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Unveiling the antibacterial potential and antibiotic-resistance breaker activity of *Syzygium jambos* (Myrtaceae) towards critical-class priority pathogen *Klebsiella* isolates

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Abstract

Background: *Klebsiella* has developed multiple-drug resistance (MDR) to a wide range of medicines, including carbapenems and third generation cephalosporins which are often regarded as the most effective drugs against MDR bacteria. The present study examined the anti-klebsiella, modes of action, and antibiotic-resistance reversal potential of *Syzygium jambos* (Myrtaceae) leaf (SJL) and bark (SJB) methanol extracts towards a panel of sixteen MDR *K. pneumoniae* and *K. oxytoca* strains and clinical isolates.

Methods: The anti-klebsiella potential of SJL and SJB was assessed by determining the minimal inhibitory (MIC) and minimal bactericidal concentrations (MBC) using broth microdilution. Extracts were tested alone, in combination with an efflux pump inhibitor (PaβN), and in association with conventional antibiotics at their sub-inhibitory concentrations. Effects of SJL were also evaluated on bacterial kinetic growth, H^+-ATPase-mediated pump, and cell membrane integrity, using standards.

Results: SJL and SJB were shown to have anti-klebsiella action, with MICs ranging from 64 to 2048 g/mL. SJL was found to be more effective, acting on all tested pathogens with 100 ≤ MIC ≤ 2048 µg/mL, indicating considerable to moderate activity and generating bactericidal effects on more than half of the MDR *Klebsiella* strains investigated. SJL also produced a remarkably active effect (MIC ≤ 100 µg/mL), with MIC of 64 µg/mL against *K. pneumoniae* KP26. In the presence of PaβN, SJL and SJB activity rose significantly, demonstrating the involvement of active efflux machinery as MDR mechanisms. SJL displayed significant MDR reversal potential, as evidenced by an enhanced efficacy of conventional antibiotics, when in association. The activity of doxycycline and levofloxacin was improved on 100% of studied MDR pathogens. Interestingly, SJL also significantly enhanced the efficacy of the last resort drugs cefixime (cephalosporin) and imipenem (carbapenems) at more than 75% at MIC/2. Exposure of *K. pneumoniae* KP63 to SJL at 0.5×MIC, MIC, and 2×MIC for 20 h produced a concentration-dependent trend toward greater bacterial killing, extending the latent phase. In addition, SJL showed pronounced inhibition of the H^+-ATPase-mediated pump and mildly disrupted the cytoplasmic membrane.

Conclusion: This work provides a solid experimental foundation for considering *S. jambos* leaf extract as a viable treatment option for MDR *Klebsiella*-related illnesses.

Keywords: Antibiotic-resistance modifying agents; efflux pump inhibitor; food plants; Klebsiella infections; multidrug resistance; *Syzygium jambos*.

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Background

The multiple-drug resistance (MDR) expresses by microbial pathogens continues to compromise the ability to treat prevalent infectious diseases. MDR infections have been linked to more than a million deaths annually, and if nothing is done now, this already concerning figure will be ten times higher by 2050 [1, 2]. The WHO has created a list of antibiotic-resistant "priority pathogens" to direct and support the development of new antibiotics as part of its efforts to combat the rising worldwide antimicrobial resistance (AMR). Several Enterobacteriaceae, including *Klebsiella*, are among the most dangerous MDR bacteria [3]. These bacteria have developed resistance to a wide range of antibiotics, including carbapenems and third generation cephalosporins, which are known as the best available antibiotics for treating MDR bacteria. They can cause serious and frequently fatal infections such as bloodstream infections and pneumonia [4]. The present MDR issue is driving the quest for viable antibiotic alternatives, particularly medicines that may reverse MDR processes and thereby re-sensitize MDR bacteria to conventional therapies. Due to their wide variety, plant secondary metabolites are leading this search. Several studies have shown that plant extracts and phytochemicals are effective antibacterial agents against MDR phenotypes [5-15]. Recent comprehensive analyses have identified medicinal plants as potential sources of novel antimicrobial compounds that could be created to help the fight against antibiotic resistance [16-19]. Additionally, recent book chapters compiled information on proven plant-based antibacterial compounds [20-24]. According to the research, the use of medicinal plants and related products to treat difficult-to-treat illnesses has a promising future. Food plants have also received significant attention due to their outstanding effectiveness against MDR microorganisms. Some examples of food plants with potent MDR reversal activity include *Rubus fellatae*, *Passiflora edulis* and *Manihot esculenta* [25], *Adansonia digitata*, *Aframomum sp*, *Anonidium mannii*, *Hibiscus sabdarifa*, *Ocimum gratissimum,* and *Tamarindus indica* [26], *Sesamum indicum* and *Aframomum kayserianum* [27], *Xanthosoma mafaffa* and *Passiflora edulis* [28], *Tristemma hirtum* and *Raphia hookeri* [29], *Theobroma cacao* and *Phaseolus vulgaris* [30], *Cymbopogon citratus*, *Moringa oleifera*, *Garcinia lucida*, and *Azadirachta indica* [31], *Persea americana*, *Psidium guajava*, *Mangifera indica*, *Citrus sinensis*, *Passiflora edulis*, *Garcinia kola*, and *Artocarpus heterophylus, Capsicum annuum* [32-36]. Another example of a beneficial food plant is *Syzygium jambos*. Also known as rose apple, *S. jambos* is a membership of the Myrtaceae family, and has a long history as an important traditional medicine with a wide range of applications in numerous cultures, from Ayurveda (India) to TCM (Traditional Chinese Medicine), through Western Medicine (South America). In Ayurveda, the fruits and leaves are used for brain and liver health, to promote diuresis, and as a febrifuge [37], whereas the seeds are used to treat dysentery, diarrhea, and catarrh, and the flowers are said to alleviate fever [38]. In South American culture, leaves are used as a diuretic, expectorant, and anesthetic, and to treat diabetes and rheumatism, whereas the bark is used to treat asthma, bronchitis, and hoarseness [37]. Each plant organ is used to treat digestive tract and dental issues in TCM, as well as hemorrhages, syphilis, leprosy, wounds, ulcers, and lung disorders [39]. The plant reveals a varied set of secondary metabolites and extracts that have shown significant susceptibility to many health issues, including stress-related and inflammatory disorders [37]. Additionally, it has been demonstrated that plant chemicals and extracts have analgesic, antiviral, anti-dermatophyte, anticancer, and hepatoprotective properties in addition to their antibacterial and anti-inflammatory effects [13, 40-54]. Polyphenols, flavonoids, tannins, and sterols have been found in numerous organs of *S. jambos*, according to reports [37]. The leaves of *S. jambos* are mostly rich in phenolic compounds. Flavonoids, ellagitannins, phloroglucinols, and phenolic acids are examples of them [37, 40]. Pentacyclic triterpenoids are prevalent throughout the plant, particularly in the leaves and stem bark. They are classified as oleanane, ursane, lupane, and friedelane; the most important were betulinic acid and friedelin [55]. The volatile sesquiterpenes in the plant leaves, such as δ-cadinene, cumaldehyde, β-himachalene, isocaryophyllene, and β-cedrene, make up the majority of the essential oil [56]. The majority of studies screened phytoconstituents and extracts in line with the plant's traditional applications encountered over the world. *S. jambos* extracts and chemicals have mostly demonstrated antifungal, antibacterial, hepatoprotective, analgesic, antioxidant, anti-inflammatory, antidiabetic, anticancer, and antipyretic properties [37]. The present study examined the activity of *S. jambos* leaf and bark methanol extracts against a critical MDR pathogen *Klebsiella* and its antibiotic-resistance modulating efficacy.

