-shaped rod. A total of two hundred fifty-five (255) sterile disks and fifty-one (51) sterile petri dishes were prepared. Each petri dish contained 5 disks that were soaked in the abaca extracts; roots, corm, leaf, flower, and pseudostem sap, as well as the positive control which was streptomycin, and the sterilized water acting as the negative control. It was then sealed with masking tape and the growth inhibition was monitored and measured for 24 hours.

Treatment Application - The different parts of the Abaca served as the treatments of the study. The following treatments are as follows. The experiment was arranged in Completely Randomized Design (CRD). The abaca plant extracts were subjected to the bacteria *Escherichia coli* at rates of 15,000, 10,000, and 5,000 ppm, with three replications per ppm. Each abaca plant extract was then individually tested in vitro. The set-up was incubated for 48 hours to collect the zone of inhibition using a ruler. After the experiment, the set-up was properly disposed of following the standard protocol of the Plant Disease Diagnostic Laboratory (PPDL) for *E. coli.*

T_1 = Negatice Control (H_2O)	
T_2 = Roots	T_2C_1 = Roots (5,000ppm) T_2C_2 = Roots (10,000ppm) T_2C_3 = Roots (15,000ppm)
T_3 = Pseudostem	T_3C_1 = Pseudostem (5,000ppm) T_3C_2 = Pseudostem (10,000ppm) T_3C_3 = Pseudostem (15,000ppm)
T_4 = Flower	T_4C_1 = Flower (5,000ppm) T_4C_2 = Flower (10,000ppm) T_4C_3 = Flower (15,000ppm)
T_5 = Corm	T_5C_1 = Corm (5,000ppm) T_5C_2 = Corm (10,000ppm) T_5C_3 = Corm (15,000ppm)
T_6 = Leaves	T_6C_1 = Leaves (5,000ppm) T_6C_2 = Leaves (10,000ppm) T_6C_3 = Leaves (15,000ppm)
T_7 = Positive Control (Streptomycin)	

Antimicrobial Assay of Abaca Against R. solanacearum - The tomato seedlings were inoculated with *R. solanacearum* using the method of Singh et al. (2018). The Abaca Plant Extracts were subjected to the bacterial wilt pathogen of *S. lycopersicum* at the same rates of 15,000, 10,000, and 5,000 ppm. The tests were done at the Plant Disease Diagnostic Laboratory.

Planting of Tomato Seedlings - Two hundred tomato seedlings were prepared and soaked for two days straight. It was then transferred into sterile plastic containers that had three (3) layers of sterilized paper. This was sprayed daily with distilled water and was monitored for seven (7) days.

Figure 2. Tomato seedlings soaked in water

Treatment Application - The *R*. *solanacearum* was provided by the Plant Disease Diagnostic Laboratory (PDDL). Two methods were used, preventive and curative, to see how effective the extracts were against the bacterial wilt. The different parts of Abaca were used as the treatments of the study. The following treatments are as followed for both methods:

T_1 = Negatice Control (H_2O)	
T_2 = Roots	T_2C_1 = Roots (5,000ppm) T_2C_2 = Roots (10,000ppm) T_2C_3 = Roots (15,000ppm)
T_3 = Pseudostem	T_3C_1 = Pseudostem (5,000ppm) T_3C_2 = Pseudostem (10,000ppm) T_3C_3 = Pseudostem (15,000ppm)
T_4 = Flower	T_4C_1 = Flower (5,000ppm) T_4C_2 = Flower (10,000ppm) T_4C_3 = Flower (15,000ppm)
T_5 = Corm	T_5C_1 = Corm (5,000ppm) T_5C_2 = Corm (10,000ppm) T_5C_3 = Corm (15,000ppm)
T_6 = Leaves	T_6C_1 = Leaves (5,000ppm) T_6C_2 = Leaves (10,000ppm) T_6C_3 = Leaves (15,000ppm)
T_7 = Positive Control (Streptomycin)	

Preventive Method - The preventive method used seedlings dipped in the bacterial suspension and with a minute dipping interval into the extract then placed into the microtube. The experiment was arranged in Completely Randomized Design (CRD). The abaca plant extracts were subjected to the bacteria *Ralstonia solanacearum* at rates of 15,000, 10,000, and 5,000 ppm, with four (4) replications per ppm. Each abaca plant extract was then individually tested in vitro. The mortality rate of each test subject was monitored every 24 hours for 2 days.

