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Evaluation of *In-Vitro* Antibacterial Activity of Aqueous and Ethanolic *Syzygium aromaticum L.*, Extracts against Carbapenem-Resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a common nosocomial pathogen that causes a wide range of infections, particularly in immunocompromised patients. Carbapenems are considered the last resort antibiotics for the treatment of severe infections caused by Gram-negative bacteria, including *P. aeruginosa*. Significant infections with *P. aeruginosa* have been managed. Due to side effects, unaffordability, patient ideologies coupled with traditional beliefs, most people in Uganda depend on medicinal plants for health care needs including *S. aromaticum*. *S. aromaticum* was collected, authenticated, pulverized and extracted by maceration. 80% ethanol was used as a solvent. The sensitivity test, MIC (7.81 mg/ml and 15.6 mg/ml for ethanolic and aqueous extracts of *S. aromaticum* respectively) and MBC (125 mg/ml for both extracts against CRPA) were done. The qualitative phytochemical screening revealed the presence of alkaloids, tannins, terpenoids, flavonoids, saponins and phenolic compounds. The MIC was done using the agar well diffusion according to CLSI guidelines 2022. This study therefore evaluated the efficacy and phytochemical analysis of *S. aromaticum* that are helpful in the authentication and can be used as a reference standard in the preparation of a monograph. The study has also established the MIC and MBC of *S. aromaticum* buds in-vitro and has validated the folkloric claims of the plant in carbapenem-resistant *P. aeruginosa* infections.

Keywords: *Antibacterial activity, Pseudomonas aeruginosa, Carbapenems, Antibiotics, Phytochemical analysis, S. aromaticum*

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1. Introduction

Pseudomonas aeruginosa is a common nosocomial pathogen that causes a wide range of infections, particularly in immunocompromised patients (Reynolds and Kollef, 2021). Carbapenems are considered the last resort antibiotics for the treatment of severe infections caused by Gram-negative bacteria, including *P. aeruginosa*. Antimicrobial resistance is a complex and concerning phenomenon where microorganism, such as bacteria evolve to withstand the effects of antimicrobial agents (Pulingam *et al*., 2022; Aslam *et al.,* 2021). Antibiotic resistance is as a result of over and misuse of multiple antibiotics in different conditions leading to persistent infections and increased risks of the spread of disease (Pulingam *et al.,* 2022). Carbapenem-Resistant *P. aeruginosa* (CRPA) is a major public health concern due to its high resistance to antibiotics and its ability to cause severe infections in vulnerable populations (Ahmed *et al*., 2021). Nosocomial infections, also known as healthcare-associated infections, are infections that patients acquire within 48-72 hours of receiving treatment in a healthcare facility. The sources of such infections can be due to actions of a source of endogenous infection like normal flora or microorganisms coming from water, food or another carrier (Plata-Menchaca and Ferrer, 2022)

The worldwide prevalence of CRPA has been reported to range from 2% to 44%, depending on the geographical location and healthcare settings (Codjoe and Donkor, 2017; Hu *et al.,* 2019). The emergence of antibiotic-resistance bacteria has led to the search for new antimicrobial agents, and herbal products have shown promise as potential sources of novel drugs (Balandrin *et al*., 1985). The World Health Organization (WHO) estimates that more than 80% of people worldwide, mainly in developing countries, use multiple plant extracts and their active molecules in conventional drugs (Tran *et al*., 2020).

In Uganda, 60% of the population relies on medicinal plants, with the rural areas indicating a higher consumption as compared to the urban areas (Ssenku *et al.,* 2022). Medicinal plant species belonging to different families are used by some communities to treat bacterial infections (Ntulume *et al.,* 2019; Namukobe *et al.,* 2021). *Syzygium aromaticum L.*, is one of the medicinal plants known for its folkloric use in bacterial infections by locally renowned community traditional health practitioners (El-Maati *et al*., 2016). Ethanolic and aqueous extracts of *S. aromaticum* have also been reported to have antibacterial activity against various bacterial strains, including *P. aeruginosa* (Study *et al*., 2021). However, the antibacterial activity of *S. aromaticum* extracts against CRPA has not been extensively studied.

Therefore, this study aims to evaluate the in vitro antibacterial activity of aqueous and ethanolic *S. aromaticum* extracts against CRPA in view of its establishment as a novel herbal remedy.

