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Assessing the Antibacterial Properties of *Aloe vera* (Linn) and *Aloe schweinfurthii* (Baker) against some Plant Pathogenic Bacteria

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Abstract

The study aimed to assess the effectiveness of Aloe vera and Aloe schweinfurthii leaf extracts against plant pathogenic bacteria. Aqueous (cold and hot) and ethanolic extracts from both plants were tested on Xanthomonas axonopodis, Pseudomonas syringae, and Pseudomonas aeruginosa. Results showed antibacterial properties in both extracts, with varying inhibition zones ranging from 0.01 to 14.06mm. The most effective was the ethanolic extract from powdered A. schweinfurthii at 0.05g/ml, while hot water extracts from both species were the least effective. Phytochemical screening revealed alkaloids, tannins, saponins, flavonoids, cardiac glycosides, phytates, and oxalates in both extracts. The study concluded that A. schweinfurthii outperformed A. vera in managing the plant pathogenic bacteria, suggesting potential new antibacterial agents.

Keywords: Aloe vera, Aloe schweinfurthii, Leaf extract, Pathogenic bacteria, Antibacterial properties

INTRODUCTION

One of the main issues in many developing nations is the acute food shortage. According to reports, it results in the deaths of over 100 million people (Enyiukwu, 2014). Inadequate infrastructure required for proper food processing and storage has been identified as one of the major reasons responsible for this problem (Salami and Popoola, 2007; Kana *et al.*, 2012). On-field and post-harvest losses, occasioned by the activities of plant pathogenic organisms, have further compounded this problem in the tropics. The environmental condition of this region favours most microbial pathogens in crops. Consequently, infection and pathogenesis occur and progress effortlessly. Bacteria constitute an important group of microorganisms that cause diseases in crop plants worldwide. Annual yield loss from bacterial infection on crops has been put at 10-15% (FAO, 2005) at a monetary evaluation that runs into several millions of dollars (Abd-Alla et al., 2011). Unfortunately, managing most bacterial diseases in plants is a serious challenge. This study was designed to evaluate aqueous and ethanolic extracts from *Aloe* species for antibacterial properties against three common bacterial pathogens of crops, namely, *Xanthomonas axonopodis*, *Pseudomonas syringae*, and *Pseudomonas aeruginosa*. *Xanthomonas axonopodis* is a major pathogen that infects several crops. Bacterial canker of orange, bacterial pustule of soybean (Zinsou et al., 2015), bacterial leaf spot of *Euphorbia pulcherima*, and bacterial blight of onion are some of the diseases incited by it. *Pseudomonas syringae* is known to have several host-specific pathovars that infect beans, tomatoes, ornamental plants, and rice. It is the causative organism of bacterial canker in wild berry and stone apple, apical necrosis in mango, and blister bark in apple. In Nigeria, it is a major seed-borne pathogen of Kenaf. Infection from the bacterium has been reported to interfere with the DNA of the host plant (Block et al., 2008), resulting in mutation. *Pseudomonas aeruginosa* has a broad host range. It incites soft rot in onion bulbs in storage (Abd-Alla et al., 2011) and bacterial root rot in *Panax ginseng* (Gao et al., 2014). This is in addition to its being an opportunistic pathogen in humans, especially in immunocompromised individuals. The symptoms of infection from these bacteria on crops can manifest as mosaic patterns on leaves, stems, and fruits, liquid ooze on leaves and stems, smelly rots, spots, and the death of infected plants (Vidaver and Lambrecht, 2004).

Chemical control agents are usually only effective in preventing bacterial infection in plants but are largely ineffective as curative agents. This issue is made worse by the ability of bacteria to develop resistance to antibiotic formulations within a short period of time (Sosa, 2007). Cultivars resistant to infection by some of these bacteria have been developed, but they are often unavailable to rural farmers in most developing countries. Since bioactive chemicals from plants can increase the efficacy of antibiotics, they have been suggested as possible sources of modifying agents. Over the years, several investigations have been done on *A. vera* by researchers. This was an attempt to determine its applicability as a source of biorationality to address issues related to human health (Danish *et al.*, 2020). The majority of these efforts have produced fruitful results. For example, it has been established that extracts from *A. vera* contain broad-spectrum active components that can be utilised to treat a variety of human ailments (Salisu and Shema, 2019). *A. vera* gel's efficiency against microbial infection has also been demonstrated in other research, pointing out its potency as an antiseptic against human wounds and skin conditions (Arbab *et al.*, 2021). In addition, studies have demonstrated the plant's significance in daily life (Kumar *et al.*, 2017) and its superior antibacterial activity over conventional antibiotics (Oshomoh and Emeka-Katandu, 2014). On the other hand, not much has been reported about the benefits of other kinds of *Aloe*, particularly those native to Nigeria. Limited information is currently available regarding the potency of *Aloe schweinfurthii* leaf rind extract against plant pathogens, despite reports of significant growth inhibitory and antimicrobial bioactivities (Salawu *et al.*, 2017), which make it a viable material for the production of anticancer drugs (*Salawu et al.*, 2020). Although Alejo *et al.*, (2019) conducted a comparative assessment of the extracts of *A. vera* and *A. schweinfurthii* against some plant fungal pathogens, it is still necessary to determine the *Aloe* extracts' effectiveness against plant pathogenic bacteria. Using the agar-well diffusion method, the antibacterial activity of two species of aloe (*Aloe vera* and *Aloe schweinfurthii*) against *Xanthomonas axonopodis*, *Pseudomonas syringae*, and *Pseudomonas aeruginosa* was assessed *in vitro* in this study.

