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Antibacterial Activity of *Vernonia amygdalina* and *Vernonia cinerea* Leaf Extracts through Microwave-Assisted Extraction Technique

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ABSTRACT

The occurrence of multi-drug-resistant bacteria has been a great challenge facing public health care in recent times. Therefore, this study investigated the antibacterial activity of extracts from two species of Vernonia (Vernonia amygdalina and Vernonia cinerea) against Bacillus cereus, Enterococcus faecalis, Pseudomonas aeruginosa, and Escherichia coli. The results obtained showed that the tested bacteria were sensitive to extracts from both plant samples in the concentration range of 6 to 30 mg/mL. Higher sensitivities were seen at a concentration of 30 mg/mL. Moreover, the sensitivities were more in the extract from V. amygdalina leaf as compared to V. cinerea leaf extract. In addition, the minimum inhibitory concentration (MIC) showed a higher susceptibility of B. cereus and P. aeruginosa to V. amygdalina and V. cinerea leaves extracts, respectively. The plant extracts exhibited antibacterial effects against the tested organisms with a potential of being used as antibiotics.

1. INTRODUCTION

The discovery of effective antibiotics has reorganized the public health sector and has enabled rapid improvements in medical health care. The emergence of antibiotics impacts the growth of pharmaceutical establishments and has led to a reduction in the use of herbal medicines consumed earlier by people of ages

in treating several diseases (Cook *et al.*, 2022). Antibiotics have been effective in treating many microbial infections; however, the overdosage and abuse of antibiotics have significantly increased, and have resulted in the emergence of microbial resistance to the conventional antibiotics (Fair and Tor, 2014). Moreover, the occurrence of multi-drug-resistant bacteria has been a great challenge facing public health care (Exner *et al.*, 2017). However, sourcing alternative therapy amidst natural sources is inevitable as suggested by the World Health Organization (Moloney, 2016).

Vernonia amygdalina and *Vernonia cinerea* are promising medicinal plants that can be found in different parts of the world, especially tropical areas such as Africa and Asia. They belong to the *Asteraceae* family (Order: *Asterales*; Genus: *Vernonia*) which contains about 1000 species (Yeap *et al.*, 2010; Ijeh and Ejike, 2011). These plants have been traditionally used as an indispensable medicinal plant for centuries, particularly among the people of sub-Saharan Africa (Yeap *et al.*, 2010). These species are endowed with fats, proteins, fibres, minerals, amino acids, carbohydrate, and vitamins (Lakshmi, 2015; Alara *et al.*, 2017). More so, the consumption of the extracts of these plants had been reported to have no adverse effect.

Several studies have been conducted on the antibacterial activity of the extracts from both plant samples based in different geographical locations (Akinpelu, 1999; Gupta *et al.*, 2003; Danish Rizvi *et al.*, 2011; Adetunji et al., 2013; Qing et al, 2014; Sonibare *et al.*, 2016; Gashe and Zeleke, 2017). However, a study had reported that geographical location can influence the biological activity of plant extracts (Muraina *et al.*, 2010). More so, extraction techniques play an important role in the recovery of effective and quality extract from plant samples. Reduced effectiveness has been associated with conventional extraction techniques (Azwanida, 2015). Nowadays, unconventional techniques are being employed due to the faster extraction time, reduced solvent for the extraction, recovery of higher and quality yields of extracts (Azwanida, 2015). Nevertheless, microwave-assisted extraction (MAE) technique has been reported to be more effective over other unconventional techniques due to its recovery of higher yields in a shorter irradiation time (Tatke and Jaiswal, 2011).

Thus, this study focused on the evaluation of antibacterial activities of *V. amygdalina* and *V. cinerea* leaves extracts obtained through MAE technique against four selected bacteria (*Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 19433, *Pseudomonas aeruginosa* ATCC 10145, and *Escherichia coli* ATCC 10536) which have been implicated in several human disease conditions.

2. MATERIALS AND METHODS

2.1. Material Collection, Preparation and Sample Extraction

Fresh leaves of *V. amygdalina* and *V. cinerea* were obtained from Gambang, Malaysia. These plant samples were identified by one of the authors (Prof. Abdurahman). The leaves were rinsed in water to remove dirt, dried in the shade until a stable weight was achieved. After drying, the sample was pulverized with a grinder and sieved with a mesh of average particle 105 µm. The powdered plant samples were stored in different dark containers and kept in a 4 ºC refrigerator before extraction.

The nutrient broth and nutrient agar were bought from Oxoid Thermo-Scientific (UK), while gentamicin sulphate was bought from Life Technologies (USA). The gram-positive (*Bacillus cereus* ATCC 11778 and *Enterococcus faecalis* ATCC 19433) and gram-negative (*Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 10536) bacteria strains used in this study were obtained from the Faculty of Industrial & Technology microbiology laboratory, Universiti Malaysia Pahang, Malaysia.