1.1. Problem Statement

Antimicrobial resistance is a major public health concern worldwide, especially in developing countries where the misuse and overuse of antibiotics are rampant resulting in an elevation of antibiotic use by 65% (Dadgostar, 2022; Hu *et al*., 2020). *P. aeruginosa* is a common nosocomial pathogen that causes severe infections particularly in immunocompromised patients (Reynolds and Kollef, 2021).Carbapenem-Resistant *P. aeruginosa* (CRPA) strains have become a significant challenge for clinicians due to limited treatment options and treatment failure (Brink, 2019). CRPA is often resistant to other types of antibiotics such fluoroquinolones, cephalosporins and monobactams. Infections caused CRPA will therefore likely fail empiric treatment regimens. CRPA infections also add to considerable cost to health care since health care professionals are forced to use antibiotics that are more toxic and frequently expensive after the failure of the first-line and second-line antibiotic treatment options (Nwobodo *et al.*, 2022).

Resistance due to antibiotic use and side effects reported from conventional medicines has led to people depending on medicinal plants as alternative primary healthcare. In many areas of Uganda including Kawempe, medicinal plants such as pilau masala have been used by the population and native healers to

achieve antimicrobial treatment in severe ailments (Nsibirwa *et al.*, 2020). Therefore there is a need to explore alternative therapeutic options, such as herbal extracts, to combat antibiotic-resistant pathogens (Hu *et al.*, 2020).

1.2. General Objectives

This study aims to evaluate the in-vitro antibacterial activity of aqueous and ethanolic *S. aromaticum* extracts against carbapenem-resistant *P. aeruginosa*.

1.3. Specific Objectives

The objectives of the study are to:

i) Determine the antibacterial activity of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa*.

ii) Determine the minimum inhibitory concentration of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa*.

iii) Determine the minimum bactericidal concentration of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa*.

1.4. Research Questions

This study has been guided by the following research questions

i) What is the anti-bacterial activity of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa*?

ii) What is the minimum inhibitory concentration of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa*?

iii) What is the minimum bactericidal concentration of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa*?

1.4.1. Justification

S. aromaticum with the local name *pilau masala* as known to the community in Uganda, is one of the medicinal plants generally used among the Traditional Medical Practitioners for bacterial infection and is very effective and readily available among the users. However, the scientific basis for antibacterial activity of aqueous and ethanolic extracts of *S. aromaticum* against carbapenem-resistant *P. aeruginosa* are not available. Therefore, for this plant to be accepted for general use, its efficacy has to be evaluated. In this study, the ethno-medicinal claim of *S. aromaticum* was investigated with a view to serving as quality control and quality assurance of the plant for commercialization.

1.4.2. Ethical Approval

The report was approved by the faculty research committee, Kampala International University Committee Institution review committee. All research activities were conducted in accordance with ethical guidelines and regulations ensuring the protection and well-being of participants involved in the study.

1.5. Medicinal Importance of Plants

The use of medicinal plants in managing multidrug-resistant bacterial infections has gained attention due to the potential of plant-derived compounds to combat antibiotic-resistant strains. Several medicinal plants

with antibacterial activity and their active compounds relevant in combating the spread of multidrug resistant microorganism are listed in the Table 1 below.

Table 1: Medicinal Plants with Antimicrobial Activity

Plant Sources	Class of Compound	Compound	Mechanisms	Susceptible Microorganism
Rauwolfia serpentine	Alkaloid	Reserpine	EP inhibitor	*Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp.,
Piper nigrum		Piperine	EP inhibitor	MRSA, *Staphylococcus aureus*
		Conessine	EP inhibitor	*Pseudomonas aeruginosa*
		Berberine	Protein and DNA synthesis inhibitor	*Escherichia coli*, *Candida albicans*
Berberis vulgaris		Tomatidine	ATP synthetase inhibitor	*Listeria, Bacillus Staphylococcus spp.*
	Phenolic compound / polyphenols	Rhamentin	EP inhibitor	*Staphylococcus aureus*
Camellia sinensis		Epigallocatechin gallate	Beta-ketoacyl-reductase	*Escherichia coli*
		Chebulinic acid	DNA gyrase	*Mycobactrium tuberculosis*
		3-p-Trans-coumaroyl-2-hydroxyquinic acid	Cell membrane damage	*Staphylococcus aureus*
Cedrus deodara		Apigenin	d-Alanine:d-alanine ligase	*Helicobacter pylori*, *Escherichia coli*
Allium sativum	Sulfur-containing compounds	Allicin	Protein and DNA synthesis inhibitor	*Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*
Rubus ulmifolius		Ajoene	Sulphydryl-dependent enzyme inhibitor	*Cambylobacter jejuni*, *Streptococcus*, *Staphylococcus Escherichia coli*
		Sulforaphane	Destruction of bacterial membrane, Protein and DNA synthesis inhabitor, ATP synthase inhibitor	*Escherichia coli*
		Alyssin		*Helicobacter pylori*
Raphanus sativus		Allyl isothiocyanate Benzyl isothiocyanate Phenethyl isothiocyanate		*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Enterobacter cloacae*, *Escherichia coli*