MATERIALS AND METHODS

Collection and identification of plant materials

Young plantlets of *Aloe vera* and *Aloe schweinfurthii* were obtained from the phytomedicinal garden of the Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. The two plants were identified through the assistance of the herbarium service of the Center for Research and Development (CERAD), FUTA. The two species of aloe were grown in the Department of Crop, Soil and Pest Management, FUTA's screen house, until they reached maturity and were suitable for harvesting and application. The plants were grown in humus-rich soil.

Isolation, identification, and characterization of the three bacterial pathogens

The three bacterial pathogens were isolated at the Department of Microbiology, FUTA. The medium of isolation was nutrient agar, and it was prepared following the standard recommendation of 28g/litre of sterile distilled water. The prepared growth medium and all glass wares were sterilized in an autoclave at 1.05Kg/cm^3 (15psi) pressure and 121°C for 15 minutes. Sterilized media was amended with Nystatin antifungal for inhibition of fungal growth. The sources of inocula for the pathogens were orange fruits showing symptoms of canker (*Xanthomonas axonopodis*), onion bulb showing symptoms of soft rot (*Pseudomonas aeruginosa*), and leaves from a mango plant showing symptoms of apical necrosis (*Pseudomonas syringae*). Infected plant samples were surface sterilized in 0.1% Sodium hypochlorate for 30 seconds and rinsed with four changes of sterile distilled water. Small segments, ranging from 0.3mm x 0.4mm to 0.5mm x 0.6mm, of infected tissues were obtained from each plant sample with sterile scalpels and inoculated separately on gelled nutrient ager in Petri-dishes. Incubation was done at 37°C, while sub-culturing was done 24 hours after inoculation and incubation. The pure cultures of the isolated bacteria were confirmed through the characteristics of discrete colonies.

Preparation of extracts

The procedure described by Jothi *et al.* (2014) was adopted with some modifications. Leaves collected from each specie of aloe were cleaned in sterile distilled water and sliced. The sliced pieces of the species were further separated into two parts. The first part was blended for about 5 minutes (to get a fresh sample), while the second part was oven-dried at 65°C for 72 hours. The oven-dried samples were ground into powder with a sterile mortar and pestle. Aqueous (cool and hot) and ethanolic extraction were done separately for the two *Aloe* spp. The cold and hot aqueous extractions were accomplished by adding 50g of powdered and 200g of blended fresh aloe leaf samples to 500 ml and 50 ml of sterile water at 25^{0}C (cool) and 65^{0}C (hot).

Filtration, with a sterile muslin cloth, was done after 24 hours. The filtrate was dried in an ovum at 65ºC while the residue was discarded. Following drying, the solid extract was scrapped (with a sterilized scalpel) and weighed into 20mg, 50mg, and 100mg portions. Each portion was separately dissolved in 1ml sterile distilled water to provide three concentrations: 20 mg/ml, 50 mg/ml, and 100 mg/ml. The same procedure described above was adopted for ethanolic extraction, the only difference being that the aloe leaf powder/ethanol suspension was allowed to stand for 72 hours before filtration.

Phytochemical Screening

Phytochemical screening was done to find the bioactive ingredient included in the extracts from the two aloe species. The presence of oxalate, alkaloids, and saponins was screened for. Tannins, phytates, cyanides, and flavonoids were also assessed in the extracts. The evaluation techniques outlined by Ogbuewu (2008) and Sofowora (1993) were used.