The extraction of *V. amygdalina* leaves was carried out using an enclosed ethos microwave extractor of capacity 1000 W and Frequency 2450 MHz (Milestone, Italy). The extractor works based on a 3-level heating process: preheating of the sample for 2 min at a temperature of 70 ºC, irradiation of plant sample for 4 min and cooling of the extractor to 30 ºC for 2 min. A plant sample of 10 g was mixed with aqueous ethanol (76% of ethanol concentration) of 100 mL and the mixture was irradiated at a microwave power level of 558 W based on the result obtained from an optimization process previously carried out (Alara *et al.*, 2018a;

Alara *et al.*, 2020). However, the extract from *V. cinerea* leaves was obtained at an irradiation time of 2 min, extraction temperature of 70 ºC, solute/solvent of 1:14 g/mL, and microwave power of 447 W as earlier reported (Alara et al., 2018b; Alara et al., 2018c). After the extraction process, the mixture was filtered and concentrated using a rotary evaporator (Buchi Rotavapor R-200 coupled with Buchi Vac V-500 pump, Switzerland). The experimental process was repeated thrice, and the extracts were stored in a 4 ºC refrigerator prior to the analyses.

2.2. Evaluation of the Antibacterial Activities of the Extracts

The extracts of *V. amygdalina* and *V. cinerea* leaves were tested for antibacterial activity against four species of bacteria, namely *B. cereus*, *E. faecalis*, *P aeruginosa*, and *E. coli* based on the method described by Akinyele *et al.* (2014) with slight modification. The nutrient agar was poured on sterile Petri dishes, left to cool and solidify. The organisms were cultured on the plate before using a sterile cork-borer to punch 6 mm diameter wells into the nutrient agar, then, plant extracts or gentamicin dissolved in sterile water at different concentrations of 30, 24, 18, 12, and 6 mg/mL was used to fill the wells. Thereafter, the plates were incubated at 37 ºC for a day. The zone of inhibition of bacteria by the extracts was measured using a meter rule based on the diameter of the clear zones around the wells. This procedure was carried out in triplicate and the mean values were recorded.

2.3. Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration (MIC) of the extracts on the selected gram-positive and negative bacteria was determined according to the outlined method of Shah et al. (2018). The dried extracts of *V. amygdalina* and *V. cinerea* leaves or gentamicin were dissolved in sterile water to obtain a stock solution of 30 mg/mL each. Thereafter, 1 mL of the plant extracts prepared at different concentrations through double dilution in sterile water (30, 15, 7.5, 3.75, and 1.875 mg/mL) were mixed with 4 mL of nutrient broth in sterile test tubes. About 1 mL of the selected microbes previously grown overnight in nutrient broth was added to the individual set of test tubes containing the extracts. However, Miles and Misra's method was employed in the count of viable bacteria. The control was prepared by mixing sterile water, test bacteria, and sterilized broth at the ratio of 1:1:5, respectively. Thereafter, all the test tubes were left to incubate at 37 ºC for a day. After the incubation, the growth inhibitions of the broth cultures were determined at 600 nm. The percentage of bacterial inhibition was evaluated using Equation (1). These procedures were repeated thrice, and the mean values were recorded.

$$Percentage\ of\ bacterial\ inhibition = \frac{A_c - A_0}{A_c} \times 100\% \quad (1)$$

where A_c denotes absorbance of the mixture of sterile water, test bacteria, and sterilized broth, A_0 represents absorbance of the mixture of test bacteria, sterilized broth, and plant extract.

2.4. Data Analysis

The experimental procedure and analyses were repeated thrice, and the results obtained are presented as a mean ± standard deviation. Paired t-test in the Microsoft Excel 2013® was used to evaluate the significance of the results at $p < 0.05$ level.

3. RESULTS AND DISCUSSION

The antibacterial activities of *V. amygdalina* and *V. cinerea* leaves extracts against two gram-positive (*B. cereus* ATCC 11778 and *E. faecalis* ATCC 19433) and two gram-negative (*P. aeruginosa* ATCC 10145 and *E. coli* ATCC 10536) bacteria strains are shown in Figure 1. The Petri dishes show the zones of inhibition of the extracts/gentamicin sulfate on the tested bacteria strains. It can be clearly observed that both plant extracts and gentamicin sulfate possess anti-bacterial activities against the four tested bacteria strains. The results obtained indicated that *V. amygdalina* leaf extract inhibited all the tested organisms within a concentration range of 24 to 30 mg/mL. However, *E. coli* was not sensitive to *V. cinerea* leaf extracts at any

