

ACTIVITIES OF LEAVES EXTRACTS OF *VERNONIA AMYGDALINA* AND *ABRUS PRECATORIUS* AGAINST SELECTED ANTIBIOTIC RESISTANT BACTERIAL PATHOGENS

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ABSTRACT

Ethanol and methanol extracts of Vernonia amygdalina and Abrus precatorius leaves were screened respectively against multi-antibiotic-resistant clinical isolates of Klebsiella pneumoniae, Salmonella enterica var. Typhi, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pneumoniae by agar-well diffusion method using graded concentrations of extract (200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL). Inhibition zone sizes (IZDs) produced by decreasing concentrations of V. amygdalina were recorded as Klebsiella pneumoniae (20 mm, 20 mm, 13 mm, 10 mm and 0 mm); Salmonella enterica var. Typhi (18 mm, 18 mm, 13 mm, 0 mm and 0 mm); Pseudomonas aeruginosa (18 mm, 18 mm, 13 mm, 0 mm and 0 mm); Staphylococcus aureus (15 mm, 15 mm, 10 mm, 0 mm and 0 mm); Streptococcus pneumoniae (17 mm, 17 mm, 15 mm, 0 mm and 0 mm). A. precatorius produced IZDs against Klebsiella pneumoniae (15 mm, 11 mm, 10 mm, 0 mm and 0 mm); S. enterica var. Typhi (16 mm, 13 mm, 9 mm, 0 mm and 0 mm); Pseudomonas aeruginosa (13 mm, 11 mm, 10 mm, 0 mm and 0 mm); Staphylococcus aureus (13 mm, 11 mm, 9 mm, 0 mm and 0 mm); Streptococcus pneumoniae (17 mm, 13 mm, 10 mm, 0 mm and 0 mm). The results suggest that extracts of V. amygdalina and A. precatorius leaves exert broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. These findings support the widespread use of the plants as local remedy for a variety of ailments, and indicate the potential roles of the plants in drug development programs of the pharmaceutical industry.

Keywords: *Vernonia amygdalina*, *Abrus precatorius*, antibiotic-resistant bacteria, agar-well diffusion.

INTRODUCTION

Vernonia amygdalina Del is a perennial plant with height between 1 m and 6 m. It is a soft wooded, multipurpose and rapid regenerating shrub with a bitter taste which informs its

common name ‘bitter leaf’ (Nwosu et al. (2013). *V. amygdalina* is found in nature close to rivers and lakes, in forests margins, woodland and grassland up to 2800 m altitude, in areas with mean annual rainfall 750–2000 mm. The plant can tolerate drought although

humid environment is more suitable for its growth (Ndaeyo, 2007).

V. amygdalina is a common and edible garden vegetable eaten as soup vegetable as well as raw for medicinal purposes. The leaves have been used in traditional medicine around the world for the treatment of a host of illnesses including heart disease (Nguemfo et al. 2023), and widely reported to have multiple bioactivities including antibacterial and antioxidant (Ovenseri and Johnbull, 2023), antifungal (Yusoff et al. 2020), antimalarial (Abay et al. 2015), liver protection (Tokofai et al. 2021), cytotoxic effects (Joseph et al. 2021), anti-inflammatory (Nguyen et al. 2021), antidiabetic and anthelmintic (IfedibaluChukwu et al. 2020), and as sheep feed supplement (Adugna et al. 2023) which are beneficial to health.

Phytochemical components of *V. amygdalina* responsible for its ethnobotanical uses include alkaloids, tannins, saponins, flavonoids, polyphenols, alkaloids, anthraquinones, edotides, xanthonenes, coumarins and sesquiterpenes, which have been identified in the plant (Alara et al. 2017).

With increasing antimicrobial resistance exhibited by most pathogenic bacteria, the need for alternative and preferably natural antimicrobials has never been more urgent. Research on the prospects of *V. amygdalina* as a source of antimicrobial compounds is on the increase. In a study conducted in Benin, Nigeria, the ethanolic extract of *V. amygdalina* produced inhibition zones ranging from 7.0 ± 0.0 mm at 25 mg/ml to 14.5 ± 2.5 mm at 200 mg/ml against *E. coli*; 6.5 ± 0.5 mm at 100 mg/ml to 9.0 ± 2.0 mm at 200 mg/ml against *S. aureus*; 11.0 ± 1.0 mm at 50 mg/ml to 16.5 ± 5.0 mm at 200 mg/ml; 7.5 ± 1.5 mm at 25 mg/ml to 11.5 ± 0.5 mm at 200 mg/ml. Inhibition zones by aqueous extract ranged from 8.0 ± 2.0 mm at 25 mg/ml to 12.5 ± 1.5 mm at 200 mg/ml against *P. aeruginosa*; 9.0 ± 1.0 mm at 50 mg/ml to 15.0 ± 1.5 mm at 200 mg/ml