Methods

Collection of plant material, identification, and extraction

The leaves and stem barks of *Syzygium jambos* (Myrtaceae) were harvested in Dschang (West Region, Cameroon), in January 2021. Plants samples were further identified and authenticated at the Cameroon National Herbarium (HNC, Yaounde) by M. Nana Victor (Botanist), where a voucher specimen number was provided (30458/HNC). Fresh plant samples were collected and air-dried for two weeks before being ground to produce a fine powder. Next, 200 g of the plant powder was macerated in methanol 95°C (1:3 w/v) for 48 hours. The mixture was stirred every six hours to boost the solvent's extraction capacity. Following that, the powder-solvent mixture was filtered using Whatman paper grade 1. The same procedure was followed with the filtering residue, then the filtrates were then concentrated using a rotary evaporator (BÜCHI R-200) at reduced temperature (40°C) and pressure to obtain the crude extracts. After drying the extracts for residual solvent evaporation, the extraction yield was calculated as the ratio of the weight of the crude extract produced to the weight of the starting powder extracted. As a result, the yields for leaves (SJL) and bark (SJB) were 14.26% and 15.57%, respectively. All extracts were kept in dark sterile vials at 4°C for future use.

Chemical substances and sample solution preparations

para-Iodonitrotetrazolium chloride (INT) was used as the bacterial growth indicator. Dimethyl sulfoxide (DMSO) served to solubilize plant extracts. Classes of conventional antibiotics used include cephalosporins (cefixime, ceftriaxone), phenicol (chloramphenicol), fluoroquinolones (levofloxacin, ciprofloxacin), penicillins (ampicillin, augmentin), cyclines (tetracycline, doxycycline), carbapenem (imipenem), and polypeptide (polymixin B). These antibiotics were chosen based on their widespread usage in the treatment of bacterial illnesses, particularly *Klebsiella* infections. Phenylalanine arginine beta naphtylamide (PAβN) was utilized as the efflux pump inhibitor. Sigma-Aldrich (Germany) supplied all of the mentioned chemicals. The plant extract and antibiotic solutions were prepared at concentrations of 8192 µg/mL and 1024 µg/mL, respectively.

The test extracts (SJL and SJB) were dissolved in DMSO (the final concentration of DMSO was less than 2.5%, to ensure its innocuity) and the volume was adjusted with the culture media. The PAβN was prepared at the concentration of 30 µg/mL.

Microorganisms and inoculum preparation

Sixteen strains and laboratory collection isolates of *Klebsiella pneumonia* and *Klebsiella oxytoca* were used. Their resistance features are given in Table 1. The microorganisms were confirmed using eosin methylene blue (EMB). Mueller Hinton Agar (MHA, Liofilchem, Italy) was used for the pre-culture of tested bacteria, whereas Mueller Hinton Broth (MHB, Titan Biotech LTD, India) was used for broth microdilution testing, to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of plant extracts. These culture media were prepared under aseptic stringent sterile conditions according to the manufacturer's instructions. Bacterial inoculum was prepared from 18 to 24-hour-old bacterial culture colonies. After ensuring that the culture was free of contamination, young colonies of bacteria were picked and placed in 10 mL of sterile distilled water. The resulting solution was compared to the turbidity of a standard McFarland 0.5 ($1.5x10^8$ CFU/mL).

Minimal inhibitory concentration (MIC) and minimal bacterial concentration (MBC) assessment using INT colorimetric assay

MIC determination

The MIC of SJL and SJB was determined using a rapid microplate dilution technique, as previously described, using INT as a bacterial growth indicator [30, 57-59]. In each well of a microplate (96 wells) was introduced 100 µL of MHB, then in the rows of the upper wells was introduced 100 µL of the stock solutions of test extracts (SJL and SJB) and antibiotics at 8192 µg/mL and 1024 µg/mL, respectively. The serial 2-fold dilutions were carried out followed by the introduction into each well of 100 µL of bacterial inoculum at a concentration of 2×10^6 CFU/mL for a final volume of 200 µL per well. The microplates were sealed and incubated at 37 °C for 18-24 hours. The neutral control included only MHB, the negative control contained bacterial inoculum in addition to MHB, and the positive control contained MHB, inoculum, and a reference antibiotic (chloramphenicol). Bacterial growth was revealed after 24 hours of incubation at 37 °C following the addition of 40 µL of 0.02% INT into each well [57]. The MIC of an extract was defined as the least concentration of extract that inhibits any apparent growth of a bacterial strain after 18 to 24 hours of culture at 37°C (the smallest concentration for which no color change is observed). Each experiment was repeated three times in triplicate.

MBC determination

The MBC was assessed by adding 50 µL aliquots of the preparations that did not display bacterial growth after incubation during the MIC experiments, to 150 µL of MHB. These preparations were then incubated for 48 hours at 37°C. The MBC was considered the lowest concentration of a sample that did not elicit a color change with the addition of INT [60, 61].