Evaluation of extracts from the two *Aloe* spp. for antibacterial properties

The process that Adamu *et al*. (2007) outlined was used. Each of the three bacterial isolates was made into a nutritional broth two days before usage according to the standard protocol. As previously mentioned, the growth medium, nutrient agar, was also prepared at the manufacturers rate and sterilized. Pour plating of sterile nutrient agar was done when it cooled to about 45°C at 15ml/Petri-dish. Exactly 5ml bacterial broth from each bacterial pathogen was then introduced into Petri-dishes with gentle swerving to ensure proper mixing of bacterial broth and the nutrient agar. Each of the three bacterial isolates underwent a different technique that was repeated four times. Following the media's solidification, six wells per Petri dish were punched out of the media using a sterile, 5mm cork borer.

Each well received a separate aseptic introduction of approximately 0.5ml of each of the three strengths of extracts from the two *Aloe* species. Hot, cool, and ethanolic extracts from the two plant samples at the three different concentrations were evaluated separately, such that the hot extract from the two plants was evaluated at the same time for each bacterial isolate. The same was done for cool and ethanolic extraction. The control consisted of Celexin powder at 0.2g, 0.5g, and 1.0g per 10ml of distilled water, respectively. Incubation was at 37°C for 24 hours, after which the culture was observed to inhibit bacterial growth. The zones of inhibition were measured with the aid of a Vernier caliper.

Statistical Analysis

Date were collected on the proportion of bioactive chemicals present in *Aloe* spp. extracts and their ability to inhibit bacterial growth. Data on the inhibition of bacterial growth was subjected to statistical analysis using Minitab software, while means were separated using Tukey's test at a 5% probability level.

RESULTS

Phytochemical constituents of the leaf of the two *Aloe* species

Aloe species' leaves were found to contain alkaloids, tannins, saponins, flavonoids, cardiac glycosides, phytates, and oxalates, according to phytochemical screening.

However, as shown in Table 1, the proportions of the phytochemicals in the two aloes were significantly different.

Table 1: Quantitative examination of the two *Aloe* species' phytochemical components

Aloe Species	Oxa (mg/100g)	PA (mg/100g)	HCN (mg/100g)	Tn (mg/100g)	Alk (%)	Sap (%)	Flav (%)
A. shw	0.36a	7.41b	0.84a	1.12b	1.80b	3.57a	1.67a
A. vera	0.18b	8.24a	0.68b	4.33a	6.88a	1.77b	0.98b

Key: Oxa = Oxalate, PA = Phytate, HCN = Cyanide, Tn = Tannins, Alk = Alkaloid, Sap = Saponin, Flav = Flavonoids, *A. shw* = *A. schweinfurthii*, *A. vera* = *A. vera*

NOTE: The same column's mean values separated by different letters differ significantly ($p<0.05$).

Effect of leaf extracts of the two *Aloe* species on the growth of three bacterial plant pathogens

The activity of the leaf extracts against the three different bacteria pathogens evaluated showed zones of inhibition, which ranged from 0.01 to 14.06mm. Though the results show that there is no significant difference amongst most of the leaf extracts in terms of their effect on the bacteria, ethanolic extracts from powdered and fresh samples of *A. schweinfurthii* at 0.05g/ml (EPS2) and 0.10g/ml (EFS3) respectively; and cold water extract from powdered sample of the same *Aloe* specie at 0.05g/ml (CPS2) exhibit the highest inhibitory effect on the bacteria (3.17-14.06mm).

Conversely, when tested against *Xanthomonas axonopodis*, *Pseudomonas syringae*, and *Pseudomonas aeruginosa*, the hot water extract from powdered *Aloe vera* at 0.02g/ml (HPA1) showed minimal effectiveness (0.02-0.07mm). The same applied to ethanolic extract from fresh *Aloe schweinfurthii* at 0.02g/ml (EFS1) when tested against the three bacteria *(0.03-0.13mm)*. Some of the results are depicted on Plates I, II & III.