Table 1 (Cont.)

Ferulago campestris	Coumarin	Aegelinol	DNA gyrase inhibitor	*Salmonella enterica serovar Typhi*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Staphylococcus aureus*
		Agasyllin	DNA gyrase inhibitor	*Salmonella enterica serovar Typhi*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Helicobacter pylori*
Prangos hulusii		4′-senecioiloxyosthol	DNA gyrase inhibitor	*Bacillus subtilis*
		Osthole	DNA gyrase inhibitor	*Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, MRSA
Mesua ferrea		Bergamottin epoxide	EB inhibitor	MRSA
Thymus vulgaris	Terpene	Furnesol	Cell membrane disturbance	*Staphylococcus aureus*
		(4R)-carbone	Cell membrane disturbance	*Cambylobacter jejuni*, *Enterococcus faecalis*, *Escherichia coli*
		Thymol	Cell membrane (H+)-ATPase inhibition, Cell membrane disturbance, EP inhibitor	*Candida albicans*, *Candida glabrata*, *Candida crusei*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*
		Carvacrol	Cell membrane disturbance, EP inhibitor	*Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimarium.* *Aspergillus niger*, *Aspergillus fumigatus*, *Epadosporium spp.*, *Rhizopus oryzae*

The link between medicinal plants and their potential as alternatives in the management of various conditions lies in the diverse bioactive compounds present in these plants. These compounds often exhibit therapeutic properties that can be beneficial for health. Medicinal plants contain a wide array of phytochemicals such as alkaloids, flavonoids, terpenoids, and polyphenols. These compounds possess diverse biological activities with potential therapeutic effects (Anyanwu and Okoye, 2017).

2. Materials and Methods

2.1. Study Design

The study is a laboratory based in-vitro experimental design that was conducted at the Pharmacognosy and Microbiology laboratory at Kampala International University, situated along Kasese road Bushenyi Uganda. The *S. aromaticum* also known as pilau masala extract was evaluated for antibacterial activity.

2.2. Plant Collection, Identification and Authentication

Dry *S. aromaticum* were collected from Nakasero market in Kampala district Uganda and placed in black polythene bags. The plant and associated indigenous information for its antibacterial properties was provided by a locally renowned Traditional Medical Practitioner (TMP) by local people. An open exhaustive interview was held with the TMP to obtain in-depth information about the plant. The identification and authentication of the plant was done by a taxonomist at Mbarara University of Science and Technology.

2.3. Preparation of Aqueous Plant Extract

The dry *S. aromaticum* is pulverized using a mortar and pestle to coarse powder grade. Powdered material of 200 g and 1000 mL of distilled water were added into two separate conical flasks. The flasks were covered with a wooden cork and the contents of the flasks mixed thoroughly. The flasks were placed in a shaker adjusted at 100 rpm overnight. The mixtures were then be filtered through a muslin cloth and centrifuged at 2000 rpm for 5 min and the supernatant transferred into a sterile falcon tube after filtration and stored at 4 °C in the refrigerator until use (Liu *et al*., 2021).

