Original Research Article

ENDOPHYTIC *BACILLUS AEROPHILUS* FROM THE LEAVES OF *AZADIRACHTA INDICA* AS A POTENTIAL BIOCONTROL AGAINST *STAPHYLOCOCCUS AUREUS*.

ABSTRACT

Aims: The study entailed isolation and identification of *Bacillus aerophilus* in the leaves of young *Azadirachta indica* plants, establishing the antimicrobial activity of the endophyte against *Staphylococcus aureus*, and screening for phytochemicals. The study investigated the potential of the endophytic *Bacillus aerophilus* in pathogen inhibition and phytochemical screening for bioactive compounds produced by the endophyte that can be used in drug development. The research contributes to the wider scientific goal of curbing rising cases of antibiotic resistance.
Place and Duration of Study: Sample: collected from Kanyoonyoo in Kitui County, between August 2022 and June 2023.
Methodology: The bacteria were aseptically isolated and sub-cultured on nutrient agar. For identification, the bacteria underwent biochemical tests and molecular characterization. The 16S rRNA region was amplified and sequenced using universal primers 27F and 1492R. Bacterial isolate K1L003 was identified as *Bacillus aerophilus*. For antimicrobial testing, the agar disk diffusion method was used against pathogen*Staphylococcus aureus*on Mueller Hinton Agar plates (MHA).The controls were doxycycline and vancomycin.
Results: The endophytic isolate only inhibited *Staphylococcus aureus* (*P*<.001), with an inhibition mean similar to doxycycline (26 mm), against the pathogen. The endophytic isolate produced terpenoids, saponins, alkaloids, and flavonoids.
Conclusion: Endophytic *Bacillus aerophilus* has better antimicrobial inhibition potential than vancomycin, against *Staphylococcus aureus* and produces diverse bioactive secondary metabolites. The endophyte has the potential to produce compounds that are useful in treating drug-resistant Staphylococcal infections.

Keywords: Bacillus aerophilus, Staphylococcus aureus, Azadirachta indica, doxycycline, vancomycin, bioactive compounds

UNDER PEER REVIEW

1. INTRODUCTION

Microbes exist virtually everywhere and have advantages and disadvantages depending on their interactions with themselves and other organisms within the ecosystems [1]. Research has shown that microbes exist in plants and animals. According to Deng et al. [2], plants and animals exist in the world of microbes. These microbes have adapted to living in various habitats and have coevolved alongside other organisms over time, forming relationships, including mutualism, parasitism, and commensalism [3]. Endophytes are among the mutualistic and beneficial microbes. Deng et al. [2] states that the endophytes are found in plant tissues and have no adverse effects on the plants. They include both bacteria and fungi. There are diverse endophytic communities within the plant that have proven beneficial to the plants.

According to Deng et al. [2], some endophytes play crucial roles in plant protection and plant growth. The ways in which these endophytes confer resistance to plants against pathogens has triggered curiosity amongst scientists.

The *Bacillus* sp. is common endophytic bacteria. They have been isolated from all types of plants, including *Zea mays*, where several *Bacillus subtilis* species were isolated and shown to inhibit *Botrytis cinerea*, a phytopathogen causing significant economic losses [4]. *Bacillus* sp. have been isolated from apple buds and shown to enhance cell development, protein metabolism, and shoot growth in apples [5]. These bacteria species have been found in medicinal plants. Zhao et al. [6] conducted a study in which they isolated endophytic bacteria from *Lonicera japonica*, a medicinal plant. *Bacillus* strains were among the isolated endophytes that inhibited *Fusarium oxysporum* and promoted wheat growth. *Lonicera japonica* has antibacterial, anti-inflammatory, antiviral, antifungal, and fever-reducing effects [6]. According to Miller et al.[7], *Bacillus* strains are among the prominent bacteria forming the endophytic community of medicinal plants and produce bioactive substances such as phytotoxins and antimicrobials. The beneficial impacts of *Bacillus* sp. include protection of plants against phytopathogens, nematodes, and insects, promoting growth in plants, and eliciting resistance [8]. Saxena et al. [9] reports that *Bacillus* sp. fix nitrogen, solubilize phosphorus, produce siderophores, phytohormones, promote uptake of nutrients, and act as biocontrol agents. These bacteria are a potential source of probiotics. Mazkour et al. [10] showed that the administration of *Bacillus subtilis* and *Bacillus coagulans* in rats significantly reduced coliform counts, improved weight gain, improved intestinal probiotic microbiota, and reduced feed consumption. They concluded that the bacteria were beneficial intestinal microbiota in the rats. More research can lead to the bacteria being used as human food supplementation in the future and possible sources of novel antimicrobial compounds.