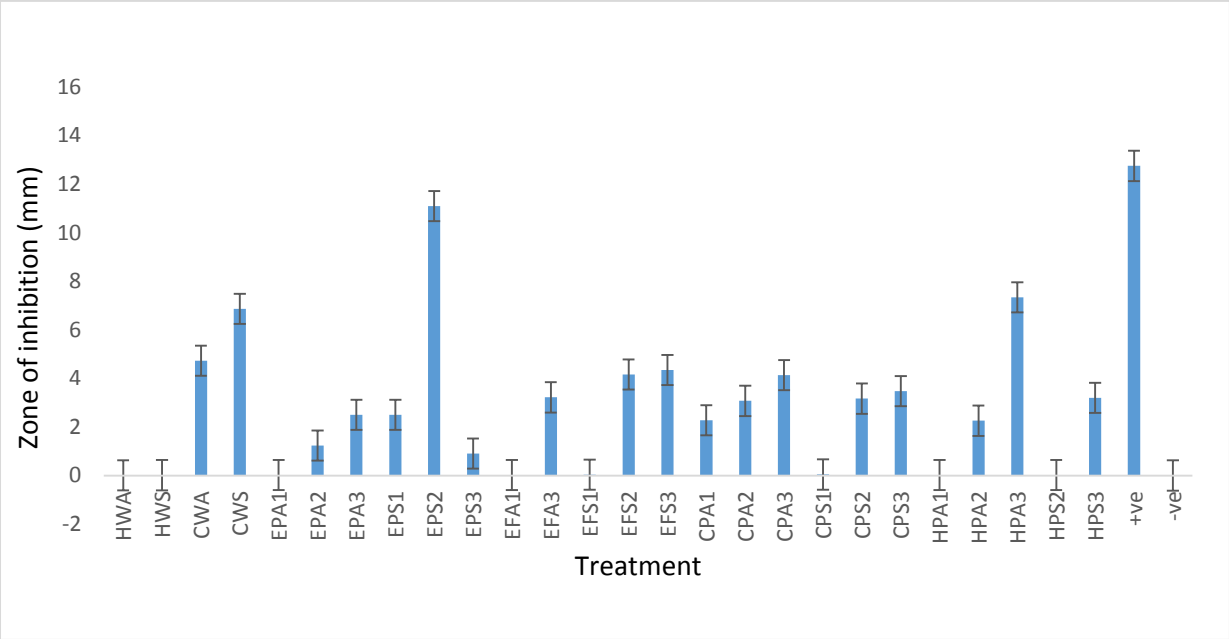

Figure 1: Effects of the extracts on *X. axonopodis* after 24 hours

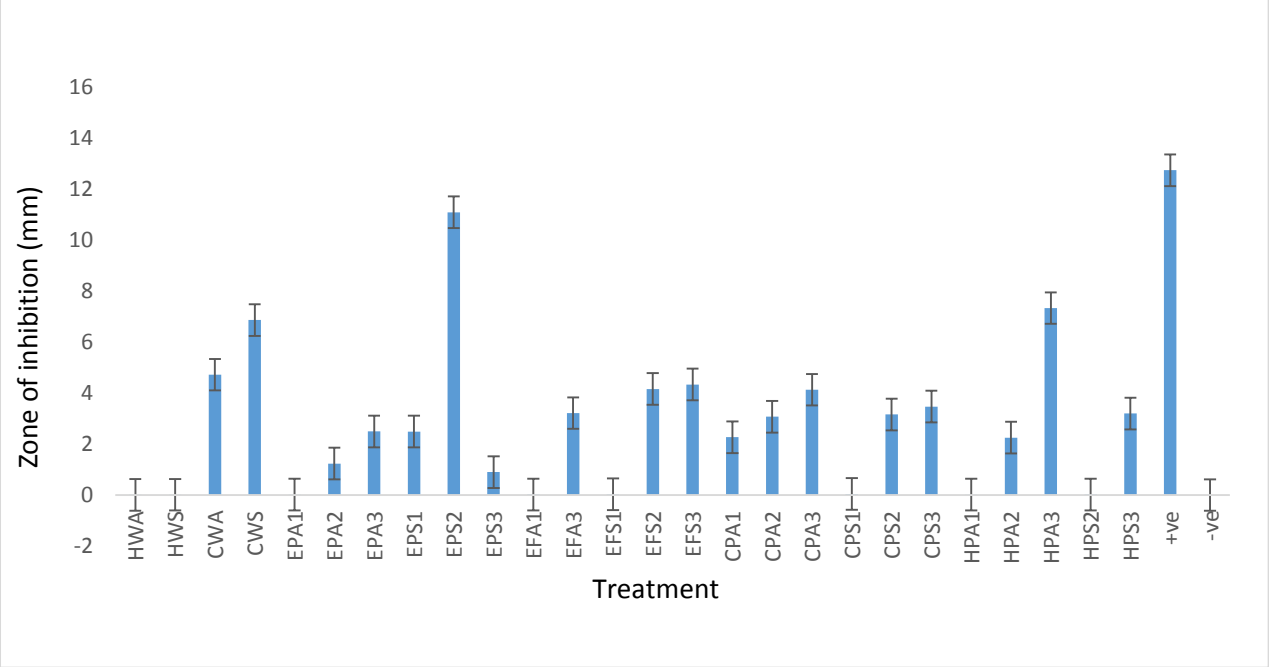

Figure 2: Effects of the extracts on *P. syringae* after 24 hours

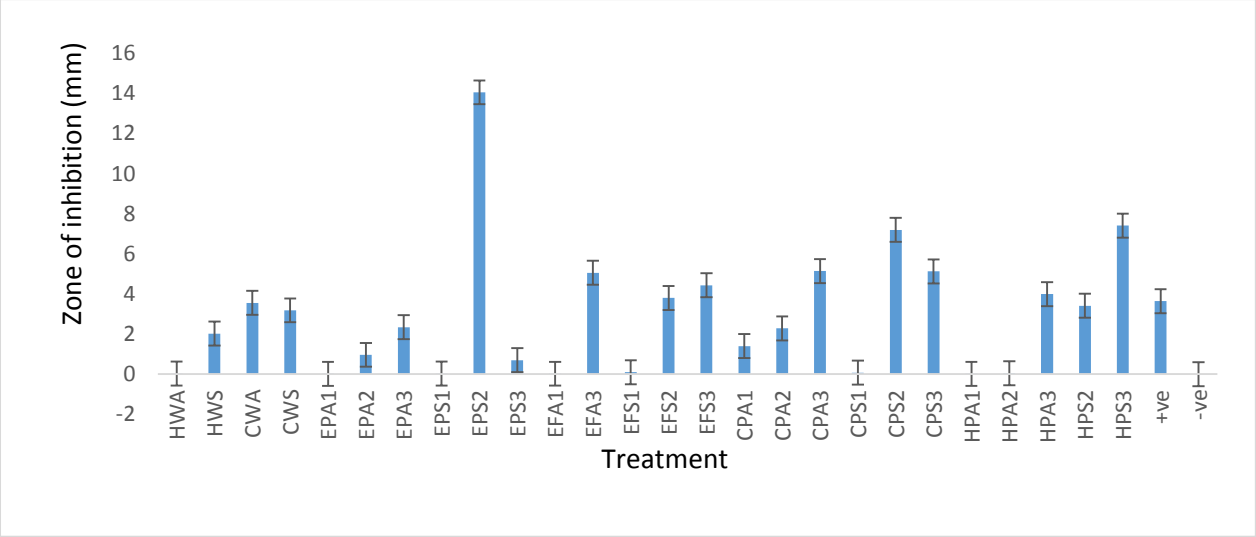

Figure 3: Effects of the extracts on *P. aeruginosa* after 24 hours

Key: HWA=Hot water extract from fresh *Aloe vera*, HWS=Hot water extract from fresh *Aloe schweinfurthii*
CWA=Cold water extract from fresh *Aloe vera*, CWS=Cold water extract from fresh *Aloe schweinfurthii*
EPA1=Extract of ethanol from 0.2g of *Aloe vera* powder, EPA2=Extract of ethanol from 0.5g of *Aloe vera* powder
EPA3=Extract of ethanol from 1.0g of *Aloe vera* powder,
EPS1=Extract of ethanol from 0.2g of *Aloe schweinfurthii* powder,
EPS2=Extract of ethanol from 0.5g of *Aloe schweinfurthii* powder,
EPS3=Extract of ethanol from 1.0g of *Aloe schweinfurthii* powder,
EFA1=0.2g of fresh *Aloe vera* ethanol extract, EFA2=0.5g of fresh *Aloe vera* ethanol extract,