2.4. Preparation of Ethanolic Plant Extract

Initially, 200 g of *S. aromaticum* powder were placed in a conical flask of 1000 ml capacity. Ethanol with a concentration of 80% was added and the flask periodically shaken for one hour a day for three days. After three days, the mixture was filtered through Whatman No. 1 filter paper and ethanol evaporated by heating it in a hot air oven at 40 °C. Finally, the resulting concentrated crude extract was transferred to a container, appropriately labeled, and stored in a refrigerator at a temperature of 4 °C until use. The percentage yield of *Syzygium aromaticum* extract was calculated using the following formula (Kakande *et al*., 2019).

$$\text{Yield of extract} = \frac{Weight\ of\ the\ extract}{Weight\ of\ powder} \times 100$$

2.5. Sterility Test of Extracts

The sterility of all the extracts was examined on nutrient agar. 1 ml of each extract was inoculated in nutrient agar plates and incubated for 24 hours at 37 °C. The plates were checked for growth of bacteria after incubation. No growth on the plates would indicate that the extracts are sterile (Liu *et al*., 2021).

2.6. Phytochemical Screening

2.6.1. Test for Tannins

To 5 ml of extract, few drops of neutral 5% ferric chloride solution were added. Formation of dark green color indicated the presence of tannins.

2.6.2. Test for Terpenoids

1 ml of the extract was treated with chloroform, acetic anhydride followed by three drops of Sulfuric acid. The formation of dark green color indicated the presence of terpenoids.

2.6.3. Test for Saponins

0.2 g of the extract were dissolved in the 5 ml of distilled water in a test tube and then heated to boil. Frothing which persisted on warming indicated the presence of saponins.

2.6.4. Test for Flavonoids

The extract was heated with concentrated sulfuric acid resulting into the formation of orange color. This indicated a positive result for flavonoids.

2.6.5. Test for Alkaloids

To 1 ml of extract, three drops of Wagner's reagent were added. A reddish-brown precipitate indicated presence of alkaloids.

2.6.6. Test for Phenolic Compounds

To 2 ml of the extract, 2 ml of lead acetate solution were added. Formation of precipitate indicated the presence of phenolic compounds.

2.7. Test Organism

The Carbapenem-resistant *P. aeruginosa* was used to evaluate the antibacterial activity of the ethanolic and aqueous extracts of *S. aromaticum*. The Carbapenem-resistant *P. aeruginosa* was obtained from the Kampala International University Microbiology Laboratories.

2.8. Determination of Zone of Inhibition of Ethanolic and Aqueous Extracts of S. aromaticum

The agar well diffusion technique was used to determine the antibacterial activity of *S. aromaticum* extracts. Initially, Muller Hinton agar was prepared by diluting 10 g of Mueller Hinton agar in 250 ml of distilled water and sterilized at 121 ^{o}C for 15 minutes. The medium was cooled to about 45 ^{o}C, then ten milliliters of MHA were poured onto sterile petri dishes.

A prepared suspension of carbapenem-resistant *P. aeruginosa* standardized to McFarland standard were spread on the surface of Mueller Hinton Agar using a sterile cotton swab. Final extract concentrations of 500 mg / ml and 100 mgD ml were prepared for both the aqueous and ethanolic extracts of *S. aromaticum* and kept until use (Khan *et al*., 2017).

Using sterile glass cork borers, four wells of 6 mm diameter were carefully made on the agar plates without distorting the media and sufficiently spaced.

Two wells made in Mueller Hinton agar plate were each be filled with 50 µl of 500 mg / ml and 100 mg / ml concentration of *S. aromaticum* ethanolic extract. The third well were filled with 50 µl of colistin as positive control and the fourth with 50 µl of 10% sterile DMSO as negative control. The culture plates were left for 30 minutes to allow the extracts to diffuse through the media and then incubated for 24 hrs. After 24 hours of incubation, the diameters of the zones of inhibition were measured for all petri dishes using a metric ruler in millimeters (mm) and the average were calculated for each extract (Ntulume *et al*., 2019).

2.9. Experimental Outcomes

From the experiment, the zones of inhibition of the ethanolic and aqueous *Syzygium aromaticum* extract against the Carbapenem-resistant *P. aeruginosa* were measured. We also determined the Minimum Inhibitory Concentration and the Minimum bactericidal concentration of ethanolic and aqueous *S. aromaticum* extract against Carbapenem-resistant *P. aeruginosa*.

2.10. Determination of Minimum Inhibitory Concentration (MIC) of Ethanolic and Aqueous Extracts of S. aromaticum

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. The micro broth method dilution was used to determine the extract concentration. Stock concentrations of both extracts of the *S. aromaticum* at 1000 mg / mL were prepared. The stock concentration of extract was added to the first wells of the micro titre plate.