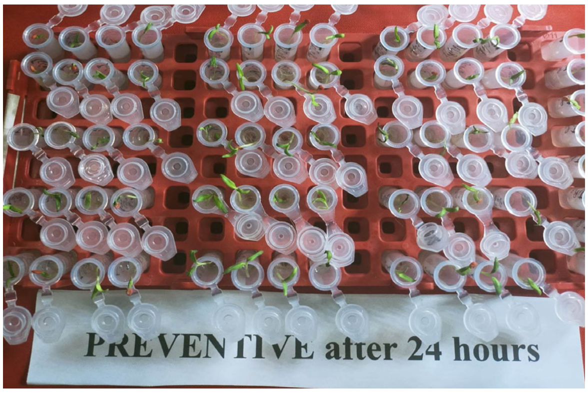

Figure 3. Tomato seedlings afflicted by R.*solanacearum* at 24 hours after treatment application using preventive method

Figure 4. Tomato seedlings afflicted by R.*solanacearum* at 48 hours after treatment application using preventive method

Curative Method - The curative method used seedlings dipped in the different abaca extracts and with a minute dipping interval into the bacterial suspension then placed into the microtube. The experiment was arranged in Completely Randomized Design (CRD). The abaca plant extracts were subjected to the bacteria *Ralstonia solanacearum* at rates of 15,000, 10,000, and 5,000 ppm, with four (4) replications per ppm. Each abaca plant extract was then individually tested in vitro. These were monitored every 24 hours for 2 days.

Figure 5. Tomato seedlings afflicted by R.*solanacearum* at 24 hours after treatment application using curative method

Figure 6. Tomato seedlings afflicted by R.*solanacearum* at 48 hours after treatment application using curative method

Toxicity Test of Abaca Plant Extracts - Test solutions were prepared for the toxicity tests according to Table 1. The exact amounts of each liquid component were measured using a 5-mL micropipette. The contents of each tube were gently shaken to homogenize and the reaction time began after the addition of methylene blue and yeast as the mixture was shaken. The tubes were allowed to sit undisturbed for about 30 minutes while observing the color change. As the blue color fades, the time estimate necessary to reach a color matching that of the control color is the end of the test. Each test sample was performed in triplicates.

Table 1
Sample solutions prepared for the toxicity test

	Vol. of water, mL	Vol. of extract, mL.	Vol. of yeast/sugar mixture, mL	Vol. of methylene blue, drop	Vol. of mineral oil, mL
Blank	11.0	-	-	1.0	1.0
Control	1.0	-	1.0	1.0	1.0
Root Extract	-	0.5	5.0	0.5	0.5
Pseudostem Sap	-	1.0	1.0	1.0	1.0
Flowers Extract	-	1.0	1.0	1.0	1.0
Corm Extract	-	1.0	1.0	1.0	1.0
Leaves Extract	-	1.0	1.0	1.0	1.0

Total Flavonoid Content (TFC) - The Total flavonoid content was determined according to the most commonly used procedure for total flavonoid determination, the Spectrophotometric Assay, of Christ and Müller (1960).

Preparation of standard samples and ethanolic extracts - Of the test solution (standard or sample), 1 mL was mixed with 0.3 mL of $NaNO_2$ (5 %, *w*/*v*) and after 5 min, 0.5 mL of $AlCl_3$ (2 %, *w*/*v*) was added. Flavonoid standard solutions of 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 500 µM were used. The sample was mixed and 6 min later was neutralized with 0.5 mL of 1 M NaOH solution. The mixture was left for 10 min at room temperature and then subjected to spectral analysis in the range of 300–600 nm against the blank, where $AlCl_3$ solution was substituted by water. Catechin (in the 50–500-µM concentration range) was the standard of choice for the expression of results at 510 nm.