concentration ranging from 6 to 30 mg/mL. The resistance of *E. coli* to the extract may be attributed to the species of the organism as different species of the same organism could present plasmid-mediated resistance against certain antimicrobial agents (Shah et al., 2018). The sensitivity of *V. cinerea* leaf extracts on the test bacteria strains was lower within the considered range of concentrations as compared to *V. amygdalina* leaf extracts, likely due to specie-mediated differences in the phytochemical content of the two species. Nevertheless, gentamicin sulfate, a broad-spectrum antibiotic showed wider inhibition zones against the organisms compared to the extracts. However, the sensitivity of the organisms to the extracts may increase if the concentration of the extracts is further increased above 30 mg/mL. As earlier reported, higher concentrations of plant extracts and observable time limit might enhance bactericidal influence on the organisms (Adetunji et al., 2013). These zones of inhibitions were measured and recorded as presented in Table 1.

Table 1 presented the zones of inhibition for the anti-bacterial activities of *V. amygdalina* and *V. cinerea* leaves extracts compared to that of gentamicin sulfate. The maximum zones of inhibition were observed for both extracts at a concentration of 30 mg/mL except against *E. coli* where there was no sensitivity against *V. cinerea* leaf extract probably due to species-induced resistance. The potential of the plant extracts declined as the concentration decreased from 30 mg/mL to 6 mg/mL. *V. amygdalina* leaf extracts showed the highest inhibition zone (16 mm) against *B. cereus* and *P. aeruginosa* at the concentration of 30 mg/mL. However, the highest zone of inhibition of *V. cinerea* leaf extract (10 mm) was observed against *E. faecalis*. In a similar study, *B. cereus* showed an inhibitory zone of 8 mm using *V. cinerea* leaf crude extract at a concentration of 2 mg/well (Danish Rizvi *et al.*, 2011). Although, gentamicin sulphate showed higher zones of inhibition against all the four tested bacteria at any concentration (6 to 30 mg/mL) compared to the plant extract, nevertheless, it should be noted that gentamycin is a broad-spectrum antibiotic which has proven antibacterial effects against both gram-positive and gram-negative bacteria. An increase in the concentration of the extracts beyond 30 mg/mL could as well increase their effects on the organisms (Adetunji *et al.*, 2013).

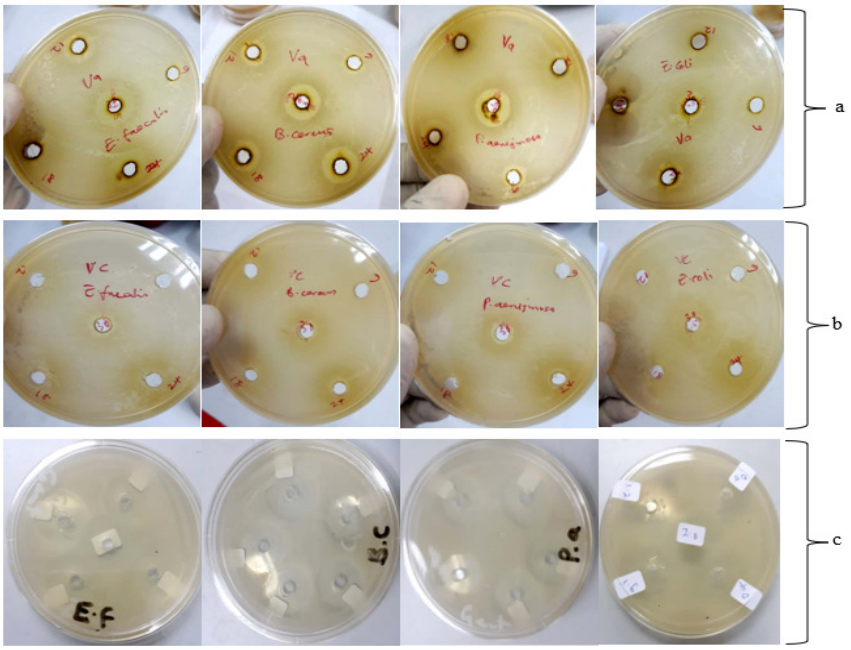

Figure 1: Pictorial images of anti-bacterial activities of *Vernonia amygdalina* leaf extract (a); *Vernonia cinerea* leaf extract and gentamicin sulfate (c) against *Enterococcus faecalis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli.*

Table 1: The zone of inhibition (mm) for the anti-bacterial activities of *Vernonia amygdalina* and *Vernonia cinerea* leaves extracts