Antibacterial activity of Syzygium jambos extracts in the presence of an efflux pump inhibitor

The antibacterial activity of SJL and SJB in the presence of PaβN, an efflux pump inhibitor, was evaluated according to the method described by Kuete et al. (2010). The MIC was determined in the same manner as previously reported, with the difference that 50 µL of the PAβN solution was added first, followed by the addition of 50 µL of bacterial inoculum (4.10^6 CFU/mL) for a total volume of 200 µL for each well. Two negative controls were used: one produced by wells containing the inoculum, MHB, and inhibitor, and the other by wells containing the inoculum, MHB, and DMSO 2.5%. The neutral control consisted of just MHB-containing wells, while the positive control consisted of a reference antibiotic (chloramphenicol). The work was done in duplicate and repeated thrice.

Antibiotic-resistance modifying activity of Syzygium jambos extracts

To assess the extracts' antibiotic-resistance modifying action, a preliminary experiment was carried out to measure the MICs of antibiotics in the absence and presence of SJL and SJB extracts using the broth microdilution technique, as previously described [57, 62]. For preliminary experiments, *K. pneumoniae* KP55 was utilized, and plant extracts were examined at various sub-inhibitory doses (MIC/2, MIC/4, MIC/8, and MIC/16). The results allowed us to choose SJL and the sub-inhibitory doses of MIC/2 and MIC/4 for future investigations on *K. pneumoniae* and *K. oxytoca* strains. In brief, following successive dilution of the antibiotic, the test extract was applied to each well at its sub-inhibitory concentration, and bacterial inoculation was done; the MIC was then measured. The antibiotic MICs were determined in rows that received antibiotic dilutions without the test extract. The modulation factor was defined as the ratio of the MIC of the antibiotic alone versus that of the antibiotic in the presence of extract. Modulation factor (AMF) ≥ 2 was set as the cut-off for the biological significance of antibiotic-resistance modulating effects [63].

$$AMF = MIC_{\text{antibiotic alone}}/MIC_{\text{antibiotic in combination}}$$

Anti-Klebsiella modes of action of Syzygium jambos leaf extract

Action on bacterial cell cycle

The effect of SJL extract on the bacterial cell cycle was assessed on *K. pneumoniae* KP63 through the reading of the optical densities (OD) at 600 nm [64]. Briefly, the bacterial suspensions were prepared at a concentration of 10^8 CFU/mL in the corresponding flasks. They were subsequently treated with SJL at 0.5×MIC, MIC, and 2×MIC. The vials were incubated at 37°C under an orbital shaker (REMI) at 200 rpm. The positive and negative controls consisted of the vials containing ciprofloxacin at MIC as well as the vial containing MHB and the bacterial suspension, respectively. A volume of 500 µL was then collected at different time intervals (0 h, 1 h, 2 h, 4 h, 6 h, 10 h, 12 h, 14 h, 16 h, 18 h, and 20 h) and the OD was read using a spectrophotometer (Thermo Scientific, Langenselbold, Germany) at 600 nm. Each test was repeated three times. The OD values were used to plot the OD = f(T) using Microsoft Office Excel 2016.

Action on bacterial H^+-ATPase-mediated pump

The ability of SJL extract to inhibit the H^+-ATPase-mediated proton pumping of *K. pneumoniae* KP63 was evaluated by controlling the acidification of the bacterial growth medium as described previously [65], with minimal modifications. Briefly, 100 mL of bacterial suspension was grown in MHB for 18 hours at 37 °C. The

resulting culture was centrifuged at 3000 rpm for 10 minutes at 4 °C. Next, the pellet was washed twice in distilled water, then in 50 mM KCl, and suspended in 50 mL of KCl (50 mM). The cell suspension was then incubated overnight (18 h) at 4 °C, to induce glucose starvation. To 4 mL of the cell medium, 0.5 mL of the crude extract (0.5×MIC, MIC, and 2×MIC) and antibiotic (Ciprofloxacin) were added. The pH was adjusted to 6.4 with 1 M HCl or 0.1 M NaOH. After 10 min of pre-incubation at 37 °C, acidification of the medium was initiated by the addition of 0.5 mL of glucose 20%, followed by pH measurement every 10 min for 1 h using a pH meter (Thermo Scientific, USA). The negative control was constituted by replacing the extract with DMSO. The recorded pH values were used to plot pH=f(T).

Action on the bacterial cell membrane

The intracellular content of *K. pneumoniae* KP63 after treatment with different concentrations of the crude extract (0.5×MIC, MIC, and 2×MIC) and polymixin B was evaluated by measuring the absorbance of the supernatant at 260 nm as described earlier [66, 67], with slight modifications. Bacterial young colonies were harvested from a fresh culture on MHA medium and a suspension of 1 x 10^6 CFU was prepared. Bacterial cells were then treated with SJL (at 0.5×MIC, MIC, and 2×MIC), and incubated at 37°C for 12 h. The samples were centrifuged, and the absorbance of the supernatant was measured at 260 nm for control and treated cells, using a spectrophotometer (Thermo Scientific, Langenselbold, Germany). The tube containing the inoculum and DMSO was used as a negative control. Each assay was performed in triplicate. An increase in absorbance at 260 nm indicated a release of intracellular contents (DNA, RNA) by the destruction of the integrity of the plasma membrane at the different concentrations tested (0.5×MIC, MIC, and 2×MIC), compared to negative controls.

Results

Antibacterial efficacy of Syzygium jambos extracts

The antibacterial activity of the *S. jambos* extracts (SJL and SJB) was evaluated by determining the MIC and MBC towards clinical MDR isolates of *K. pneumoniae* and *K. oxytoca*. The MBC/MIC ratio was used to conclude the bactericidal or bacteriostatic effect of test extracts. The results obtained are summarized in Table 2. The activity of the extracts varied according to the plant part used and the tested bacteria strain, with MIC recorded ranging from 64 to 2048 µg/mL. SJL and SJB displayed a broad spectrum of inhibition, SJL inhibited 100% of tested bacteria, whereas the stem bark (SJB) was effective on 15 out of 16 studied pathogens (94%). Interestingly, the reference antibiotic used (CHL), tested at 256 µg/mL, was effective only on 3 (19%) studied bacteria. SJL displayed an increased potency (most of the MIC values ranged between 64 and 512 µg/mL) compared to SJB. The MIC < 100 µg/mL, the best activity, was registered with SJL against KP26 (MIC = 64 µg/mL). Furthermore, each of the test extracts showed a bactericidal effect (MBC/MIC ≤ 4) on at least two studied bacteria.