Total Phenolic Content (TPC) - The total phenolic contents of the ethanolic abaca extracts were estimated using the Folin-Ciocalteau reagent as described by Singleton and Rossi (1965).

Preparation of standard samples - Blank sample, 1.0, 0.1, 0.01, and 0.001 µg/mL Gallic acid solutions with 5.0 mL of Folin-Ciocalteau reagent (diluted tenfold) and 4.0 mL of sodium carbonate solution (75 g/L) were used as the standard. The absorbance was measured after 30 min. at 765 nm.

Preparation of ethanolic extracts - For the ethanolic extracts (1 g/ 100 mL), 1 mL was mixed separately with the same reagents, as performed for constructing the standard samples. After 1 h, the absorbance was measured to determine the total phenolic contents in both extracts separately using the formula, C= C1 x V/m; where C= total phenolic content in mg/g, in GAE (Gallic acid equivalent), C1= concentration of Gallic acid established from the standard samples in mg/mL, V= volume of extract in mL, and m= the weight of the plant extract in g (Siddiqui et al., 2017).

Statistical Design and Analysis - Data was consolidated and analyzed using ANOVA through Statistical Tool for Agricultural Research (STAR). Treatment means were compared using Turkey's Honest Significant Difference (Turkey's HSD).

4. Results and discussion

4.1 Percent Recovery of the M. textilis Plant Extracts

After vacuum drying, the extracts of the different components of the abaca plants were collected and determined as percentage yield per fresh weight of the different plant samples. Table 2 shows the amount of the collected extracts from the individual plant component and each corresponding percent yield of recovered extracts. The determined percent yield of the recovered extracts can be used to estimate the amount of extract that can be collected from a predetermined amount of fresh abaca plant sample required for a series of tests. The results show that the abaca roots had the highest amount of recovered extract per weight sample prepared, followed by the abaca flowers, leaves, and corm. This suggests that more abaca fresh corm were required to recover and extract comparable to that of the abaca roots.

Table 2
The percent yield of crude abaca extracts per weight of fresh plant samples

Samples	Weight of fresh plant sample (g)	Weight of dry plant sample (g)	Moisture content of plant sample (%)	Weight of collected extract (g)	Percent Yeild of Extract (%)
Roots	250.00	125.00	50.00	23.60	9.44
Leaves	750.00	250.00	66.67	38.20	5.09
Flowers	500.00	250.00	50.00	36.30	7.26
Corm	2000.00	500.00	75.00	46.80	2.34
Pseudostem	-	-	-	-	-

4.2 Antimicrobial Activity of Abaca Plant Extracts Against E. coli

Escherichia coli is a gram-negative bacterium responsible for numerous diarrheal diseases like traveler's diarrhea and dysentery. It is the primary pathogen causing uncomplicated cystitis and contributes to various extraintestinal conditions such as pneumonia, bacteremia, and abdominal infections like spontaneous bacterial peritonitis (Mueller and Tainter, 2013). Its growth inhibition against abaca extract was observed for 48 hours and measured every 24 hours.

Inhibition Zone of E. coli 24 Hours After Treatment Application - At 5,000 ppm, none of the treatments exhibited similarities with the positive control (streptomycin). However, as illustrated in Table 1, both the pseudostem sap and corm extract did not significantly differ from the negative control (H_2O). This suggests a resemblance between the pseudostem sap, corm extract, and the H_2O treatment. All treatments showed similarities, with the root extract that displayed a higher level of variance. Overall, the root treatment demonstrated the highest inhibition measurement compared to the other extracts at this concentration. At a higher concentration of 10,000 ppm, the measurement of the inhibition zones of the pseudostem sap, corm extract, and leaves extract increased, indicating similarities among them. The pseudostem sap no longer shared any similarities with the H_2O treatment, while the leaves extract showed the highest inhibition measurement and variance compared to the other treatments. However, the root extract maintained its inhibition measurement and did not significantly differ from the corm extract, sharing some similarities with the remaining extracts.