against *S. aureus*. The minimum inhibitory concentration of ethanolic extract ranged from 25 mg/ml for *S. aureus*, *P. aeruginosa*, *B. subtilis* and *K. pneumoniae* to 50 mg/ml for *E. coli*. Minimum bactericidal concentration of the ethanol extract was 50 mg/ml in *P. aeruginosa* and *K. pneumoniae* and 100 mg/ml for *E. coli*, *S. aureus* and *B. subtilis*. MBC of 200 mg/ml was observed for *B. subtilis*, *S. aureus* and *P. aeruginosa* in the aqueous fraction of the plant (Evbomwan et al. 2018).

In another study at Ozoro, Delta State, Nigeria, aqueous extract of *V. amygdalina* exhibited broad-spectrum activity against bacteria isolated from wounds. Inhibition of the target organisms by *V. amygdalina* extract (2 mL), gentamicin and amoxicillin respectively were as follows: *Corynebacterium* species 30 mm, 22 mm, 11 mm, *Enterobacter aerogenes* 14 mm, 16 mm, 25 mm, *Enterococcus* species 15 mm, 24 mm, 0 mm, *Lactobacillus* species 0 mm, 0 mm, 0 mm, *Mycobacterium* species 14 mm, 19 mm, 12 mm, *Staphylococcus aureus* 20 mm, 13 mm, 25 mm (Oshilim, 2017)). In another study at Akure, Nigeria, the aqueous extract inhibited *Escherichia coli* with inhibition zones of 10.333 mm and 36.667 mm for 25 mg/mL and 100 mg/mL concentrations respectively, while the ethanolic extract inhibited *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium* sp. *Enterococcus faecalis* and *Pseudomonas aeruginosa* with inhibition zones ranging from 7.000 mm to 30.333 mm (Olusola-Makinde et al. 2021).

Abrus precatorius Linn. is a woody twinning plant belonging to the family Fabaceae (Leguminosae) with characteristic toxic red seeds with a black mark at the base. It is native to India, at altitudes up to 1200 m on the outer Himalayas, and is now naturalized in all tropical subtropical regions such as Nigeria and China (Acharya et al. 2004; Mensah et al. 2011).

A. precatorius is widely reported to have numerous biological activities including anti-diabetic activity (Boye et al. 2020), anti-oxidant activity (Kaur et al. 2022), neuroprotective effect (Premanand and Ganesh, 2010), anti-convulsant (Ranju et al. 2009; Shenoy et al. 2012), memory enhancer activity (Zambenedetti et al. 1998), and antimicrobial activities (Adelowotan et al. 2008; Bobbarala and Vadlapudi, 2009).

Antibacterial activity of *A. precatorius* was demonstrated by Sunday et al. (2016) against clinical isolates of *Salmonella typhi* and *Shigella dysenteriae*. The pathogens were susceptible to aqueous extracts of the leaf, stem and root of *A. precatorius* at 50 mg/mL, while their susceptibilities at concentrations of 40, 30 and 20 mg/mL varied. Qualitative study reveals that tannins, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols were present in all of the plant parts. The leaf has the highest quantities of tannin and phenol. The root generally showed the lowest quantity of all the compounds (Sunday et al. 2016).

In a study carried out in Umuahia, South East Nigeria, the ethanol extract of *A. precatorius* had minimum inhibitory concentrations of 12.5 mg/mL, 25 mg/mL and 12.5 mg/mL against *S. aureus*, *Escherichia coli* and *Candida albicans* respectively. The hot water extract had minimum inhibitory concentrations of 50 mg/mL, 50 mg/mL and 25 mg/mL against *S. aureus*, *E. coli* and *C. albicans* respectively while cold water extract produced 100 mg/mL, 100 mg/mL and 50 mg/mL respectively against the isolates (Akubuenyi, 2022).