Antibacterial efficacy of Syzygium jambos extracts in the presence of PAβN

The efficacy of *S. jambos* extracts (SJL and SJB) was tested against selected *Klebsiella* strains, in association with an efflux pump inhibitor (PAβN), to demonstrate the expression of efflux pump machinery in studied bacteria as well as how it affects the antibacterial activity of the extracts. Results are depicted in Table 3. A significant improvement in the activity of both extracts (SJL and SJB) was noted against all selected MDR *Klebsiella* strains, in the presence of PAβN. The enhancement factor (ratio of the MIC alone/MIC in association) ranged from 4 to more than 64.

Antibiotic-resistance modifying activity of Syzygium jambos leaf extract

The ability of SJL (the most active extract) to modulate the resistance of studied *Klebsiella* strains was done through its combination with selected conventional antibiotics. A preliminary test of the combination was carried out on the most resistant strain *K. pneumoniae* KP55, at sub-inhibitory concentrations of plant extracts (MIC/2, MIC/4, MIC/8, and MIC/16) (Table 4). The test allowed the selection of sub-inhibitory concentrations of MIC/2 and MIC/4 of SJL as having the best potentiating effects against studied MDR strains. Then, further testing was performed on extended strains at these selected concentrations. The results obtained are shown in Table 5. The findings (Table 5) revealed an improvement in the efficacy of chosen antibiotics combined with SJL at MIC/2 and MIC/4, with the antibiotic-resistance modulating factor (AMF) ranging from 2 to 32. More pronounced effects were obtained at MIC/2, with a percentage of potentiation recorded above 62% of the MDR bacteria. SJL potentiated the activity of doxycycline and levofloxacin on 100% of tested bacteria. Interestingly, SJL also significantly enhanced the efficacy of the last resort drugs cefixime (cephalosporin) and imipenem (carbapenems) at more than 75% at MIC/2.

Effect of Syzygium jambos leaf extract on bacterial cell cycle

The ability of SJL to affect the kinetic of bacterial cell growth was examined at 0.5×MIC, MIC, and 2×MIC, against a control containing the inoculum without any bioactive agent (negative control). The results are depicted in Figure 1. SJL induced a concentration-dependent decrease in bacterial counts as compared to the control over 20 hours of incubation. At all concentrations, a prolonged latent phase was recorded, up to 4 hours for extracts and 6 hours for the reference drug ciprofloxacin. Also, a significant reduction in the exponential phase of bacterial growth was registered.

Effect of Syzygium jambos leaf extract on bacterial H^+-ATPase pump

The influence of SJL on H^+-ATPase-mediated proton pumping was examined towards *K. pneumoniae* KP63 at 0.5×MIC, MIC, and 2×MIC. From the plot displayed in Figure 2, it is observed that the SJL prevented, in a concentration-dependent manner, the acidification of the milieu, within 60 min of incubation as compared with the negative control. The extract concentration of MIC and 2×MIC displayed better effects, as well as the reference drug ciprofloxacin.

Effect of Syzygium jambos leaf extract on the bacterial cell membrane and release of intracellular components

The action of SJL on the *K. pneumoniae* KP63 cell membrane was determined, and the results are presented in Figure 3. After treatment with SJL at 0.5×MIC, MIC, and 2×MIC, the OD increased up to 0.16 from 0.08. The effect was concentration dependent.