Bacillus aerophilus isa rod-shaped, gram positive, spore-forming bacterium, first isolated from cryogenic tubes that were used in collecting air samples from high altitudes [11]. According to Saxena et al. [9], *Bacillus aerophilus* has been shown to fix free or atmospheric nitrogen. *Bacillus aerophilus* is useful to both flora and fauna. A study by Ramesh et al. [12] showed that *Bacillus aerophilus* used in dietary supplementation increased disease resistance and boosted immunity in fish (*Labeorohita*), by inhibiting the pathogen *Aeromonas hydrophila* that affected freshwater fish. According to Ramesh et al. [12], the use of antibiotics against the pathogen has led to increase in drug resistance and persistence of substances that are harmful to humans upon consuming the fish, leading to concerns on food safety. Thus, using probiotics is a much safer and more effective route. *Bacillus aerophilus* has great probiotic potential in aquaculture [12]. *Bacillus aerophilus* have not been widely researched as an endophyte and a potential antimicrobial agent in medicinal plants. This study aims to isolate the bacteria from *Azadirachta indica* and determine its antimicrobial potential and its ability to produce phytochemicals.

Scientists now target endophytes in medicinal plants to determine whether the medicinal properties of these plants are attributable to their endophytes. *Azadirachta indica* is a well-known herbal plant whose products are useful in disease treatment and prevention [13]. Alzohairy [13] states that *Azadirachta indica* was harnessed for use in Chinese, Unani, and Ayurvedic remedies. The pharmacological uses of the plant include, as an antioxidant, anticancer activity, antimalarial, antidiabetic, antiviral, antibacterial, antifungal, anti-inflammatory, neuroprotective, immunomodilatory, and wound healing properties [13]. Some of the chemical constituents that are part of the bioactive metabolites of the plant include isoprenoids, such as azadirachtin, terpenoids, tertranorterpenoids, steroids, and limonoids, phenolics, tannins, flavoglycosides, fatty acid derivatives, hydrocarbons, and sulphur compounds [14]. The use of extracts from *Azadirachta indica* has no adverse effects, however daily consumption of extreme amounts of the extracts can lead to organ toxicity, with symptoms, such as Reye reported in India [14]. The plant was used in ayurvedic medicine to treat the skin, eye, and gastrointestinal problems [14]. The plant oil extracts inhibited many Gram negative and Gram positive bacteria in vitro, including *Staphylococcus aureus, Mycobacterium tuberculosis, Vibrio cholera, Klebsiella pneumonia,* and *Streptococcus* sp. [14].

Staphylococcal strains are significant pathogenic strains because they are prominent sources of food poisoning, skin conditions, and secondary infections such as meningitis, cystitis and peritonitis [14]. According to Tembe-Fokunang et al. [14], these bacteria have developed strains resistant to antibiotics, such as the methicillin-resistant *Staphylococcal aureus* (MRSA), and are major contributors in hospital-acquired infections. MRSA is a major source of community-acquired infections [15]. The pathogenic bacteria have an increasing resistance against lincosamides, macrolides, and fluoroquinolones [16]. Some mechanisms of drug resistance in *Staphylococcus aureus* include inactivating the antibiotic using enzymatic action, altering the drug target and decreasing affinity for the drug, efflux pumps and trapping the antibiotics [17]. These bacteria cause serious illnesses such as necrotizing fasciitis, septic arthritis, osteomyelitis, necrotizing pneumonia, and pyomyositis [16]. Thus, it is vital to find effective therapy to curb the antibiotic resistance.