Meanwhile, the inhibition measurement of the flower extract decreased, no longer significantly varying from the H_2O treatment. At 15,000 ppm, the root extract's measurement of inhibition significantly increased, becoming the highest in both variance and inhibition measurement among all the extracts. The inhibition measurements of the flower and leaves extracts also increased but did not significantly differ from each other. Conversely, the corm extract's inhibition measurement decreased, no longer varying significantly from the pseudostem sap and flower extract, while showing minimal similarities with the leaves extract. The pseudostem sap maintained its inhibition measurement while continuing to share no similarities with the H_2O treatment. The consistent increase in inhibition measurements across most extracts indicates that the antimicrobial efficacy of abaca is concentration-dependent, with higher concentrations yielding more efficient results. Additionally, extracts with higher water content show improved outcomes at higher concentrations, reducing their resemblance to the H_2O treatment. It is also observed that the higher the inhibition measurement, the more significantly varied it becomes from the rest of the treatments.

Table 3

Average Inhibition Zone of E.coli affected by different abaca extracts at 24 hours after treatment applications

Treatments	24 HOURS INHIBITION ZONE		
	Concentrations		
	5,000 ppm*	10,000 ppm*	15,000 ppm*
H_2O	0.71^{e}		
Roots Extract	1.32^{bcd}	1.32^{bcd}	1.73^{b}
Pseudostem Sap	1.13^{cde}	1.23^{cd}	1.23^{cd}
Flower Extract	1.22^{cd}	1.08^{de}	1.32^{bcd}
Corm Extract	1.11^{de}	1.36^{bcd}	1.28^{cd}
Leaves Extract	1.18^{cd}	1.29^{cd}	1.54^{bc}
Streptomycin	4.12^{a}		
CV (%)	9.83		

* Means in a column followed by the same letter are not significantly different at 5% HSD

Inhibition Zone of E. coli 48 Hours After Treatment Application - As shown in Table 3, after 48 hours, the inhibition measurements of the treatments showed a decrease. At 5,000 ppm, the extracts did not exhibit significant variation from each other or the H_2O treatment. However, there was a slight deviation observed in the pseudostem sap and leaves extract compared to the other treatments, with the leaves extract displaying the highest inhibition measurement followed by the pseudostem sap. It's worth mentioning that at this concentration, the leaves extract showed the highest inhibition measurement. Moving on to 10,000 ppm, most of the extracts demonstrated an increase in inhibition measurement, except for the flower and leaves treatments, which showed a decrease. The variance remained consistent, with the pseudostem sap and leaves extract showing slight variation from the other extracts but not significantly varying from each other.

Additionally, the inhibition measurements of the extracts did not significantly vary from the previous concentration of 5,000 ppm. At 15,000 ppm, all extracts exhibited higher inhibition measurements compared to those at lower concentrations. The significance of variation was not observed among the extracts, except for the leaves extract, which showed slight variance from the other extracts but no longer resembled the H_2O treatment. The extracts at higher concentrations also displayed slight variation from the measurements at lower concentrations. This further indicates that the higher the concentration the more varied it becomes from the rest of treatments. The longer the time continues to pass, the less effective the antimicrobial properties become, especially at lower concentrations, meaning that higher concentrations should be used to maximize the efficacy of extracts.