Discussion

The rise of drug resistance to routinely administered antibiotics is a major worry, driving research and development of novel antibiotic substitutes or substances capable of reversing resistance. Herb extracts, with a focus on edible plants, are well recognized as one of the most abundant sources of potent antibacterial agents against MDR bacteria [20, 68, 69]. *Klebsiella sp.* is a critical priority pathogen with significant resistance to which special emphasis should be made in the quest for and development of novel antibacterial medicines [3]. This explains the current study, which looked at the anti-Klebsiella potential of *S. jambos* extracts (SJL and SJB), focusing on their capacity to reverse *Klebsiella* drug resistance to standard antibiotics. In addition, the possible modes of action were determined on bacterial kinetic growth, cell membrane, and H^+-ATPase pump. The test extracts (SJL and SJB) showed pronounced antibacterial activity against studied *K. pneumoniae* and *K. oxytoca*, with MICs recorded ranging from 64 to 2048 µg/mL. Based on the interpretive criteria on the antibacterial efficacy of edible plants previously established [70], SJL was found very active (MIC ≤ 100 µg/mL) against *K. pneumoniae* KP26 with a recorded MIC of 64 µg/mL. Furthermore, MICs were recorded on all the 16 pathogens tested when exposed to SJL, and the latter also displayed significant activity (100 ≤ MIC ≤ 512 µg/mL) against the majority of MDR *Klebsiella* isolates and strains tested. Besides, SJB inhibited 15 of the 16 microorganisms examined, and the MICs observed in most cases were lower than those obtained with SJL. As a result, SJL appeared to be the more active plant part against the studied *Klebsiella*. According to Popović et al. [71], study on *Gentiana pneumonanthe* plant parts, the quantity and quality of bioactive secondary metabolites vary depending on the plant sections employed, which might explain why different parts of the same plant have different levels of activity. The results obtained with SJL and SJB within the framework of this work corroborate those of Wamba et al. [72] who showed that the methanolic extract of the barks and leaves of *S. jambos* was endowed with significant antibacterial properties against Gram-negative MDR bacteria over-expressing efflux pumps, including some *K. pneumoniae* strains. These data support *S. jambos*' anti-Klebsiella activities against MDR strains. Djipa et al. [73] discovered that the aqueous and acetone extracts of *S. jambos* bark had antibacterial activity, with MICs above 1000 µg/mL against *K. oxytoca* vero, *K. oxytoca* 118, and *K. pneumoniae*. However, the MICs recorded in the present study with the methanol extract of the bark (SJB) were below 1024 µg/mL in most cases. The variation can be attributed to the kind of solvent employed in the extractions as well as the intrinsic features of the *Klebsiella* isolates investigated. Antibacterials are commonly classified as bactericidal or bacteriostatic. A substance is deemed bactericidal if the MBC/MIC ratio is minimal (<4-6), and it is possible to produce drug concentrations that will kill 99.9% of the organisms exposed. If the MBC/MIC ratio is high, it may be impossible to safely deliver quantities of the antibiotic that kill 99.9% of the bacteria, and the drug is classified as bacteriostatic [74]. SJL and SJB displayed bactericidal effects on 10 and 7 *Klebsiella* isolates, respectively. Gram-negative bacteria can withstand antibiotics by over-expressing efflux pumps (EPs). Kuete et al. [75] revealed that EPs reduce the intracellular concentration of chemical compounds and hence their action. To emphasize this resistance phenomenon in our isolates (the most resistant were chosen), the anti-Klebsiella effectiveness of SJL and SJB was further evaluated in the presence of PAβN, an efflux pump inhibitor (EPI). The findings of this study revealed that the activity of all extracts tested in the presence of PAβN increased against all MDR *Klebsiella*. This suggests the expression of EPs as a resistance strategy by studying MDR *Klebsiella*. The increased antibacterial activity of SJL and SJB in the presence of PAβN would be owing to these inhibitors being the favored substrate of EPs, creating a rise in intracellular concentration and so restoring the extracts' activity [76]. Antibiotics are losing their effectiveness against MDR bacteria. Combining plant extracts or phytochemicals with commonly prescribed antibiotics is a potential strategy for fighting MDR and restoring classical antibiotic potency [77]. The findings of the current work demonstrated the ability of SJL (at sub-inhibitory concentrations of MIC/2 and MIC/4) to improve the efficacy of ten antibiotics of different classes (ampicillin, penicillin, augmentin, ceftriaxone, cefixime, doxycycline, tetracycline, levofloxacin, imipenem, and chloramphenicol) at different extend, with the percentage of potentiation ranging from 50 to 100 % of MDR *Klebsiella* isolates tested, in most cases. The activity of doxycycline and levofloxacin was improved on 100% of studied MDR *Klebsiella* pathogens. *Klebsiella* has developed resistance to a wide range of antibiotics, including carbapenems and third generation cephalosporins, which are known as the best available antibiotics for treating MDR bacteria. They can cause serious and frequently fatal infections such as bloodstream infections and pneumonia [4]. Interestingly, SJL enhanced the efficacy of cefixime (cephalosporin) and imipenem (carbapenems) at more than 75% at MIC/2, suggesting the potent antibiotic-resistance breaker (ARB) activity of SJL. Previous works corroborate the ARB activity of *S. jambos* extracts against MDR bacteria. For instance, Wamba et al. [72] revealed the ability of *S. jambos* extracts to improve the efficacy of chloramphenicol against bacteria strains showing MDR phenotypes. At MIC/2, plant leaf and bark extracts exhibited up to 70% antibiotic-modulating efficacy against MDR *Staphylococcus aureus* strains. Similar effects were achieved when tetracycline, ciprofloxacin, and erythromycin were used against Gram-negative bacteria, including *Escherichia coli* strains (AG100ATet, AG102), *Enterobacter aerogenes* (EA27, EA289), *K. pneumoniae* (KP55, KP63), *Providencia stuartii* (PS299645, NEA16) and *Pseudomonas aeruginosa* (PA01, PA124). SJL, in theory, generates its synergistic effects by blocking EPs. Indeed, Braga et al. [78, 79] said that compounds capable of potentiating antibiotic action on more than 70% of bacteria are classified as efflux pump inhibitors (EPIs). Resistance mechanisms to these antibiotics have also been revealed in the context of our work with PAβN. Plant-derived components, due to their chemical and structural variety, operate on several targets in bacterium cell structure, including membrane, cell wall, and/or molecular targets (ions or protons, proteins, DNA/RNA) via various methods of action [80]. The action of SJL was investigated against a strain of *K. pneumoniae* (KP63). The effects on the bacteria kinetic depicted a significant decrease of bacterial counts over 20 h when compared to the starting inoculum (Figure 1), with increasing sample concentrations, a concentration-dependent trend towards greater bacterial killing was observed with an extension of the latent phase. This suggests that the test extracts can hinder *K. pneumoniae* from efficiently using the nutrients necessary for development. Furthermore, the extracts can alter cell division, restricting fast proliferation. The inhibition of *K. pneumoniae's* H^+-ATPase-mediated pump was noted after treatment with SJL (Figure 2). Bacterial ion exchange systems are linked to ATP energy generation, and any obstruction of their functioning (resulting in less acidification of the medium) might be deleterious to their survival. At acid pH, bacteria cells' cytoplasmic pH is controlled by proton extrusion through the respiratory chain and K^+ influx, whereas cation/proton antiporter regulates pH in alkaline states [81]. Every agent that disrupts the control of

ATPase, which is responsible for maintaining ion homeostasis and osmotic stability inside the cell, is termed a proton pump target. As shown in Figure 2, SJL induced inhibition of the glucose-induced acidification of the external medium by *K. pneumoniae* KP63 in a time- and concentration-dependent manner. At concentrations of MIC and 2×MIC, SJL exerted a constant and total inhibition of the KP63 proton pumps, suggesting that the H^+-ATPase of *K. pneumoniae* is a potential cellular target of SJL. The ability of SJL to alter the cell membrane of the *K. pneumoniae* strain (KP63) was examined (Figure 3). The measurement of UV-absorbing material release at OD260 indicated a significant and permanent change in the cytoplasmic membrane. The release of intracellular material was an indicator of membrane breakdown and loss of integrity [82]. The low OD observed, together with the slight rise following SJL treatment compared to control, implies that SJL only mildly disrupts the cytoplasmic membrane, resulting in a limited leakage of KP63 intracellular components. The high tannin concentration of *S. jambos* extracts appears to be connected to its antibacterial effects [38, 73]. Furthermore, phenolic substances extracted from *S. jambos* extracts, such as quercetin and rutin, have been shown to have significant antibacterial properties [83].