Research shows that *Azadirachta indica* is capable of prevailing in controlling *Staphylococcus aureus* through producing novel compounds, better than the antibiotics currently used to control the pathogen. Furthermore, a *Bacillus* sp.compound, such as the cell-free supernatant of the *Bacillus subtilis*, has been shown to increase the susceptibility of *Staphylococcus aureus* to drugs including penicillin and reduce the pathogen burden in mouse models [18]. The supernatant suppresses the genes responsible for adhesive factors, virulence factors, biofilm formation and quorum

sensing in *Staphylococcus aureus* [18]. Piewngam et al. [19] showed that *Bacillus* bacteria had the ability to inhibit the colonization of *Staphylococcus aureus* via inhibiting quorum sensing systems of the pathogen. The study concluded that *Bacillus* strains can be used to combat drug resistant *Staphylococcus aureus* species and can be applied and safe and simple probiotic decolonization agents against *Staphylococcus aureus*. The research aims at uncovering the potential of endophytic *Bacillus aerophilus* from *Azadirachta indica* as a potential antimicrobial agent against pathogenic *Staphylococcus aureus* and a potential source of bioactive compounds against the pathogen.

2. MATERIAL AND METHODS

2.1 Sampling

The samples were collected in August 2022 during the dry season from Kitui County (1°11'3 "S, 37°49'11 "E). The samples collected comprised leaves from young plants of *Azadirachta indica*. The young plant leaves sampled were purplish or reddish. The samples were transported to Kenyatta University Microbiology Laboratories in labelled plastic bags and processed within 24 hours.

The samples were washed in running tap water and allowed to air dry. The samples were surface sterilized by immersing them in 70 % ethanol for two minutes, then immersed in 3.5 % sodium hypochlorite for one minute, and rinsed five times using double distilled water. The samples were dried using sterile blotting paper in a laminar flow cabinet and cut aseptically using a sterile blade into 1 cm by 1 cm segments[20]. The pieces were aseptically macerated using a mortar and pestle and 6 mL of normal saline. The suspension was inoculated on Nutrient Agar (NA) plates and incubated for 24 hours at 37 °C. Colonies of bacteria were differentiated based on their morphology. Each individual colony was sub cultured in NA Petri dishes to purify the colonies. Every Petri dish contained a single microorganism from the plates containing the mixed cultures [20, 21]. For confirmation of surface sterilization, 1mL of the sterile-distilled water from the final rinse during sterilization was spread on control nutrient agar plates and inoculated for 24 hours at 37 °C [22]. There was no growth on the control plates indicating successful surface sterilization and zero contamination.

2.2 Biochemical Identification of Endophyte *Bacillus aerophilus*

2.2.1 Gram Test

For Gram staining, colonies of pure cultures of bacteria were fixed on microscope slides, after which, they were stained using crystal violet. After forty-five seconds, the stain was washed off with distilled water. Three drops of Gram's iodine solution were added to each slide. After 1 minute, the iodine solution was washed off with distilled water. Next, the decolouriser was added to each slide and washed off after five seconds. A few drops of safranin, the counterstain, were added onto each slide and washed off with distilled water after thirty seconds. The slides were then blot-dried and observed under a light microscope [23].

2.2.2 Oxidase Test

The pure bacterial colonies were smeared on commercially-prepared HIMEDIA oxidase discs. The appearance of a deep-purple colour indicated a positive result for the oxidase test [24].

2.2.3 Catalase Test

Drops of hydrogen peroxide were added to the pure colonies and observed for the presence of gas bubbles which indicated a positive result [24].

2.2.4 Starch Hydrolysis Test

The pure cultures were aseptically inoculated in starch agar by making single streaks of the cultures and incubated for 48 hours at 37 ℃. The agar surfaces were flooded with iodine solution using a dropper. After thirty seconds, the excess iodine was poured off and the cultures examined for presence of clear zones around the cultures. The zones indicated a positive result [24].