EFA3=1.0g of fresh *Aloe vera* ethanol extract, EFS1=0.2g of fresh *Aloe schweinfurthii* ethanol extract,
EFS2=0.5g of fresh *Aloe schweinfurthii* ethanol extract, EFS3=1.0g of fresh *Aloe schweinfurthii* ethanol extract,
CPA1=Extract of cold water from 0.2g of *Aloe vera* powder,
CPA2=Extract of cold water from 0.5g of *Aloe vera* powder,
CPA3=Extract of cold water from 1.0g of *Aloe vera* powder
CPS1=Extract of cold water from 0.2g of *Aloe schweinfurthii* powder,
CPS2=Extract of cold water from 0.5g of *Aloe schweinfurthii* powder,
CPS3=Extract of cold water from 1.0g of *Aloe schweinfurthii* powder,
HPA1=Extract of hot water from 0.2g of *Aloe vera* powder,
HPA2=Extract of hot water from 0.5g of *Aloe vera* powder,
HPA3=Extract of hot water from 1.0g of *Aloe vera* powder,
HPS1=Extract of hot water from 0.2g of *Aloe schweinfurthii* powder,
HPS2=Extract of hot water from 0.5g of *Aloe schweinfurthii* powder,
HPS3=Extract of hot water from 1.0g of *Aloe schweinfurthii* powder,
+ve=Standard, -ve = No treatment