The foregoing is a testament that *Vernonia amygdalina* and *Abrus precatorius* extracts have significant activity against antibiotic resistant as well as hospital acquired pathogens including *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus*

aureus (MRSA). The global trend of escalating resistance to antimicrobials emphasizes the need for continued search for new therapies. The development of resistance in bacterial microbes is inevitable since antibiotic resistance is a survival strategy and a key evolutionary mechanism. The need to be many steps ahead of the ever-evolving pathogens drives the unrelenting efforts in antimicrobial research and discovery (R&D)

The present study aims to screen *Vernonia amygdalina* and *Abrus precatorius* extracts against new strains of multiple antibiotic-resistant (MAR) bacteria in order to update existing data generated by previous researchers with regard to the potentials of the plants as sources of novel antimicrobials.

MATERIALS AND METHODS

Study Areas and Collection of Plant Materials

Leaves of *Vernonia amygdalina* were collected at Ilupeju quarters in Ondo town, Ondo State, Nigeria on 23rd March 2021 in a home garden. *Abrus precatorius* leaves were collected from the natural veld around a village of Erinje, Okitipupa, Okitipupa Local Government, Ondo State on 25th June 2021. Ondo State experiences average annual rainfall range of between 2000 and 1150 mm per annum, with the period between May and August recording the heaviest rainfall which becomes scanty and unevenly distributed between September and January.

The plant materials were taken to the Herbarium Unit of Department of Biological Sciences, Olusegun Agagu University of Science and Technology Okitipupa, Ondo State, for proper identification and authentication, and was assigned the Herbarium numbers: *Vernonia amygdalina* (OAUSTECH/H/546) and *Abrus precatorius* (OAUSTECH/H/0338).

Drying, Pulverization and Storage of Plant Materials

Fresh leaves of *V. amygdalina* and *A. precatarius* were dried at room temperature (25°C) for 2 weeks and then pulverized into dry powder using a mill. The powdered materials were stored in labeled clean, air-tight Amber bottles and kept in dark cupboards until further analyses.

Bacterial Samples

The target bacteria used in the study were MAR bacterial pathogens isolated from hospitalized patients obtained from Microbiology laboratory of Lagos University Teaching Hospital (LUTH). They included *Klebsiella pneumoniae*, *Salmonella enterica* var. *Typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Extraction of *V. amygdalina* and *A. precatarius*

Fifty grams (50g) of pulverized *V. amygdalina* leaves was soaked in 250mL of 70% ethanol for 24 hours. The extract was filtered using Whatman filter paper and the filtrate was then centrifuged at 2,000 r.p.m. for 5 minutes after which it is decanted to obtain the filtrate. The final filtrate was air dried to get the crude extract. The crude extracts were stored in labeled amber bottles at 4°C until required. The pulverized leaves of *A. precatarius* was macerated in 95% methanol with a ratio of 1:3 (w/v), and perfectly submerged for 72 hours and the maceration results were filtered and the filtrate concentrated using a rotary evaporator and finally dried in air.

Serial dilution of extracts

A stock solution of 200mg/mL of each extract was prepared by introducing 2g of extract into 10mL of sterile distilled water in a universal bottle and stirring with a glass rod until it dissolved. This resulted in a concentration of 200 mg/mL. Two-fold dilution was carried out

by transferring 5ml of the stock solution into an equal amount of sterile distilled water in another universal bottle. This gave a concentration of 100mg/mL. Two-fold dilution was continued to obtain 50mg/mL, 25mg/mL and 12.5mg/mL of the extract.

Antimicrobial screening of extract by agar-well diffusion method

Antibacterial activity of leaf extract of *Abrus precatarius* was determined against the test organisms using agar-well diffusion method as previously reported (Nmema and Anaele, 2013).

Twenty-five millimeters (25mL) of sterile Mueller-Hinton (MH) agar was poured aseptically into each labeled Petri dish and allowed to solidify on the bench. A sterile cotton swab was immersed in the bacterial suspension standardized with 0.5 McFarland standard (containing 1.5×10^8 CFU/mL). Excess fluid was expressed by rotating the swab against the inside wall of the test tube. This was used to swab the surface of the Mueller-Hinton agar plates while rotating the plate anticlockwise until the entire surface has been swabbed.

Five wells were bored into each MH agar plate with a cork borer of 8mm and the wells were labelled with the different concentrations of extract (200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL). Then 0.1ml of each concentration of the extract was introduced into appropriately labeled wells. The plates were allowed to stand on the bench for 1 hour for proper diffusion to take place, and then incubated at 35°C for 18- 24 hours. The diluent was used as a negative control. After incubation, the plates are examined for zones of inhibition and inhibition zone diameters are measured with a transparent ruler and recorded in millimeters.