A twofold serial dilution of stock of each plant extract was made by transferring and picking 50 µL from the subsequent wells up to the final well where 50 µL is discarded. A pre-prepared organism suspension equivalent to 0.5 McFarland standard of a 24 h clinical culture of carbapenem-resistant *P. aeruginosa* was diluted to obtain 1.0×10^{6} CFU / ml and 100 µL of this concentration was added into each of the tubes that contains serially diluted extract. This resulted in decreasing final dilution extract concentration of 1000 mg / ml, 500 mg / ml, 250 mg / ml, 125 mg / ml, 62.5 mg / ml, 31.25 mg / ml, 15.63 mg / ml, 7.81 mg / ml, and 3.91 mg / ml, in each of the wells, with an organism concentration of 5.0×10^{5} CFU / ml.

To ensure accuracy, triplicates of each tube concentration was prepared. Two control groups was prepared: Control 1 was contain broth and bacteria but no extract to check for media support of *P. aeruginosa* growth and viability of the organism. Control 2 with only organisms. The micro titre plate was incubated at 37 °C for 24

hours, followed by the addition of 30 µL of 0.0015% resazurin to all the test wells. The wells were then be incubated at 37 °C for 30 minutes. Each well was examined for any color change from blue to pink which was indicative of presence of bacterial growth that causes reduction of resazurin. The above experiment was done in triplicates, in order to validate the results obtained. The minimum inhibitory concentration of ethanolic and aqueous extracts of *S. aromaticum* against Carbapenem-resistant *P. aeruginosa* were determined as the lowest concentration of the extract that did not result in the color change (Ntulume *et al.*, 2019).

2.11. Determination of Minimum Bactericidal Concentration (MBC) of Ethanolic and Aqueous Extracts of S. aromaticum

To determine the minimum concentration of ethanolic and aqueous extracts of *S. aromaticum* required to kill *P. aeruginosa*, the wells that remained blue from the MIC test were plated onto sterile Mueller Hinton Agar. The plates were incubated at 37 °C for 24 hours. The minimum concentration that resulted in no colony growth on the plates were considered the Minimum Bactericidal Concentration (Ntulume *et al.*, 2019).

2.12. Statistical Analysis

2.12.1. Antibacterial Activity

The inhibition zone diameters and their corresponding concentrations were recorded in Microsoft Excel and then analyzed using SPSS-20 software. Descriptive statistics such as mean and standard deviation of the inhibition zone diameters in mm were computed using one-way ANOVA (Ntulume *et al.*, 2019).

2.12.2. Environmental Considerations

Disposal of used culture plates and inoculums tubes was done in a way that ensured the safety of the environment by packing all the used culture plates, and inoculums tubes into autoclave bags and autoclaved at 121 °C for 20 minutes. Then later the waste media was disposed of in waste bins.

3. Results

3.1. The Phytochemical Analysis of Ethanolic and Aqueous Extracts of Syzygium aromaticum

Phytochemical analysis showed the presence of alkaloids, tannins, terpenoids, flavonoids, saponins and phenolic compounds (Table 2).

Table 2: Results Obtained from the Phytochemical Screening for Alkaloids, Tannins, Flavonoids, Saponins and Phenolic Compounds

Phytochemicals	Ethanolic Extract	Aqueous Extract
Alkaloids	-	+
Tannins	+	+
Terpenoids	+	+
Flavonoids	+	-
Saponins	+	-
Phenolic compounds	+	+

Note: (+) represents the presence of the constituent; (-) represents the absence of the constituent.

3.2. Identification of Carbapenem Resistant Pseudomonas aeruginosa

The carbapenem resistant *Pseudomonas aeruginosa* strain was obtained from the microbial bank and sub cultured on MacConkey agar plate and a disc of imipenem was placed to determine its resistance. There was no zone of inhibition around the imipenem disc which confirmed the resistance of the *Pseudomonas aeruginosa* strain. The strain was also green in color which is characteristic of the virulent strain of *Pseudomonas aeruginosa*.