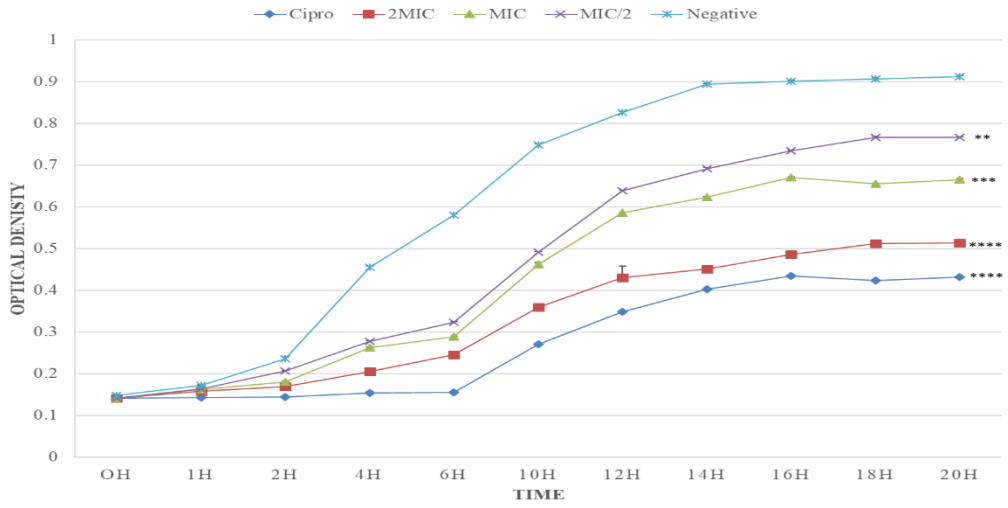

Figure 1. Effect of *Syzygium jambos* leaf extract on the bacterial cell cycle of *K. pneumoniae* KP63.

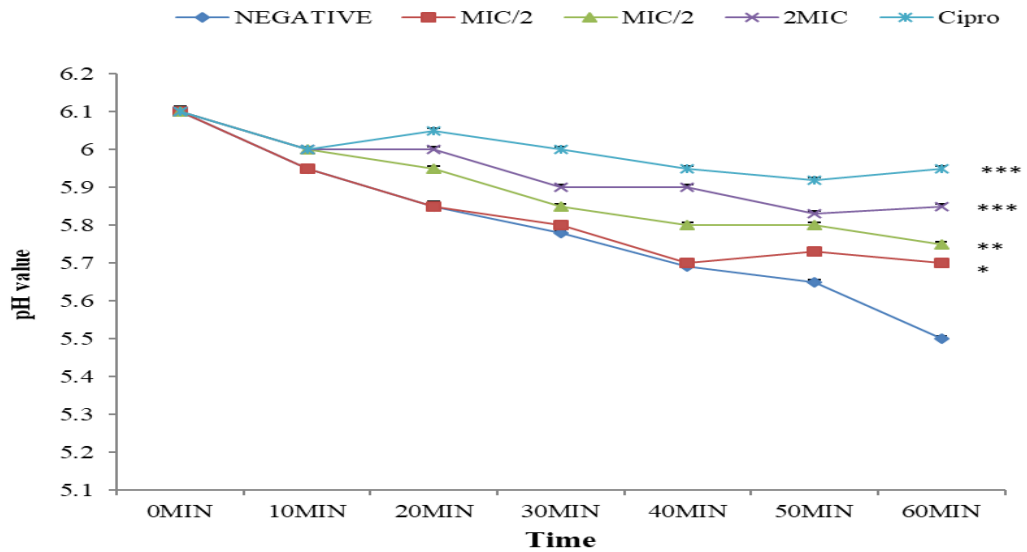

Figure 2. Effect of *Syzygium jambos* leaf extract on H^+-ATPase proton pumps of *K. pneumoniae* KP63.

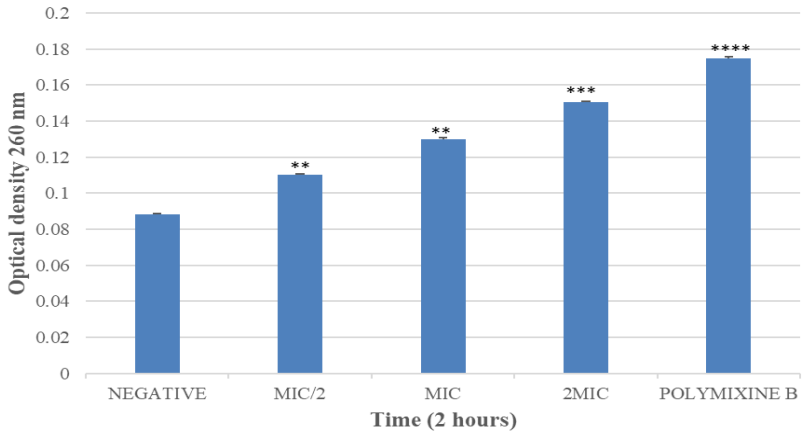

Figure 3. Effect of *Syzygium jambos* leaf extract on the cell membrane and release of intracellular components of *K. pneumoniae* KP63.

Table 1. Resistance features of isolates and strains of *Klebsiella pneumonia* and *Klebsiella oxytoca*

Bacterial strains and isolates	Features	References
Klebsiella pneumoniae		
ATCC 11296	Reference strain	[59, 75]
Kp 63	Clinical strain: Tet^r, Chl^r, Amp^r, Atm^r	[59, 75]
KP55	Clinical isolate MDR, Tet^r,Amp^r ,Atm^r,Cef^r	[59, 75]
Kp22	Clinical isolate MDR, Lev, Atm^r, Do^r, Imp^r, f^r	
KP126	Clinical isolate MDR, Amc^r	
KP96	Clinical isolate MDR, Atm^r, Mi^r, Amc^r,Cn^r,Ak^r	
Kp26	Clinical isolate MDR, Amc^r	
Kp80	Clinical isolate MDR, Prl^r, Amc^r	
KP31	Clinical isolate MDR, Ofx^r, Mi^r, Do^r, Mi^r	
Kp44	Clinical isolate MDR, Atm^r Do^r, Mi^r	
Klebsiella oxytoca		
Ko107	Clinical isolate MDR, Atm^r Do^r, Mi^r, Cip^r	
Ko109	Clinical isolate MDR, Atm^r Do^r, Mi^r, Imp^r	
Ko43	Clinical isolate MDR, Atm^r Do^r, Mi^r, Imp^r	
Ko53	Clinical isolate MDR, F^r, Atm^r,	
Ko122	Clinical isolate MDR, Lev^r, F^r, Atm^r Do^r, Mi^r, Imp^r	
Ko58	Clinical isolate MDR, Mi^r, Amc^r	