RESULTS

The extracts of *Vernonia amygdalina* and *Abrus precatorius* were screened against *Klebsiella pneumoniae*, *Salmonella enterica* var. *Typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae* using agar-well diffusion method. The results of the study revealed significant activity of *V. amygdalina* and *A. precatorius* leaves extract against the target bacteria. The extract concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL produced inhibition zones (IZD) that varied with the organisms.

Results for *Vernonia amygdalina*

Inhibition zones (IZDs) produced by decreasing extract concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL against the target bacteria were recorded as *Klebsiella pneumoniae* (20 mm, 20 mm, 13 mm, 10 mm and 0 mm); *Salmonella enterica* var. *Typhi* (18 mm, 18 mm, 13 mm, 0 mm and 0 mm); *Pseudomonas aeruginosa* (18 mm, 18 mm, 13 mm, 0 mm and 0 mm); *Staphylococcus aureus* (15 mm, 15 mm, 10 mm, 0 mm and 0 mm); *Streptococcus pneumoniae* (17 mm, 17 mm, 15 mm, 0 mm and 0 mm). Table 1. Plate 1 and Plate 2.

Table 1: Inhibition Zones (mm) produced by ethanolic extract of *Vernonia amygdalina* leaves against selected bacterial pathogens

Test organism	200 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	Control (Diluent)
<i>K. pneumoniae</i>	20 mm	20 mm	13 mm	10 mm	0 mm	0 mm
<i>S. enterica</i> var. <i>Typhi</i>	18 mm	18 mm	13 mm	0 mm	0 mm	0 mm
<i>P. aeruginosa</i>	18 mm	18 mm <td 13 mm	0 mm	0 mm	0 mm	
<i>S. aureus</i>	15 mm	15 mm	10 mm	0 mm	0 mm	0 mm
<i>S. pneumoniae</i>	17 mm	17 mm	15 mm	0 mm	0 mm	0 mm

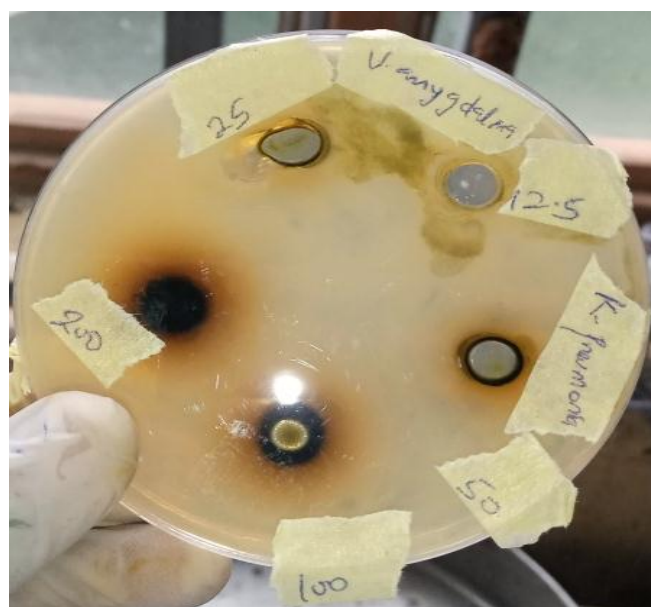


Plate 1: Inhibition zones of *Vernonia amygdalina* extract against *Klebsiella pneumoniae* at different concentrations of ethanolic extract