2.3 Molecular Identification of Endophytic *Bacillus aerophilus*

2.3.1 DNA Extraction from Bacterial Cells

UNDER PEER REVIEW

Bacterial cells of pure colonies suspected to be *Bacillus aerophilus* after the biochemical identification were transferred to sterile microcentrifuge tubes containing 500 microliters sterile normal saline. The tubes were centrifuged to pellet the bacterial cells and the normal saline decanted. The process was repeated severally to get rid of extracellular polymeric substances (EPS). In the next phase, the cells were lysed. Cell lysis buffer pH 8.0 containing (2 % w/v CTAB, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, and 0.3 % Mercaptoethanol) was added to the tubes [25]. The samples were vortexed for 1 minute and then incubated at 65 ℃ for one hour. They were then centrifuged for 5 minutes at 13,000 rpm. The liquid phase was transferred to sterile microcentrifuge tubes followed by addition of equal volume of Chloroform: Isoamyl at a ratio of 24:1 [25]. The mixture was gently mixed by inversion and incubated at -20 ℃ for 30 minutes, and centrifuged at 13000 rpm for 4 minutes and the supernatant transferred to a fresh sterile microcentrifuge tube. The supernatant contained the DNA. The DNA was precipitated by addition 500 µl of chilled absolute ethanol and centrifuged at 12,000 revs per minute for 4 minutes to pellet the DNA. The liquid phase was gently decanted off. The precipitate was mixed with 100 µl of chilled 70% ethanol and centrifuged at 12,000 rpm for 2 min. After decanting the 70 % ethanol the precipitate was dried by inversion on sterile paper towels followed by elution of the DNA using 50 mL TE buffer pH 8.0.

2.3.2 Amplification of the Extracted DNA

The bacterial DNA extracted was amplified via the 16SrRNA gene sequencing method. The 27F and 1492R Macrogen universal primers with sequences (F) AGAGTTTGATCMTGGCTCAG and (R) TACGGYTACCTTGTTACGACTT-respectively, were used in amplification as described by Sharma and Roy [26]. One Taq Quick-load 2X Master mix with standard buffer from New England BioLabs was used for the PCR process. The PCR reaction contents were composed of 1.0 µl primers (10 µM per primer), 12.5 µl 2X master mix with buffer, 10.5 µl PCR water and 1.0 µl of bacterial isolate DNA was added. This PCR reaction mixture was introduced into a PCR thermocycler which was set for 35 cycles. Initial denaturing was at 94 °C for 2 minutes. This was followed by a cycle consisting of denaturation at 94 °C for 45 seconds, annealing at 49 °C for 1 minute and extension at 72 °C for 2 minutes. The final extension was done at 72 °C for 5 minutes. The amplification was a modification of the amplification perimeters used by Moyo *et al.*[25].

After DNA amplification, the PCR products were subjected to Gel electrophoresis. DNA loading buffer (3 µl) containing SYBR GREEN dye was mixed with 3 µl aliquots of the PCR product and pipetted into the wells of the 1 % agarose gel in 0.5X Tris borate-EDTA (TBE) buffer. This was connected to source of current (80 V) and left to run for 30 minutes at low temperatures, after which the results were visualized using a UV trans-illuminator. This was to determine the quality and quantity of the PCR product [27].

2.3.3 PCR Sequencing

The PCR products were purified via column purification using the QIAquick PCR Purification Kit. After purification, the DNA fragments were amplified via Sanger sequencing using forward and reverse primers. The primers used were the same sequences as those in Section 2.3.2. The samples were sequenced at Macrogen, Europe, using theABI 3730 DNA Analyser machine, and identified using the National Centre for Biotechnology Information genome sequence (NCBI) database.

2.4 Antimicrobial Activity of the Endophytic Isolate

After identification, the antimicrobial activity of the endophyte *Bacillus aerophilus* was tested via the agar diffusion method against the pathogenic *Staphylococcus aureus*, as outlined in the Clinical Laboratory Standards Institute (CLSI) [28]. The pathogen was cultured in nutrient broth and an inoculum containing 10^6 colony forming units (cfu)/ml of the *Staphylococcus aureus* culture was spread on sterile Mueller Hinton agar (MHA) Petri dish with a sterile swab moistened with the pathogenic bacteria suspension [29]. Wells of about 6 mm were bored into the MHA using a sterile cork borer. The endophyte was centrifuged for 15 minutes at 6000 rpm and the supernatant was inoculated into the wells. Each well was filled with 100 µl (25 mg/ml) of the supernatant, allowed to diffuse for 2 hours at room temperature, and incubated in an upright position for 24 hours at 37 °C [29]. The zones of inhibition were observed and measured. The experiment was done in triplicates and compared to the standard antibiotic discs of vancomycin and doxycycline used as positive controls against the pathogen [30]. The negative controls were sterile filter paper discs dipped in normal saline and placed on MHA inoculated with the pathogenic bacteria.