3.3. Zones of Inhibition of Ethanolic and Aqueous Extracts of S. Aromaticum

Table 3: Mean Inhibition Zone Diameters of *Syzygium aromaticum* Extract against CRPA

Extract Concentrations and Controls	Mean ± SEM Inhibition Zone Diameters (mm)	
	Ethanolic Extract	Aqueous Extract
500 mg/Ml	15.33 ± 0.33[a]	9.00 ± 0.0
100 mg/Ml	12.33 ± 0.67[b]	0
Colistin 30 µg	12.33 ± 0.33[cde]	
Negative Control (normal saline)	0.000	

Note: [a]Ethanolic extract 500 mg/mL Vs. Aqueous extract 500 mg/mL= $p \leq 0.0001$; [b]Ethanolic extract 100 mg/mL Vs. Aqueous extract 100 mg/mL = $p \leq 0.0001$; [c]Colistin 30 µg Vs. Ethanolic extract 500 mg/mL = $p \leq 0.0001$; [d]Colistin 30 µg Vs. Ethanolic extract 100 mg/mL = $p \leq 0.0001$; [e]Colistin 30 µg Vs. Aqueous extract 500 mg/mL = $p \leq 0.0001$.

Table 4: Percentage Yield of Ethanolic and Aqueous Extracts of *S. aromaticum*

Extract	Weight of Powder (g)	Weight of Extract (g)	Percentage Yield (%)
Ethanolic	200	17.2	8.6
Aqueous	200	16.6	8.3

Table 5: Minimum Inhibitory Concentration of Ethanolic and Aqueous Extracts of *S. aromaticum*

	Ethanolic Extract (mg/ml)	Aqueous Extract (mg/ml)
CRPA	7.81	15.6

Table 6: Minimum Bactericidal Concentration of Ethanolic and Aqueous Extracts of *S. aromaticum*

	Ethanolic Extract (mg/ml)	Aqueous Extract (mg/ml)
CRPA	125	125

4. Discussion

4.1. Percentage Yield of the Extract

The yield of clove recovered from the two different solvents was 8.3% for aqueous and 8.6% for ethanolic extract per 200 g of dried powder of clove bud. The variations between the extraction yields from ethanol and water were attributed to the differences in polarity of constituents found in *S. aromaticum*. It has been reported previously that the water extract of different plants usually yields significantly higher amounts compared to ethanolic extracts of the same plant (Omoruyi *et al.*, 2014). However, according to the results obtained, the higher yield of ethanolic extract showed that ethanol was a better solvent for extracting constituents in *S. aromaticum* as compared to water.

4.2. Phytochemical Analysis of the Aqueous and Ethanolic Extract of Syzygium aromaticum

The antimicrobial activity of ethanolic and aqueous extracts from *S. aromaticum* against CRPA can be attributed to the presence of specific phytochemicals. The ethanolic extract, containing alkaloids, tannins, terpenoids, flavonoids and saponins, exhibits a diverse array of bioactive compounds known for their antimicrobial properties. Alkaloids are recognized for their toxicity to microorganism, while tannins possess astringent qualities that can disrupt microbial cell membranes. Terpenoids contribute to the antimicrobial potential with their diverse chemical structures, and flavonoids are known for their antioxidant and antimicrobial activities. Saponins, exhibiting surfactant properties, may disrupt microbial cell membranes. On the other hand, the

aqueous extract, lacking flavonoids and saponins, still contains alkaloids, tannins and terpenoids, which contribute to its antimicrobial efficacy, Alkaloids with their potential to interfere with microbial nucleic acid synthesis, and tannins, known for their protein-binding abilities, play key roles in the aqueous extract's antimicrobial activity. Terpenoids contribute further to this effect. The absence of flavonoids and saponins in the aqueous extract may suggest that these specific compounds are not crucial for its antimicrobial potential against CRPA.

4.3. Antibacterial Activity of Ethanolic and Aqueous Extracts of S. aromaticum

According to the study, both the ethanolic and the aqueous extracts of *S. aromaticum* exhibited antibacterial activity against CRPA as was with the studies conducted earlier (Ahmed *et al.*, 2021). The Turkey's multiple comparison test for ethanolic extract of *S. aromaticum* at 500 mg / ml against the same extract at 100 mg / ml reflected an adjusted p-value of <0.0001. This suggested a highly significant difference between the *S. aromaticum* ethanolic extracts concentrations of 500 mg / ml and 100 mg / ml in terms of antibacterial activity against CRPA. This continues to suggest that the antibacterial activity of ethanolic extract of *S. aromaticum* at 500 mg / ml is significantly more effective against CRPA compared to the 100 mg / ml concentration of the same extract. The implication is that higher concentrations of the ethanolic extract have a stronger inhibitory effect on CRPA which is important in the potential use of the extract in curbing bacterial infections.