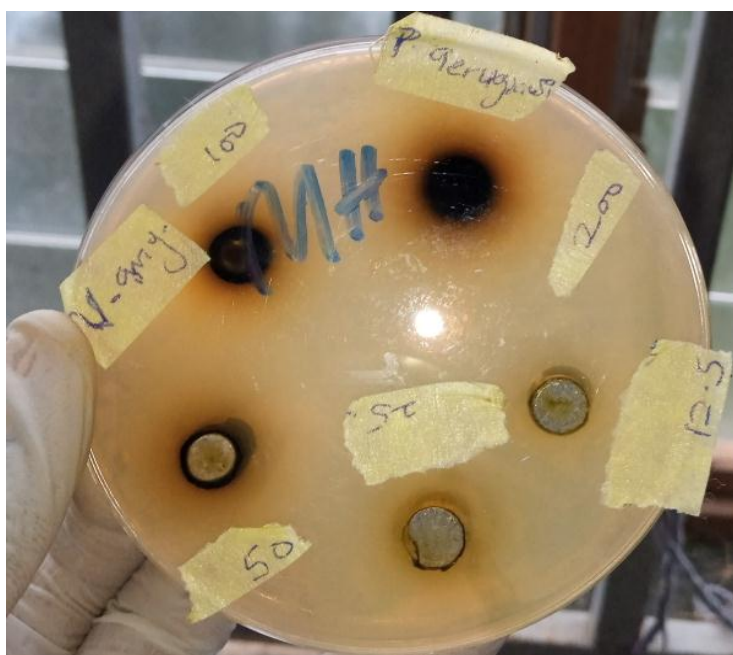


Plate 2: Inhibition zones of *Vernonia amygdalina* extract against *Pseudomonas aeruginosa* at different concentrations of ethanolic extract

Results for *Abrus precatorius*

Inhibition zones (IZDs) produced by decreasing extract concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL against the target bacteria were recorded as *Klebsiella pneumoniae* (15 mm, 11 mm, 10 mm, 0 mm and 0 mm); *S. enterica* var. *Typhi* (16 mm, 13 mm, 9 mm, 0 mm and 0 mm); *Pseudomonas aeruginosa* (13 mm, 11 mm, 10 mm, 0 mm and 0 mm); *Staphylococcus aureus* (13 mm, 11 mm, 9 mm, 0 mm and 0 mm); *Streptococcus pneumoniae* (17 mm, 13 mm, 10 mm, 0 mm and 0 mm). Table 2. Plate 3 and Plate 4.

Table 2: Inhibition zones (mm) produced by methanolic extract of *Abrus precatorius* leaves against selected bacterial pathogens

Test organism	200 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	Control (Diluent)
<i>K. pneumoniae</i>	15 mm	11 mm	10 mm	0 mm	0 mm	0
<i>S. enterica</i> var. <i>Typhi</i>	16 mm	13 mm	9 mm	9 mm	0 mm	0
<i>P. aeruginosa</i>	13 mm	11 mm	10 mm	9 mm	0 mm	0
<i>S. aureus</i>	13 mm	11 mm	9 mm	9 mm	0 mm	0
<i>S. pneumoniae</i>	17 mm	13 mm	10 mm	10 mm	0 mm	0

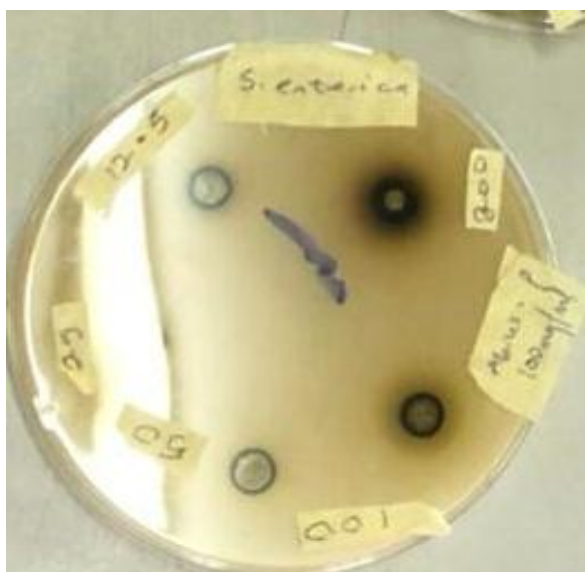


Plate 3: Inhibition zones of *Abrus precatorius* against *Salmonella enterica* var. *Typhi* at different concentrations of methanolic extract



Plate 4: Inhibition zones of *Abrus precatorius* against *Streptococcus pneumoniae* at different concentrations of methanolic extract

DISCUSSION

The ethanol extract of *V. amygdalina* produced the same inhibition zone sizes for both 200 mg/mL and 100 mg/mL which seems to suggest that the antibacterial activity was highest at 100 mg/mL and did not increase with further increase in concentration. Thereafter, decreasing concentrations produced smaller zone sizes ending with 0 mm in 12.5 mg/mL concentration for all the target bacteria. This suggests that minimum

inhibitory concentration (MIC) was between 50 mg/mL and 25 mg/mL. *K. pneumoniae* showed the highest susceptibility to the extracts, closely followed by *S. enterica* var. *Typhi*, *P. aeruginosa*, *S. pneumoniae*, and *S. aureus* in order of decreasing susceptibility (Table 1 and Plate 1 – Plate 2). These findings compare with reports by previous authors who reported an inhibition zone size of 20 mm produced by *V. amygdalina* leaves extract against *S. aureus* (Oshilim, 2017), and