4.3 Antimicrobial Assay of Abaca Against R. solanocearum

Among the soil-borne diseases, tomatoes are highly susceptible to bacterial wilt caused by which is one of the most important and widely distributed plant pathogenic bacteria in the tropical, subtropical, and warm temperate climates of the world (Hayward, 1991). It causes bacterial wilt disease on over 200 plant species in 50 families (Hayward, 2000) and remains the major biotic factor limiting the growth and development of several important solanaceous crops including potato, tomato, eggplant, pepper, and tobacco (Anith et al., 2004). The survival rate of tomato seedlings was determined using preventive and curative methods and compared to identify which method was better suited to increase the survival rate of tomato seedlings against *R. solanacearum*.

Table 4

Average Inhibition Zone of E.coli affected by different abaca extracts at 48 hours after treatment applications

Treatments	48 HOURS INHIBITION ZONE		
	Concentrations		
	5,000 ppm*	10,000 ppm*	15,000 ppm*
H_2O	0.71^{c}		
Roots Extract	0.79^{c}	0.81^{c}	0.93^{bc}
Pseudostem Sap	0.98^{bc}	1.03^{bc}	1.09^{bc}
Flower Extract	0.77^{c}	0.75^{c}	1.05^{bc}
Corm Extract	0.77^{c}	0.88^{c}	0.94^{bc}
Leaves Extract	1.03^{bc}	1.01^{bc}	1.29^{b}
Streptomycin	$4{,}16^{a}$		
CV (%)	11.17		

* Means in a column followed by the same letter are not significantly different at 5% HSD

Survival Rate of Tomato Seedlings Afflicted by R. solanacearum at 24 Hours After Treatment Application Using Curative Method - After 24 hours it was observed that the treatments do not vary significantly among each other. This could indicate that the sample size was too small leading to a more random result from the data. However, higher survival rates were observed at higher concentrations, indicating that the efficacy of the abaca extract's antimicrobial properties is dependent on its concentration. In other words, the higher the concentration of abaca extract used, the more effective the treatment becomes.

Table 5

Average survival rate of tomato seedlings 24 hours after being afflicted by R.solanacearum and treated by abaca extracts using curative method

Treatments	24 HOURS SURVIVAL RATE		
	Concentrations		
	5,000 ppm^{ns}	10,000 ppm^{ns}	15,000 ppm^{ns}
H_2O	5.36		
Roots Extract	5.36	7.69	7.69
Pseudostem Sap	5.37	5.37	10.02
Flower Extract	5.37	5.37	7.69
Corm Extract	3.04	7.69	10.02
Leaves Extract	5.36	5.36	5.36
Streptomycin	10.02		
CV (%)	70.56		

^{ns} Means in a column do not vary significantly among treatments

Survival Rate of Tomato Seedlings Afflicted by R. solanacearum at 48 Hours After Treatment Application Using Curative Method - After 48 hours, there was a slight decrease in the average survival rates of the treatments. However, it is to be noted that the treatments still do not show significant variation among each other. This observation could suggest that the sample size might have been insufficient, potentially leading to a more random outcome in the data. A larger sample size could provide a clearer picture of the treatment effects. Interestingly, higher survival rates were observed at higher concentrations of the abaca extract. This finding suggests that the efficacy of the extract's antimicrobial properties is directly proportional to its concentration. The higher the concentration of the extract, the more effective the treatment becomes in inhibiting microbial growth or enhancing survival rates. This concentration-dependent effect underscores the importance of dosage considerations when utilizing abaca extract for antimicrobial purposes.

Survival Rate of Tomato Seedlings Afflicted by R. solanacearum at 24 Hours After Treatment Application Using Preventive Method - The means do not vary significantly among treatments, as shown in the table below (Table 6), the only varying treatment is the negative control (H2O) which has a lower survival rate compared to the other treatments. All extracts had the same survival rate as the positive control, streptomycin.