An adjusted p-value of >0.999 indicated that there was no statistical difference between the ethanolic extract of *S. aromaticum* at 100 mg / ml and colistin 30 µg when it comes to antibacterial activity against CRPA. The observed difference are associated with random variability, and there is insufficient evidence to conclude that the ethanolic extract of *S. aromaticum* at 100 mg / ml performs differently compared to colistin. This suggests that the antibacterial activity of the ethanolic extract of *S. aromaticum* at 100 mg / ml does not exhibit a significantly different inhibitory effect against CRPA as compared to colistin.

An adjusted p-value of >0.999 indicated that there was no statistical difference between the aqueous extract of *S. aromaticum* at 100 mg / ml and normal saline when it comes to antibacterial activity against CRPA. The observed difference are associated with random variability, and there is insufficient evidence to conclude that the aqueous extract of *S. aromaticum* at 100 mg / ml performs differently compared to normal saline. This suggested that CRPA is less susceptible to aqueous extract of *S. aromaticum* at 100 mg / ml and below as they don't exhibit a significant antibacterial activity. The ethanolic extract of *S. aromaticum* was both bactericidal at higher concentrations and bacteriostatic at low concentration as compared to the aqueous extract of the same plant. Its efficacy at the different concentrations explored in this study explains why there are more studies done on the ethanolic extract of *S. aromaticum* as compared to its aqueous extract (El-Maati *et al.*, 2016).

5. Conclusion

The study revealed that both ethanolic and aqueous extracts of *S. aromaticum* have antibacterial activity against carbapenem resistant *Pseudomonas aeruginosa.* It also showed the presence of tannins, phenolic compounds and terpenoids, in both extracts, are responsible for the antibacterial activity against carbapenem resistant *Pseudomonas aeruginosa.* The study validated the modification of the components of the ethanolic and aqueous extracts of *S. aromaticum* in a bid to synthetically develop alternative treatments against carbapenem resistant *Pseudomonas aeruginosa.*

6. Recommendations

According to the result above, the ethanolic and aqueous extracts of *S. aromaticum* had antibacterial activity against carbapenem resistant *P. aeruginosa*, I would recommend that further qualitative and characterization studies be done on the different constituents of the ethanolic and aqueous extracts of *S. aromaticum*. This will be step towards the development of synthetic alternative treatments against Carbapenem resistant *P. aeruginosa*.

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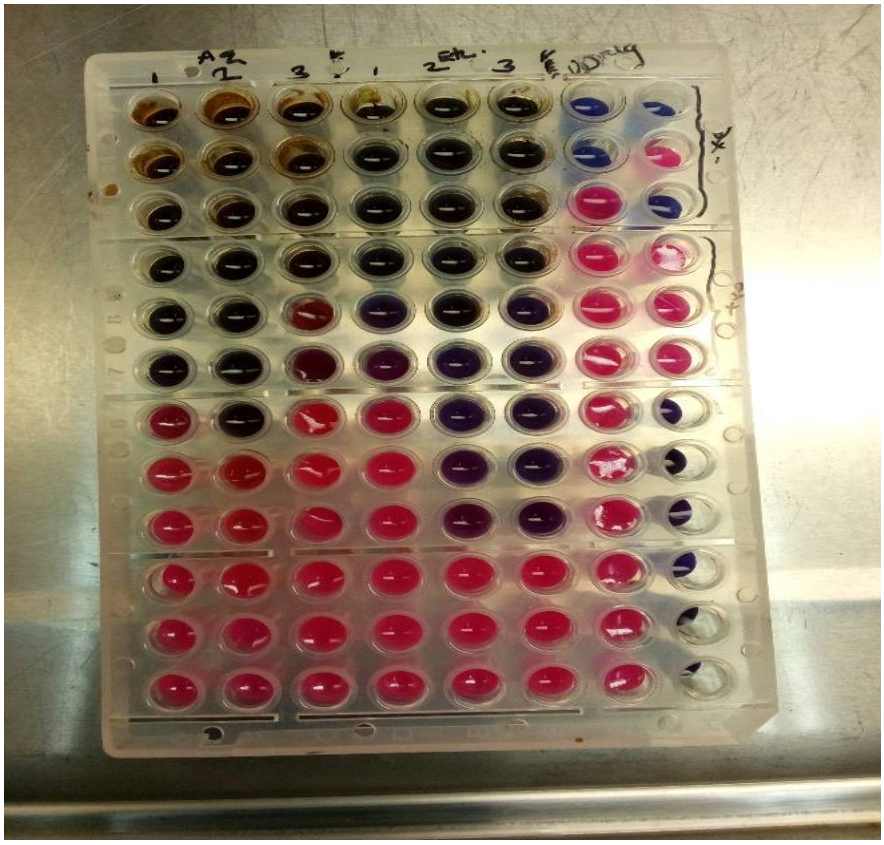
Appendices