2.5 Phytochemical Screening for Bioactive Compounds of Endophytic *Bacillus aerophilus*

2.5.1 Test for Tannins

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UNDER PEER REVIEW

A few drops of Iron (III) chloride ($FeCl_3$) solution were added to the extract of the endophytic bacteria after five days of incubation in nutrient broth. Presence of tannins was indicated by a green color precipitate formation [31].

2.5.2 Test for Flavonoids

Ten percent lead acetate solution (1 mL) was added to 3 mL of the extract. Presence of a yellow precipitate indicated a positive result [31].

2.5.3 Test for Terpenoids

Each extract (3 mL) was dissolved in chloroform (2 mL) and evaporated to dryness, after which, 2 mL of concentrated sulphuric acid was added. The solution was heated for about two minutes. A greyish colour indicated a positive test result for terpenoids [31].

2.5.4 Haemolytic Test for the Presence of Saponins

A few drops of the fresh blood were placed on a glass slide. Two drops of the extract were added to the blood on the glass slip and observed for the formation of clear zones [31].

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2.5.5 Test for Steroids

Two millilitres of the extract was mixed with an equal amount of chloroform, followed by sidewise addition of concentrated sulphuric acid (2 mL). The presence of steroids was indicated by a red colour in the lower chloroform layer [32].

2.5.6 Test for Anthraquinones

The extract was boiled with dilute sulphuric acid. Chloroform was added to the mixture, which was then filtered and eventually cooled. An equal volume of dilute ammonia was added to the filtrate and shaken. Appearance of pink to red colour indicated that derivatives of anthraquinones were present [33].

2.5.7 Keller-kilani's Cardiac Glycosides' Test

The extract was mixed with glacial acetic acid (2 mL) in test tubes and two drops of 2% iron (III) chloride solution added, followed by addition of 2 mL of concentrated sulphuric acid. The appearance of a brown ring at the interphase indicated a positive test result [32].

2.5.8 Test for Phlobatannins

Three millilitres of each of the aqueous extracts was added to 2 mL of 1% hydrochloric acid and boiled. A red precipitate deposit indicated the presence of phlobatannins [31].

2.5.9 Test for Alkaloids

Three millilitres of the aqueous extract was added to 1% hydrochloric acid (3 mL) on a steam bath, followed by addition of Mayer and Hager's reagent. The presence of turbidity in the precipitate and appearance of a yellow colour respectively, indicated a positive result test for alkaloids [31].

2.5.10 Test for Phenols

The extract was mixed with 2% Ferric chloride solution. A blue-green or black coloration indicated a positive test result for phenols [32].

2.6 Data Analysis

Consensus for sequences generated using 27F and 1492R primers were created using BioEdit Software after base calling using Chromas Lite Software. A BLAST (Basic Local Alignment Search Tool) search of the consensus sequence in the National Centre for Biotechnology Information (NCBI) database was used for molecular identification of the isolates. The isolate consensus sequences and identity sequences were aligned using MUSCLE algorithm in Mega X software [34]. The phylogenetic tree was developed using the neighbour-joining method and evolutionary distances determined using the maximum composite likelihood in the Mega X statistical tool. The R statistical tool was used to analyse enzyme

UNDER PEER REVIEW

bioassay data. Tukey's HSD was utilized to determine the significance in zones of inhibition (ZOI) among the isolates and compared to ZOI of antimicrobial drugs as well ($P < .001$).