Table 6

Average survival rate of tomato seedlings 48 hours after being afflicted by R.solanacearum and treated by abaca extracts using curative method

Treatments	48 HOURS SURVIVAL RATE		
	Concentrations		
	5,000 ppm^{ns}	10,000 ppm^{ns}	15,000 ppm^{ns}
H_2O	3.04		
Roots Extract	5.37	3.04	5.37
Pseudostem Sap	3.04	5.37	7.69
Flower Extract	3.04	5.37	5.37
Corm Extract	0.71	5.37	7.69
Leaves Extract	5.37	5.37	5.37
Streptomycin	10.02		
CV (%)	94.08		

^{ns} Means in a column do not vary significantly among treatments

Table 7

The average survival rate of tomato seedlings 24 hours after being afflicted by R. solanacearum and treated by abaca extracts using preventive method

Treatments	24 HOURS SURVIVAL RATE		
	Concentrations		
	5,000 ppm^{ns}	10,000 ppm^{ns}	15,000 ppm^{ns}
H_2O	7.69		
Roots Extract	10.02	10.02	10.02
Pseudostem Sap	10.02	10.02	10.02
Flower Extract	10.02	10.02	10.02
Corm Extract	10.02	10.02	10.02
Leaves Extract	10.02	10.02	10.02
Streptomycin	10.02		
CV (%)	5.72		

^{ns} Means in a column do not vary significantly among treatments

Survival Rate of Tomato Seedlings Afflicted by R. solanacearum at 48 Hours After Treatment Application Using Preventive Method - The Average survival rates of the treatments have slightly decreased after 48 hours however, the treatments still do not vary significantly among each other. At 15,000 ppm, most of the treatment's survival rate increased compared to lower concentrations except that of the pseudostem extract, and due to the small sample set, this may not be accurate.

Table 8

Average survival rate of tomato seedlings 48 hours after being afflicted by R.solanacearum and treated by abaca extracts using preventive method

Treatments	48 HOURS SURVIVAL RATE		
	Concentrations		
	5,000 ppm^{ns}	10,000 ppm^{ns}	15,000 ppm^{ns}
H_2O	3.04		
Roots Extract	7.69	10.02	10.02
Pseudostem Sap	7.69	7.69	3.04
Flower Extract	0.71	5.37	5.37
Corm Extract	5.37	5.37	5.37
Leaves Extract	3.04	5.37	7.69
Streptomycin	10.02		
CV (%)	44.59		

^{ns} Means in a column do not vary significantly among treatments

Although the sample set may be insufficient, it is noteworthy that the results shown in the tables (Table 7 and Table 8) may indicate that, compared to the curative method, using the preventive method may result in a better survival rate.

4.4 Toxicity Test of Abaca Plant Extracts

The result of the toxicity test conducted on the five (5) extracts of the abaca plant parts showed that after thirty minutes had passed, the addition of the methylene blue indicator into the solution had an observable change from blue to colorless. It denotes that the production of ATP (Adenosine triphosphate) has not stopped in which the electrons are continuously transferred from one chemical carrier to another. This suggests that the yeast cells did not die out after the addition of the different abaca plant extracts. The production of ATP is essential to all cellular life and anything which slows down or stops this cycle will kill the cell or at least alter its usual function. Since the test gave a negative result for all abaca plant extracts therefore all plant extracts are considered non-toxic.

Figure 7. Toxicity test of the abaca plant extracts at (a) 0 minutes, (b) after 15 minutes and (c) end-point, after 30 minutes.

4.5 Total Flavonoid Content (TFC)

Calibration Curve of Flavonoid Standard Samples - The calibration curve of the flavonoid standard samples that the 500 µM had the highest flavonoid content followed by 400, 350, 300, 250, 200, 150, 125, 100, 75, and 50 µM as shown in the graph below (figure 8).