OFX, Tet^r, Amp^r, Atm^r, Cef^r, Cip^r, Imp^r, Chl^r, Mi^r, Do^r, Cn^r, F^r Lev^r resistance respectively to Ofloxacine, Tetracycline Ampicillin, Aztreonam, Cefixim, Ciprofloxacin, Imipenem, Chloramphenicol, Minocycline, Doxycycline, Gentamicine, Nitrofurantoin. MDR : Multidrug Resistant. Kp : *Klebsiella pneumoniae*, Ko : *Klebsiella oxytoca*.

Table 2. MIC and MBC (µg/mL) of *Syzygium jambos* extracts against drug resistant *Klebsiella sp.*

Bacteria		*Syzygium jambos*						Reference antibiotic (CHL)
		Leaves			Stem bark			
		MIC	MBC	R	MIC	MBC	R	MIC
K. pneumoniae	ATCC 11296	256	2048	8	256	1024	4	-
	KP55	512	-	nd	1024	2048	2	-
	KP63	128	1024	8	256	2048	8	-
	KP31	256	1024	4	512	2048	4	-
	KP26	**64**	1024	4	256	256	4	-
	KP44	512	1024	2	1024	-	nd	-
	KP46	256	512	2	1024	-	nd	128
	KP96	512	2048	4	512	2048	4	-
	KP80	1024	-	nd	1024	2048	2	-
	KP22	512	-	nd	512	1024	2	-
K. oxytoca	KO122	1024	2048	2	-	-	nd	-
	KO107	512	2048	4	256	-	nd	-
	KO109	1024	-	nd	1024	-	nd	-
	KO126	256	1024	4	1024	-	nd	128
	KO43	512	2048	4	1024	-	nd	128
	KO58	2048	2048	1	1024	-	nd	-

KP *Klebsiella pneumoniae*, KO *Klebsiella oxytoca*, MIC Minimal inhibitory concentration, MBC Minimal Bactericidal concentration, R MBC/MIC ratio, nd non-determined, (-) MIC > 2048 µg/ml for plant extracts and > 256 µg/ml for the reference antibiotic, CHL chloramphenicol. Classification of food plant extracts activity: Very active (MIC < 100 µg/ml), Significantly active (100 ≤ MIC ≤ 512 µg/ml), Moderately active (512 < MIC ≤ 2048 µg/ml), Weakly active (MIC > 2048 µg/ml), Not active (MIC > 10 mg/ml) [70].

Table 3. Antibacterial efficacy (µg /mL) of *Syzygium jambos* extracts alone and in the presence of an efflux pump inhibitor (PAβN) against selected *Klebsiella* strains.

***Klebsiella* strains**	*Syzygium jambos*					
	Leaves			**Stem bark**		
	MIC alone	**+PAβN**	**R**	**MIC alone**	**+PAβN**	**R**
Kp22	512	64	**8**	512	128	**4**
Kp46	256	16	**16**	1024	32	**32**
Kp55	1024	32	**32**	1024	64	**16**
Kp63	256	≤8	**≥32**	256	≤8	**≥32**
Kp96	512	16	**32**	512	<8	**≥64**
KO107	512	≤8	**≥64**	256	8	**32**
KO109	1024	32	**32**	1024	32	**32**
KO122	1024	32	**32**	-	64	**≤16**

- MIC> 1024 µg /mL or not active, R Enhancement factor = MICalone/MIC in the presence of PAβN, Values in bold Significant values, PAβN Phenylalanine arginine beta naphtylamide (efflux pump inhibitor), MIC Minimal inhibitory concentration.

Table 4. Preliminary testing of the combination of *Syzygium jambos* extracts with conventional antibiotics against *K. pneumoniae* kp55

Antibiotics	***Syzygium jambos***							
	Leaves				**Stem bark**			
	MIC/2	**MIC/4**	**MIC/8**	**MIC/16**	**MIC/2**	**MIC/4**	**MIC/8**	**MIC/16**
Ampicillin	64(**4**)	128(**2**)	128(**2**)	256(1)	256(1)	256(1)	256(1)	256(1)
Chloramphenicol	128(**2**)	128(**2**)	256(1)	256(1)	256(1)	256(1)	256(1)	256(1)
Levofloxacin	2(**4**)	8(1)	8(1)	8(1)	8(1)	8(1)	8(1)	8(1)
Ceftriaxone	8(**8**)	8(**8**)	16(**4**)	16(**4**)	8(**8**)	16(**4**)	16(**4**)	16(**4**)
Cefixime	32(**4**)	64(**2**)	64(**2**)	64(**2**)	16(**8**)	32(**4**)	32(**2**)	64(**2**)
Imipenem	128(1)	256(0.5)	256(0.5)	256(0.5)	32(**4**)	256(0.5)	256(0.5)	256(0.5)
Augmentin	2(**32**)	4(**16**)	32(**2**)	64(1)	2(**32**)	2(**32**)	8(**8**)	64(1)
Penicillin	4(**16**)	4(**16**)	32(**2**)	64(1)	4(**16**)	8(**8**)	16(**4**)	64(1)
Doxycycline	0.25(**8**)	2(1)	4(0.5)	4(0.5)	0.5(**4**)	0.5(**4**)	2(1)	2(1)
Tetracycline	2(**4**)	4(**2**)	8(1)	8(1)	0.25(**32**)	8(1)	8(1)	8(1)

MIC Minimal inhibitory concentration, () Antibiotic-resistance modulating factor (AMF)

Table 5. Antibiotic-resistance modifying activity of *Syzygium jambos* leaf extract.