3. RESULTS AND DISCUSSION

3.1 Morphological and Biochemical Traits of Endophytic Isolate

The appearance of the endophytic bacteria on nutrient agar comprised raised, white, irregular, spore-forming colonies with a flat top. The isolate was Gram positive (Figure 1). It tested positive for the catalase, oxidase, and starch hydrolase enzymes (Table 1). The endophytic isolate suspected to be *Bacillus aerophilus* was labelled K1L003. K standing for Kitui, the place of origin, 1L003 was the reference number for the isolate.The endophytic *Bacillus aerophilus* is Gram positive, with colonies that appear raised, white, and irregular on nutrient agar. The bacteria are rod-shaped. These finding concur with those of Shivaji [11], who was the first isolated the bacteria from cryogenic tubes for collecting air samples at high altitudes.Endophytic *Bacillus* strains have been isolated from *Azadirachta indica*. Yoganathan& Tiwari [35] isolated *Bacillus cereus* and *Bacillus megaterium* strains from *Azadirachta indica*. According to Tiwari & Thakur [36], an endophytic *Bacillus* strain, *Bacillus amyloliquefaciens* was dominant in all the *Azadirachta indica* plant samples. This shows that the *Bacillus* sp. is present in *Azadirachta indica*. There is no mention of *Bacillus aerophilus* isolated from the leaves of the plant prior to this study.

The endophytic communities of the *Azadirachta indica* can vary from one plant to another. These variations occur due to external and internal factors. Huang [37] reported that variations of endophytic communities resulted from external factors such as the geographical elevation, precipitation, and mean annual temperature. Competitive advantage among the endophytes was also cited in the study, as a factor affecting endophytic frequency and distribution in plants. It explains why some strains of Bacillus are present in some studies and absent in others. The bacteria vary in their mechanisms for establishing commensalism within the different plants. The *Bacillus* sp. is among the most adaptive endophytic microbes. Deng et al. [2] shows how endophytic *Bacillus subtilis* produces subtilomycin which helps it evade the plant's defenses and establish itself as an endophytic microbe. The ability to adapt gives the bacteria a competitive advantage and can explain their presence in most studies on medicinal plants, including *Azadirachta indica*.

Table 1. Biochemical test results of the endophytic *Bacillus aerophilus*

Biochemical Test	Test Results
GRAM STAIN	Gram positive
CATALASE	Positive
OXIDASE	Positive
STARCH HYDROLYSIS	Positive

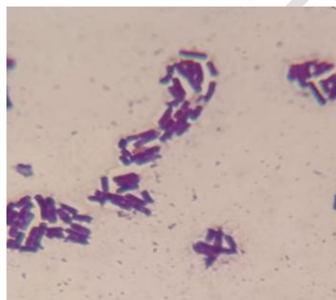

Figure 1: The microscopy image of the gram-stained endophytic isolate K1L003.

3.2 Molecular Identification

Based on nucleotide BLAST in the NCBI database, the isolate presented a 98.57 % similarity with the reference strain in the NCBI database. The reference strain was *Bacillus aerophilus* (see Table 2).

Table 2. The NCBI BLAST database similarity results of the isolate K1L003

Isolate	Accession number	Scientific Name	Query cover (%)	Percentage Identity
K1L003	ON063443	*Bacillus aerophilus*	99	98.57

3.3 Antimicrobial Activity of the Endophytic Isolate K1L003 (*Bacillus aerophilus*)

The endophytic isolate K1L003 (*Bacillus aerophilus*) inhibited *staphylococcus aureus*. The mean of inhibition against *Staphylococcus aureus* for the endophytic isolate was 26 mm (2.6 cm), which was the same as that of doxycycline, while vancomycin had a mean of inhibition of 15 mm (1.5 cm). The overall mean inhibition zone against all the pathogens for the endophytic isolate was 0.44 cm, while that of vancomycin was 0.45 cm. The antimicrobial effect of the isolate *Bacillus aerophilus* and vancomycin had no statistical difference. The mean inhibition zone of doxycycline was 0.96 cm ($P < .001$). There was a significant statistical difference between doxycycline and the endophytic isolate (see Table 3).