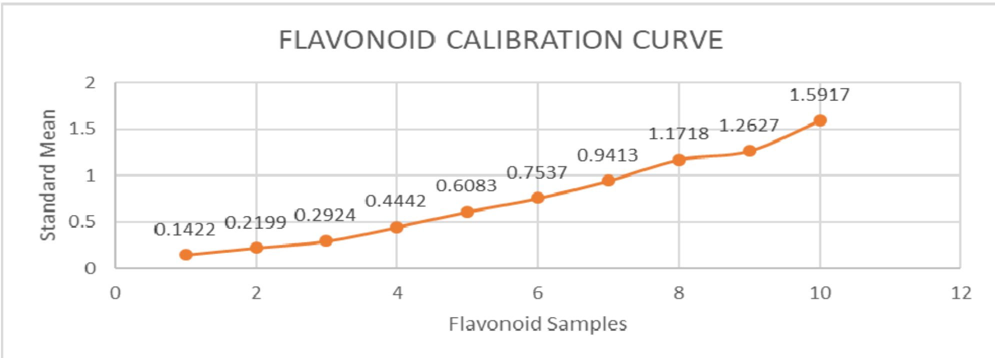

Figure 8. Flavonoid calibration curve

Flavonoid Content of Abaca Extracts - The total flavonoid content was determined using the Folin-Ciocalteu method with the abaca roots being consistently higher followed by that of the leaves, flower, corm, and, pseudostem respectively. The result of the total flavonoid content complements the result of the total phenolic content since in general, the flavonoids are one particular group of phenolics present in most plant pigments. It can be inferred that it is the roots of the abaca plant which has the potential to show the highest antioxidant activity and that it would be best to utilize predominantly the roots of this plant most of all. When comparing the flavonoid content of various abaca extracts to the calibration curve, a clear pattern emerges. The roots and leaves extract exhibit a significantly higher flavonoid content compared to the standard samples, indicating a rich concentration of flavonoids. Following closely behind are the flower extract, corm extract, and pseudostem sap, all of which fall within the range of flavonoid content observed in the standard samples. This suggests that the roots and leaves of the abaca plant are particularly potent sources of flavonoids, while the other extracts also contribute to its overall flavonoid profile, although to a slightly lesser extent.

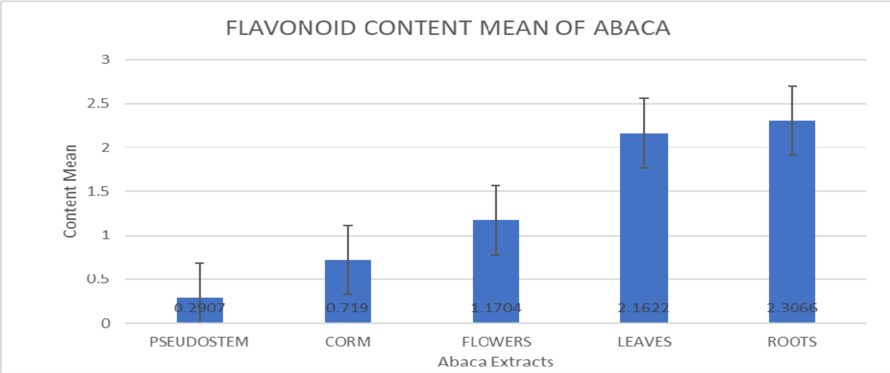

Figure 9. Flavonoid content mean of abaca extracts

4.6 Total Phenolic Content (TFC)

Average Phenolic Content of Standard Samples - The sample containing the highest level of phenols, as shown by the figure below (Figure 10) was 1.0, followed by 0.1, 0.01, and 0.001 gallic acid solution, and the blank sample contained no trace amount of phenols whatsoever. This indicates that the more a sample contains gallic acid, the higher the phenolic content is.