Antibiotics	***Klebsiella* strains/isolates**	***Syzygium jambos* leaf extract**		
Ampicillin		**0**	**MIC/2**	**MIC/4**
	KP22	32	4(**8**)	8(**4**)
	KP46	512	512(1)	512(1)
	KP63	32	4(**8**)	4(**8**)
	KP96	512	512(1)	512(1)
	KP26	512	512(1)	512(1)
	KO107	128	16(**8**)	64(**2**)
	KO109	256	128(**2**)	256(1)
	KO122	512	128(**4**)	256(**2**)
	% potentiation		**62.5%**	**50%**
Penicillin	KP22	32	4(**8**)	8(**4**)
	KP46	128	16(**8**)	32(**4**)
	KP63	32	8(**4**)	8(**4**)
	KP96	256	128(**2**)	512(0.5)
	KP26	64	32(**2**)	64(1)
	KO107	256	256(1)	256(1)
	KO109	256	4(**64**)	4(**64**)
	KO122	64	2(**32**)	2(**32**)
	% potentiation		**87.5%**	**62.5%**
Augmentin	KP22	16	2(**8**)	2(**8**)
	KP46	64	16(**4**)	32(**2**)
	KP63	16	2(**8**)	2(**8**)
	KP96	256	16(**16**)	32(**8**)
	KP26	256	256(1)	256(1)
	KO107	64	1(**64**)	1(**64**)
	KO109	64	4(**16**)	4(**16**)
	KO122	128	2(**64**)	2(**64**)
	% potentiation		**87.5%**	**87.5%**
Ceftriaxone	KP22	32	4(**8**)	8(**4**)
	KP46	64	16(**4**)	16(**4**)
	KP63	64	64(1)	64(1)
	KP96	256	8(**32**)	64(**4**)
	KP26	128	64(**2**)	64(**2**)
	KO107	128	64(**2**)	64(**2**)
	KO109	128	8(**16**)	32(**4**)
	KO122	64	2(**32**)	2(**32**)
	% potentiation		**87.5%**	**87.5%**
Cefixim	KP22	128	32(**4**)	32(**4**)
	KP46	256	64(**4**)	64(**4**)
	KP63	64	64(1)	64(1)
	KP96	128	64(**2**)	64(**2**)
	KP26	256	32(**8**)	32(**8**)
	KO107	32	8(**4**)	16(**2**)
	KO109	128	32(**4**)	64(**2**)
	KO122	128	4(**32**)	4(**32**)
	% potentiation		**87.5%**	**87.5%**

Table 5. *continuation and end*.

Antibiotics	***Klebsiella* strains/isolates**	***Syzygium jambos* leaf extract**		
Doxycycline		**0**	**MIC/2**	**MIC/4**
	KP22	2	1**(2)**	1(2)
	KP46	32	16(2)	16**(2)**
	KP63	2	1(2)	2(1)
	KP96	32	16(2)	32(1)
	KP26	16	8(2)	32(0.5)
	KO107	8	2(4)	8(1)
	KO109	16	1(16)	8(2)
	KO122	8	1(2)	1(2)
	% potentiation		**100%**	**50%**
Tetracycline	KP22	8	0.5(16)	2(4)
	KP46	8	2**(4)**	8(1)
	KP63	8	8(1)	8(1)
	KP96	16	4(4)	4(4)
	KP26	32	32(0.5)	16(2)
	KO107	4	1(4)	1(4)
	KO109	16	1(16)	4(4)
	KO122	8	0.25(32)	0.25(32)
	% potentiation		**62.5%**	**62.5%**
Levofloxacin	KP22	8	2(4)	4(2)
	KP46	8	2(4)	2(4)
	KP63	8	4(2)	8(1)
	KP96	64	4(16)	4(16)
	KP26	16	2(8)	4(4)
	KO107	16	2(8)	2(8)
	KO109	32	8(4)	8(4)
	KO122	16	0.5(32)	0.5(32)
	% potentiation		**100%**	**87.5%**
Imipenem	KP22	32	2(16)	4(8)
	KP46	256	128(2)	128(2)
	KP63	64	64(1)	64(1)
	KP96	128	128(1)	512(0.25)
	KP26	128	32(4)	32(4)
	KO107	32	4(8)	4(8)
	KO109	64	32(2)	64(1)
	KO122	32	2(16)	2(16)
	% potentiation		**75%**	**62.5%**
Chloramphenicol	KP22	512	128(4)	128(4)
	KP46	512	128(4)	512(1)
	KP63	128	128(1)	128(1)
	KP96	512	512(1)	1024(0.5)
	KP26	512	128(4)	128(4)
	KO107	256	128(2)	128(2)
	KO109	512	256(2)	256(2)
	KO122	512	16(32)	256(2)
	% potentiation		**62.5**	**62.5**

Conclusion

The leaf and bark extracts of *S. jambos* showed significant anti-Klebsiella activity against MDR *K. pneumoniae* and *K. oxytoca* isolates, with the leaf extract (SJL) being the most active. Also, SJL displayed excellent antibiotic-resistance breaker activity in association with standard antibiotics. SJL's mechanisms of action show that it has a major influence on bacterial growth and the H^+-ATPase-mediated pump, as well as a minor effect on the bacterial cell membrane. The current study provides an essential experimental foundation for considering *S. jambos* extracts, particularly the leaves, as a promising option for the development of phytomedicine against MDR *Klebsiella*-related illnesses. However, it should be highlighted that the current study was confined to in vitro testing. As a result, further in vivo and in vitro studies are needed to provide the groundwork for researching novel potentially safe anti-Klebsiella compounds derived from *S. jambos*.

Abbreviations

AMF: antibiotic-resistance modulating factor
DMSO: dimethylsulfoxide
HNC: Cameroon national herbarium
INT: paraIodonitrotetrazolium chloride
MDR: multidrug-resistant
PAβN: phenylalanine arginine beta naphthylamide
MBC: minimal bactericidal concentrations
MHA: Mueller Hinton Agar
MHB: Mueller Hinton Broth
MIC, minimum inhibitory concentrations

Authors' Contribution

VYM, JRNK, JFM, MGF, and PN carried out the experiments; AJS analyzed data and wrote the manuscript; ATM and VK supervised the study; All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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