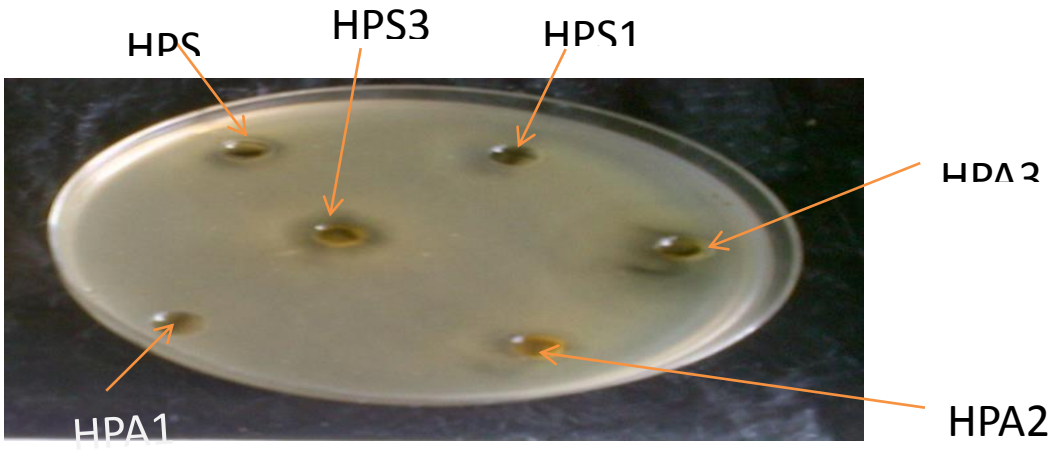

Plate I: Action of powdered *A. vera* and *A. schweinfurthii* hot water extracts against *P. syringae*

Key: HPA1 = Extract of hot water from 0.2g of *Aloe vera* powder, HPA2 = Extract of hot water from 0.5g of *Aloe vera* powder, HPA3 = Extract of hot water from 1.0g of *Aloe vera* powder, HPS1 = Extract of hot water from 0.2g of *Aloe schweinfurthii* powder, HPS2 = Extract of hot water from 0.5g of *Aloe schweinfurthii* powder, HPS3 = Extract of hot water from 1.0g of *Aloe schweinfurthii* powder

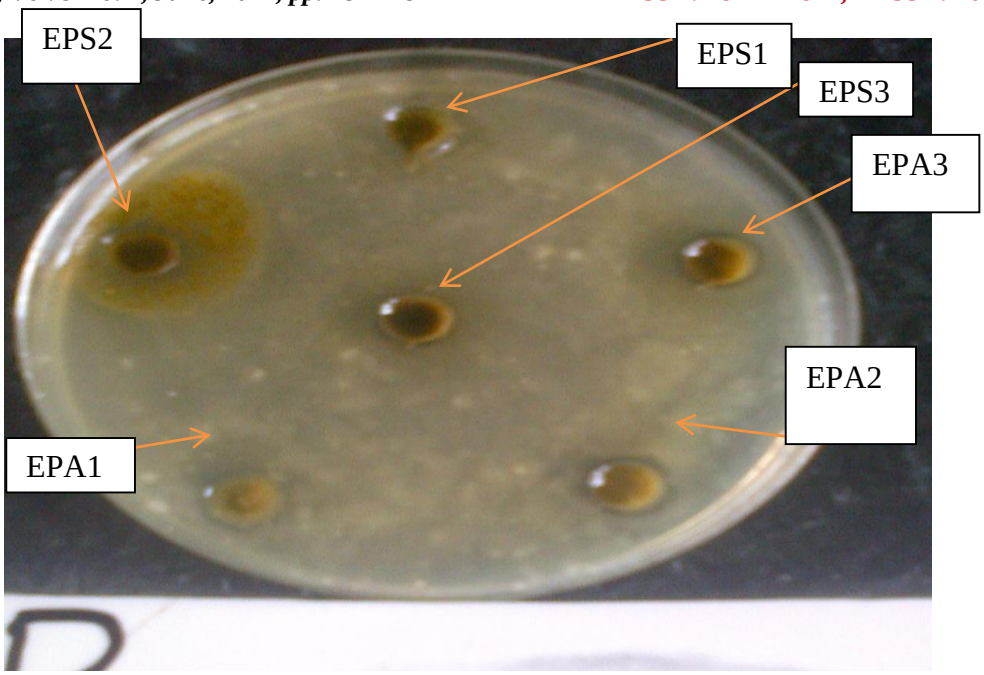

Plate II: Action of powdered *A. vera* and *A. schweinfurthii* ethanolic extracts against *P. aeruginosa*.