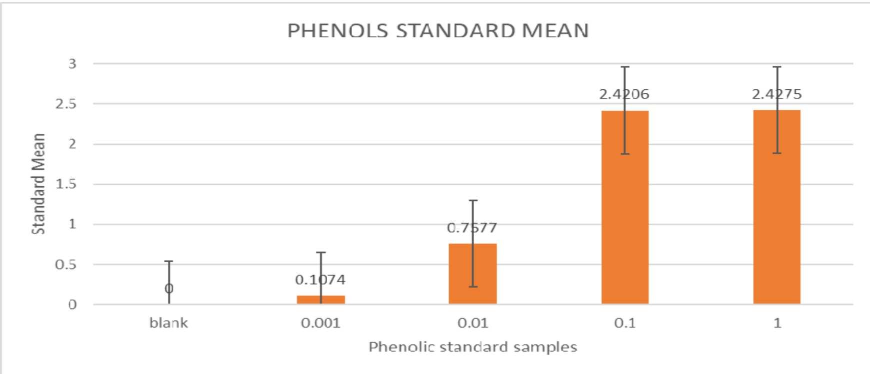

Figure 10. Phenolic content mean of standard samples

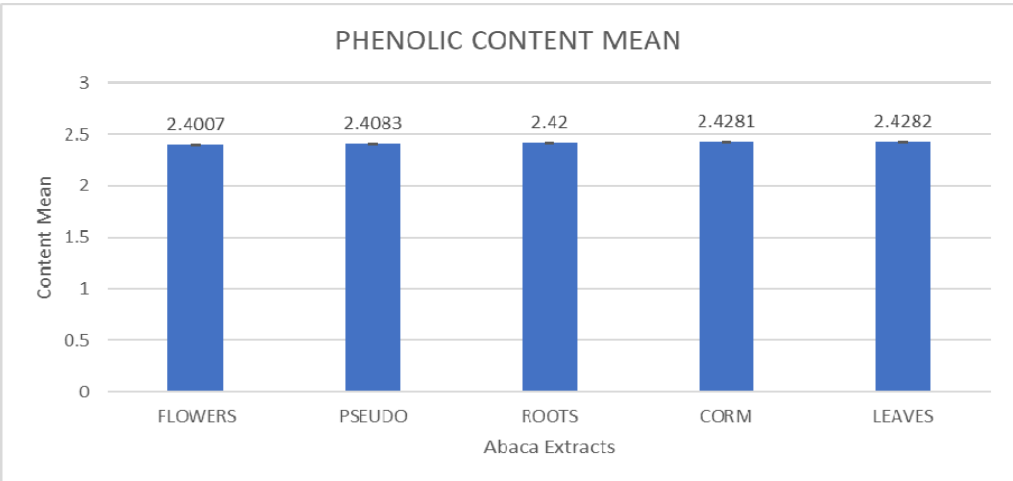

Figure 11. Phenolic content mean of abaca extracts

Phenolic Content of Abaca Extracts - Results revealed that the extract of the abaca leaves showed the highest amount of phenolic content followed by the abaca corm, roots, pseudostem, and flowers, respectively.

This suggests that the abaca leaves can be a good source of high phenol compounds which have been reported to exhibit high radical scavenging activity similar to that shown by vitamin C. Also, the result of the analysis of the total phenolic content of the different parts of the abaca plant was confirmed by the total flavonoid content of the different plant extracts. The comparison of phenolic content between the Abaca extracts and the standard samples reveals a consistent pattern. All extracts either surpass or fall within the range of 0.1 to 1.00 phenolic content found in the standard samples. This indicates that the Abaca extracts contain trace amounts of phenols, showing their presence across different parts of the abaca plant.

5. Conclusion

The results of this study conclude that the different parts of abaca are resistant against E. coli at small quantities; having used 5,000ppm, 10,000ppm, and 15,000ppm concentrations. The results show that the abaca plant extracts against *R. solonacearum* had higher rates of survival against the pathogen at higher concentrations however, the minimal variation in means among different extracts and concentrations does not provide sufficient evidence to conclude the presence of an impact related to either extract or concentration. Further research using more plant subjects, higher concentrations and different methods is necessary in order to yield better results. The different parts of abaca were tested for their total flavonoid and total phenolic content and the results that all the parts of abaca contain high amounts of flavonoid and phenols which gives solace that abaca has antimicrobial properties that can inhibit the growth of bacteria. And lastly, the toxicity test conducted had shown results that all of the different parts of abaca were found to be non-toxic. However, it is not concluded if the different parts of abaca are safe for human consumption.

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