Bacteria	*V. amygdalina*					*V. cinerea*					Gentamicin sulphate				
	30 (mg/mL)	24 (mg/mL)	18 (mg/mL)	12 (mg/mL)	6 (mg/mL)	30 (mg/mL)	24 (mg/mL)	18 (mg/mL)	12 (mg/mL)	6 (mg/mL)	30 (mg/mL)	24 (mg/mL)	18 (mg/mL)	12 (mg/mL)	6 (mg/mL)
Enterococcus faecalis ATCC 19433	13	12	10	8	0	10	9	6	0	0	25	25	25	22	20
Bacillus cereus ATCC 11778	16	15	14	11	0	8	5	0	0	0	26	24	23	21	20
Pseudomonas aeruginosa ATCC 10145	16	15	13	11	9	9	6	0	0	0	24	23	22	21	20
Escherichia coli ATCC 10536	12	11	0	0	0	0	0	0	0	0	33	31	27	25	23

Figure 2 illustrates the percentage bacterial inhibitory activities of both extracts obtained from *V. amygdalina* and *V. cinerea* leaves against *E. faecalis*, *B. cereus*, *P. aeruginosa*, and *E. coli* at a concentration range from 1.875 to 30 mg/mL. It can be observed that *V. amygdalina* leaf extract showed higher inhibitory activities against all the considered bacteria organisms (at least the concentration of 15 mg/mL). However, a concentration of 30 mg/mL showed the highest bacterial inhibitory activities against the organisms except for *V. cinerea* leaf extract which showed no activity against *E. coli* at this concentration. Moreover, the highest activity of 59% was observed from the *V. amygdalina* leaf extract against *P. aeruginosa* at a concentration of 30 mg/mL. In the previous studies, the presence of several secondary metabolites in the extracts of *V. amygdalina* and *V. cinerea* leaves, especially polyphenols and flavonoids, might be responsible for these antibacterial activities of the extracts (Alara *et al.*, 2018a; Alara *et al.*, 2018b; Shah *et al.*, 2018).

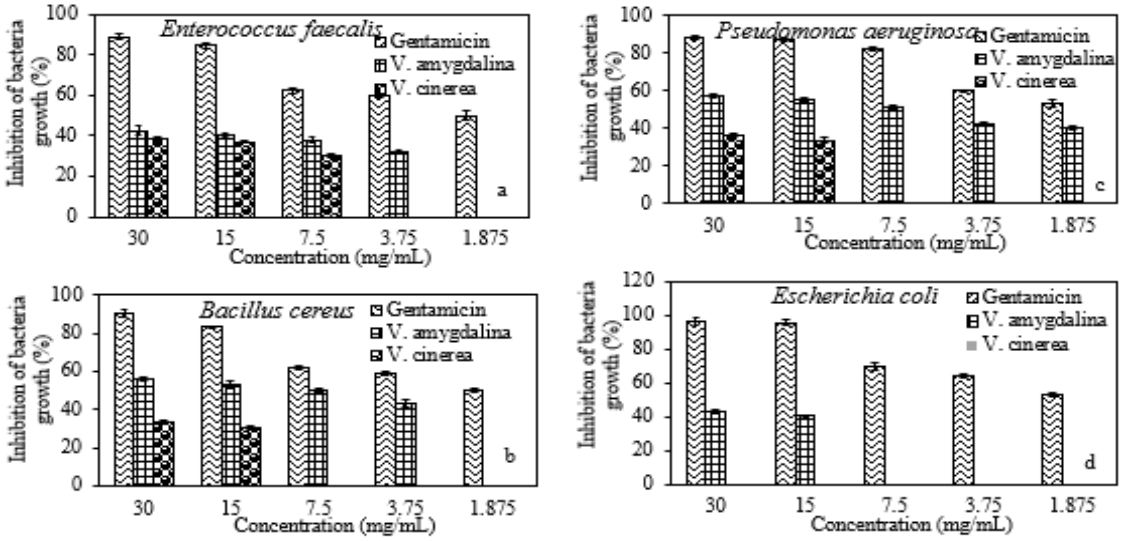

Figure 2: The percentage bacterial inhibitory capablities of *Vernonia amygdalina* and *Vernonia cinerea* leaves extracts against *Enterococcus faecalis* (a); *Bacillus cereus* (b); *Pseudomonas aeruginosa* (c); and *Escherichia coli* (d)

4. CONCLUSION

This study has outlined the anti-bacterial activities of both *V. amygdalina* and *V. cinerea* leaves extracts against four bacteria. The results clearly showed that the tested bacteria were sensitive to extracts from both plant samples at a concentration of 30 mg/mL. The potential of extracts from *V. amygdalina* leaf against the bacteria was more compared to *V. cinerea* leaf. Thus, this finding has shown the potential of *V. amygdalina* and *V. cinerea* leaves extracts as anti-bacterial agents against *B. cereus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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