Key: EPA1 = Extract of ethanol from 0.2g of *Aloe vera* powder, EPA2 = Extract of ethanol from 0.5g of *Aloe vera* powder, EPA3 = Extract of ethanol from 1.0g of *Aloe vera* powder, EPS1 = Extract of ethanol from 0.2g of *Aloe schweinfurthii* powder, EPS2 = Extract of ethanol from 0.5g of *Aloe schweinfurthii* powder, EPS3 = Extract of ethanol from 1.0g of *Aloe schweinfurthii* powder

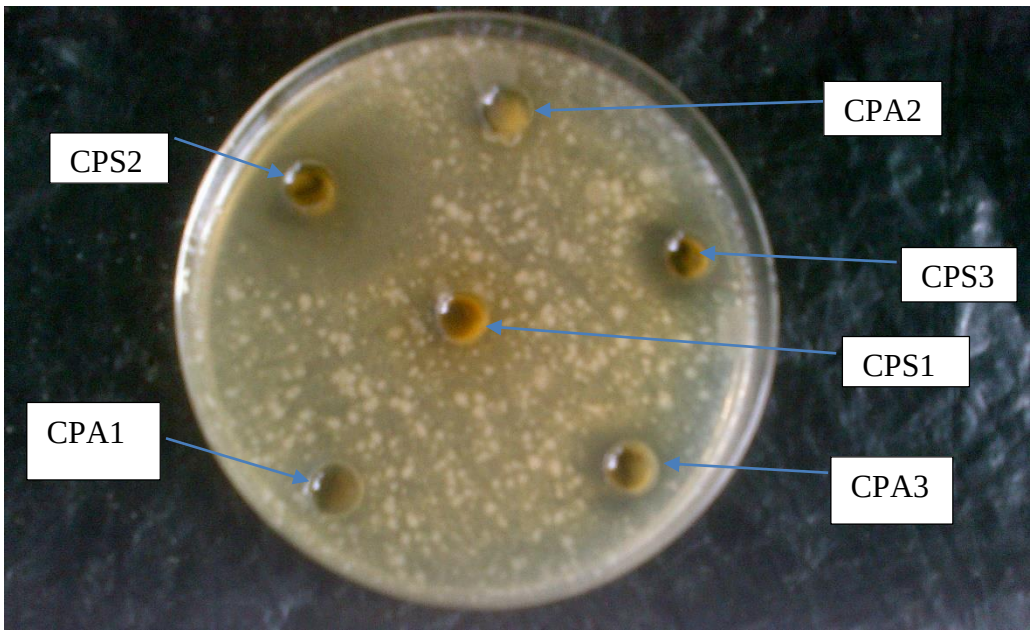

Plate III: Action of powdered *A. vera* and *A. schweinfurthii* cold water extracts against *X. axonopodis*.

Key: CPA1 = Extract of cold water from 0.2g of *Aloe vera* powder, CPA2 = Extract of cold water from 0.5g of *Aloe vera* powder, CPA3 = Extract of cold water from 1.0g of *Aloe vera* powder, CPS1 = Extract of cold water from 0.2g of *Aloe schweinfurthii* powder, CPS2 = Extract of cold water from 0.5g of *Aloe schweinfurthii* powder, CPS3 = Extract of cold water from 1.0g of *Aloe schweinfurthii* powder

DISCUSSION

The data obtained from the analysis of the phytochemical and antibacterial properties of the two aloe species provided very interesting information. The two *Aloe* species' extracts demonstrated zones of variable degrees of inhibition on plant pathogenic bacteria, ranging from 0.01 to 14.06mm. This is in line with the previous report, which showed the extracts of the two *Aloe* species to have varying inhibitory effects on the mycelial growth of some plant pathogenic fungi (Alejo *et al.*, 2019). This variation may be due to the difference in concentration and proportion of phytochemicals in the two *Aloe* species. Oxalate, saponins, flavonoids, and cyanide were in higher proportions in *A. schweinfurthii* than in *A. vera*. These higher proportions may be responsible for the marked activity of *A. schweinfurthii* extracts against the bacterial pathogens, especially because the result of the standard check compared favourably with that of ethanolic extract from 0.5g powdered *Aloe schweinfurthii* (EPS2) when tested against *X. axonopodis* and *P. syringae*. EPS2 exhibited the greatest efficacy against *X. axonopodis*, *P. syringae*, and *P. aeruginosa*. Oxalates are low-molecular-weight secondary metabolites (Grąz, 2024) that have been shown to have bactericidal properties. As demonstrated in the study by Kwak *et al.* (2016), oxalic acid from *Lentinula edodes* was found to have an inhibiting impact on *Pectobacterium carotovorum* subsp. *carotovorum*, *X. axonopodis* pv. *citri*, *X. axonopodis* pv. *glycines*, and *X. axonopodis* pv. *Vesicatoria*. It was found that oxalic acid's effectiveness against *Ralstonia solanacearum* was comparable to that of tetracyclin, a well-known antibiotic. Its capacity to provide low-pH environments, which enhance the ideal enzymatic activity for processes like biodeterioration, could have aided its efficacy (Goodell *et al.*, 1997; Gadd, 2007; Janusz *et al.*, 2017). Saponins have also been reported to be important in plants as antifeedants and in protecting plants against microbes (George *et al.*, 2002). Similarly, flavonoids have been reported (Cushmie and Lamb, 2005) to have antimicrobial properties. Cyanide is also a phytochemical found in many plants. Over the years, it has been relevant to the pesticide-producing industry because of its toxicity (US Centres for Disease Control and Prevention, USCDCP, 2022; Holstege and Kirk, 2002). Its capacity to poison cells' mitochondrial electron transport chains and prevent the body from obtaining energy from oxygen (adenosine triphosphate, or ATP) is thought to be the source of its lethality (Johns Hopkins University, Centre for Health Security, JHUCHS, 2022).