Vancomycin has been used as treatment for infections caused by *Staphylococcus aureus* for decades [38]. However, there are cases of vancomycin-resistant *Staphylococcus aureus* (VRSA), which pose a threat to public health [38]. Though the number of cases of VRSA is few, the surveillance for the VRSA strains in middle and low income countries is subpar compared to high income countries and thus the true levels of VRSA may remain undetected. According to Cong et al. [38], surveillance is crucial especially for antibiotic-resistant microbes. Doxycycline is prescribed for treatment of community-acquired MRSA [16]. Akinsanya *et al.* [39] showed that *Bacillus aerophilus* inhibited pathogens including *Streptococcus pyogenes, Candida albicans,* and *Proteus vulgaris*. The findings of this study suggest that the endophytic *Bacillus aerophilus* has the same potential in dealing with *Staphylococcus aureus* as doxycycline and a greater antimicrobial potential than vancomycin, the commonly used treatment drug. It means that the endophytic bacteria can be used as a potentially effective treatment therapy in curbing the increasing drug resistance of *Staphylococcus aureus* in vancomycin. *Azadirachta indica* has been shown to inhibit *Staphylococcus aureus* [40]. According to Sinaga et al. [41], *Azadirachta indica* is very effective in inhibiting Gram positive and Gram negative bacteria. This study suggests that the endophytes may contribute to the antimicrobial properties of medicinal plants.

Table 3. Means of inhibition zones of the controls and the endophytic isolate against pathogens

Treatment	Mean of inhibzone (cm)	Mean of tran_inhibzone (log base 10)
Doxycycline	0.96 ± 0.33^{a}	1.38 ± 0.38^{a}
Vancomycin	0.45 ± 0.16^{b}	0.83 ± 0.18^{b}
K1L003	0.44 ± 0.24^{b}	0.80 ± 0.27^{b}

******Values are means of 3 replicates ± SEM and are expressed as cm. The means with different superscript letters are statistically different at $P < .001$.*

KEY:

Mean of inhibzone – Mean of inhibition zones

Mean of tran_inhibzone – Logarithmic base mean of inhibition zones (Log_{10})

3.4 Phytochemical Screening of the Endophytic Isolate K1L003 (*Bacillus aerophilus*)

The endophytic isolate tested positive for flavonoids, terpenoids, Saponins, and alkaloids, as shown in Table 4.Nair & Padmavathy [42] state that endophytes produce compounds that benefit plants. They also provide economic benefits to humans. These compounds can be used in drug development and in food industries. The endophytes are used in bioremediation, biodegradation and nutrient recycling [42].The plant *Azadirachta indica* has been shown to contain polyphenols, alkaloids, Saponins, cardiac glycosides, phytosterols, anthraquinones, and flavonoids and activity against *Staphylococcus aureus*[41]. Sinaga et al. [41] concluded that antibacterial activity in *Azadirachta indica* resulted from bioactive compounds, namely alkaloids, flavonoids, and terpenoids present in the plant. It is possible that the endophytes are responsible or partially responsible for producing some of these compounds.

Saponins of endophytic isolates were observed to have moderate to high antimicrobial activity [43]. Jin et al. [43] reported that the endophytes co-produced saponins in plants. According to Soetan*et al.* [44], saponins possessed inhibitory activity against *Staphylococcus aureus*. The study concluded that saponins were effective against Gram positive bacteria. These endophytes produce the same compounds found within the plants. This study suggests that the bioactive secondary metabolites found within the medicinal plants are co-produced by their endophytes.

Table 4. Phytochemical screening results for bioactive compounds produced by the endophytic isolate

Test	Results
	KIL003
Tannins	-

UNDER PEER REVIEW

Flavonoids	+
Terpenoids	+
Saponins	+
Steroids	-
Anthraquinones	-
Cardiac Glycosides	-
Phlobatannins	-
Alkaloids	+
Phenols	-

4. CONCLUSION

The bacteria *Bacillus aerophilus* is part of the endophytic community in leaves of the medicinal plant *Azadirachta indica*. The endophytic isolate *Bacillus aerophilus*has the potential for use in dealing with non-drug resistant and drug-resistant Staphylococcal infections. The endophytic *Bacillus aerophilus* produces bioactive secondary metabolites, including, saponins, flavonoids, terpenoids, and alkaloids. The endophytic community is partly responsible for producing the bioactive substances that medicinal plants possess, thus contributing to their medicinal and economic value. Further research is necessary to quantify the activity of the endophytic isolate against drug resistant Staphylococcus aureus. Moreover, the bioactive metabolites of the endophytic isolate should be quantified.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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