inhibition zones ranging from 7.000 mm to 30.333 mm produced by ethanol extract *V. amygdalina* leaves *S. aureus*, *S. pyogenes*, *Corynebacterium* sp. *Enterococcus faecalis* and *P. aeruginosa* (Olusola-Makinde et al. 2021). Earlier authors had reported a high antimicrobial activity of *V. amygdalina* against oral microbes, in which the ethanol and aqueous extracts had MIC of 25 mg/mL and 55 mg/mL, respectively against *Streptococcus mutans*. They attributed this activity to a very low pH value of the extracts (Anibijuwon et al. 2012). Adetunji et al. (2013) demonstrated the activities of *V. amygdalina* against clinical isolates of *E. coli*, *S. aureus* and *P. aeruginosa*, with MIC ranging from 25 mg/mL to 200 mg/mL, and minimum bactericidal concentration (MBC) of 50 mg/mL for *P. aeruginosa* and 125 mg/mL for *S. aureus* while the extract was bacteriostatic against *E. coli* (Adetunji et al. 2013). *Vernonia amygdalina* contains bioactive agents such as terpenoids, alkaloids, tannins, saponins, flavonoids, polyphenols, anthraquinones, edotides, xanthones, coumarins and sesquiterpenes, which have been identified in the plant (Anibijuwon et al. 2012; Alara et al. 2017). Some of these components are reported to be responsible for the antibacterial activity against the target pathogenic microbes.

Abrus precatorius showed antibacterial activities against the target bacteria. *Streptococcus pneumoniae* showed the highest susceptibility to *A. precatorius* extract followed by *Salmonella enterica* var. *Typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in order of decreasing susceptibility (Table 2 and Plate 3 – Plate 4). For all the target bacteria, the zone sizes decreased gradually from the highest concentration of extract to the lowest concentration. This suggests that MIC of *A. precatorius* extract was between 50 mg/mL and 25 mg/mL. These findings compare with reports by previous authors who reported MICs between 20 mg/mL and 50 mg/mL

(Sunday et al. 2016), and MIC of 12.5 mg/ml produced by *A. precatorius* against *S. aureus* (Akubuenyi, 2022). This is lower than what is recorded in the present study. Results of the antibacterial activities of the methanol extract of *A. precatorius* is consistent with previous reports (Mistry et al. 2010; Amuta et al. 2011; Jaina and Gautam, 2011).

Oka and Nweze (2020) reported high levels of susceptibility of clinical bacterial wound isolates to ethanol extract of *A. precatorius* including *S. aureus* (87%), *E. coli* (67%), *P. aeruginosa* (60%), while *S. aureus* was susceptible to the aqueous extract (43%). The susceptibility was equally shown by multi-drug resistant strains among the isolates (Oka and Nweze, 2020).

The roots and seed essential oils of *A. precatorius* also possess antimicrobial activities. Mutmainnah et al. (2019) reported the inhibition of methicillin-sensitive *S. aureus* biofilm isolated from urine and blood by ethanol extract of *A. precatorius* roots (Mutmainnah et al. (2019).

The antibacterial activity of *A. precatorius* observed in the present study was produced through the mechanism of action of certain phytochemical compounds contained in the leaves, including tannins, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols were present in all of the plant parts. The leaf was found to contain the highest quantities of tannin and phenol (Sunday et al. 2016). The mechanism of action of alkaloids in inhibiting the growth of *S. pneumoniae*, is reported to be by inhibition of the nucleic acid synthesis as well as inhibiting the dihydrofolate reductase enzyme. The mechanism of action of flavonoids in inhibiting the growth of *S. pneumoniae* is focused on inhibiting nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane, and inhibiting the energy metabolism of the bacteria. Alkaloids and flavonoids work synergistically in inhibiting the growth of *S. pneumoniae* in

which the two compounds have almost the same mechanism and work objectives, resulting in more optimal results when the two compounds are used together compared to when they are used separately.

CONCLUSION

Overall, the results suggest that the extracts *V. amygdalina* and *A. precatorius* leaves exert broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, and literature reveal that roots and seed essential oils are active against strains of bacteria in both planktonic and biofilm form. These findings support the widespread use of the plants as local remedy for a variety of ailments. The findings of the present and previous studies confirm that *V. amygdalina* contains not only the active antibacterial molecules but also other substances that are necessary for maintaining health and physiological functions of the body without manifestation of toxicity hence will continue to play an important role in drug development programs the of pharmaceutical industry.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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