On the other hand, it was found that *A. vera* had more alkaloids, phytates, and tannins than *A. schweinfurthii*. These variations are noteworthy. Tannin is useful in treating inflammatory or ulcerated tissues as well as intestinal diseases since it has been shown to disrupt the synthesis of proteins in bacterial cells (Igbinosa *et al.*, 2009). Alkaloid has also been reported to be a painkiller (Igbinosa *et al.*, 2009). Phytates and phytic acid have been said to have therapeutic effects in preventing colon cancer due to their mineral-binding properties, which reduce oxidative stress (Vucenik and Shamsuddin, 2003). These phytochemicals could be useful in resolving the challenge of resistance often observed in bacteria in relation to antibiotics. According to Sibanda and Okoh (2007), when a dosage below the minimum inhibition concentration of plant extracts was mixed with the antibiotics to which certain bacteria were resistant, they became susceptible, indicating the synergistic effect of the extracts on the antibiotics. This effect enables the use of the respective antibiotic when it is no longer active by itself during the therapeutic treatment (Sibanda and Okoh, 2007). Plant extracts were also used by Nascimento *et al.* (2000) to treat multi-resistant pathogens such as *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

This study showed that HPA1 was not effective against the three selected bacteria. HWA and HWS were also ineffective except on *X. axonopodis*, where some inhibitory effects were observed (3.58mm and 6.20mm, respectively). When exposed to high temperatures, the unstable structure of phytochemicals may be responsible for this (Enyiukwu, 2014). Despite their growing importance in medicinal treatment, plant extracts are limited by their quick degradation by high temperatures and ultraviolet radiation (Eno, 2011; Gurja *et al.*, 2012; Enyiukwu, 2014; Alejo *et al.*, 2019).

This study showed EPS2, followed by EFS3 and CPS2, to have the highest inhibitory effect in the range 3.17–14.03mm, while HPA1, followed by EFS1 and EPA1, had the least effects against the selected bacteria in the range 0.01-0.28mm. This finding is in line with the previous report on the antibacterial activities of *A. schweinfurthii* (Salawu *et al.*, 2020). It is also consistent with the study's outcome, which showed the aqueous root extract of *A. vera* to exhibit lower antibacterial activities than its ethanolic extract (Arbab *et al.*, 2021).

CONCLUSION

In this study, the extracts of *Aloe schweinfurthii* and *A.vera* were screened for phytochemicals

and tested against three plant bacterial pathogens. While the plant extracts were observed to possess the same phytochemicals, the phytochemicals were seen to be present in different proportions. The most efficient ethanolic extract against the three chosen bacterial pathogens was found to be obtained from a powdered sample of *A. schweinfurthii* at a concentration of 0.05g/ml. Other extracts from *A. schweinfurthii*, such as ethanolic and water extracts from fresh and powdered samples (at 0.10g/ml and 0.05g/ml, respectively), were also observed to be potent against the three test organisms. Consequently, this study suggests that *A. schweinfurthii* extracts may be a good substitute and a possible complement of *Aloe vera* extracts in controlling plant diseases incited by the evaluated bacterial pathogens. It is hoped that this study's outcome will proffer a solution to the 'resistance issue' with antibiotics and encourage the utilization of indigenous plants. Further work is recommended on the isolation, purification, and characterization of active ingredients present in the extracts of the two *Aloe* species using gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC).

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