Appendix 1: Comparing Broth to 0.5 McFarland Standard

Appendices (Cont.)

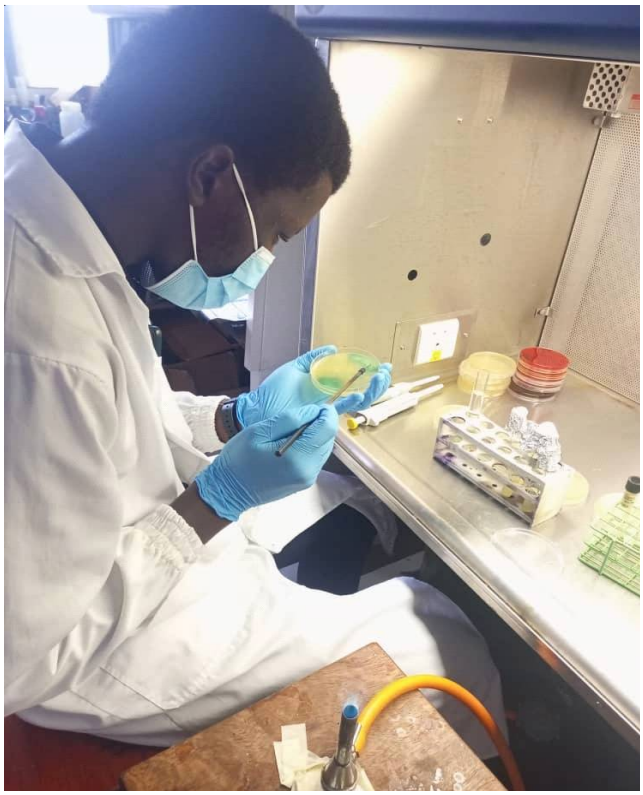


Appendix 2: Microtitre Plate Showing Results of the MIC of the Two Extracts of *S. aromaticum*

Appendix 3: Plate Showing the MBC of the Aqueous Extract of *S. aromaticum*

Appendices (Cont.)

Appendix 4: Plate Ahowing the MBC of the Ethanolic Extract of *S. aromaticum*



Appendix 5: CRPA is Picked Carefully from the Plate to Make Broth

Appendices (Cont.)

Appendix 7: *P. aeruginosa* Subculture

Appendix 8: One-Way Anova

Tukey's Multiple Comparisons Test	Mean Diff.	95% CI of Diff.	Significant?	Summary	Adjusted p-value
Ethanolic Extract					
500 mg / mL vs. 100 mg / Ml	3.000	1.738 to 4.262	Yes	****	< 0.0001
500 mg / mL vs. Colistin, 30 µg	3.000	1.738 to 4.262	Yes	****	< 0.0001
500 mg / mL vs. Negative control	15.33	14.07 to 16.59	Yes	****	<≤ 0.0001
100 mg / mL vs. Colistin, 30 µg	0.0	-1.262 to 1.262	No	ns	> 0.9999
100 mg / mL vs. Negative control	12.33	11.07 to 13.59	Yes	****	< 0.0001
Colistin, 30 µg vs. Negative control	12.33	11.07 to 13.59	Yes	****	< 0.0001
Aqueous Extract					
500 mg / mL vs. 100 mg / mL	9.000	7.738 to 10.26	Yes	****	< 0.0001
500 mg / mL vs. Colistin, 30 µg	-3.333	-4.595 to -2.072	Yes	****	< 0.0001
500 mg / mL vs. Negative control	9.000	7.738 to 10.26	Yes	****	< 0.0001
100 mg / mL vs. Colistin, 30 µg	-12.33	-13.59 to -11.07	Yes	****	< 0.0001

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