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Comparative antimicrobial study of *Vernonia amygdalina* Del. and *Lawsonia inermis* L. against microorganisms from aqueous milieu

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ABSTRACT: Limitations have been concurrent with the use of antibiotics in chemotherapy. Hence, antimicrobial potency of aqueous and ethanol leaf extracts of *Vernonia amygdalina* and *Lawsonia inermis* on some selected multiple antibiotic resistant bacteria and fungi isolated from stream were compared. The phytochemical evaluation and antimicrobial susceptibility test of MAR bacteria and fungi was achieved via CLSI reference standard of perfloxacin (10 µg) and ketoconazole (150 mg/ml) with susceptibility index (>14.00 mm and >15.00 mm, respectively) as control for bacteria and fungi respectively. Saponin and steroids were present in both *V. amygdalina* and *L. inermis* ethanol extracts but alkaloids were present in *V. amygdalina* and absent in *L. inermis* ethanol extracts. The ethanol extract of *L. inermis* had higher percentage recovery yield (16.13%) to that of *V. amygdalina* (10.78%). Synergistic effect of mixture of *V. amygdalina* and *L. inermis* ethanol extracts was displayed against *Alcaligenes faecalis* (29.00 mm) and *P. penneri* (20.00 mm). The MIC and MBC of *V. amygdalina* ethanol extract against *A. feacalis* was both 50 mg/ml. The combined mixture of *V. amygdalina* and *L. inermis* ethanol extracts showed 12.67 mm against *A. fumigatus.* This study revealed the antibacterial and antifungal potentials of *V. amygdalina* and *L. inermis* extracts in the treatment of related water-borne infections.

Keywords: *Alcaligenes faecalis* subsp. *faecalis*; *Lawsonia inermis*; Multiple antibiotic resistant; Synergistic; *Vernonia amygdalina*.

1. INTRODUCTION

Microbial effluence in marine milieu is one of the fundamental subject-matter with regard to the hygienic status of water bodies used for drinking water supply, household activities, and recreational purposes and yield of seafood; this is owing to a possible contagion by pathogenic microbial syndicate as pointed out by Aude et al. [1]. Most waterborne pathogens are introduced into drinking-water supplies in human or animal feces [2]. River water is usually contaminated by bacteria (e.g. *Escherichia coli*, *Clostridium perfringens*), viruses (e.g. adenovirus, norovirus), etc. [3].

The impact of anthropogenic activities (purposeful human activities that brings about the accumulation of chemical and biological wastes) is critically high in that water bodies have lost its self-

regeneration a great deal as depicted by Sood et al. [4]. Contaminated fresh water brings about waterborne diseases and can be innocuous in food preparation and other domestic uses to cause food-borne illness such as gastroenteritis [5]. The most pertinent criterion or determinant in water quality is the absence of potential fecal material contaminant emanating from animals and humans as detailed by Scott et al. [6]. Fecal materials associated with animals can mainly constitute a high predisposing risk factor to human health because of its tendency to harbor animal intestinal bacteria pathogens [6]. *V. amygdalina* and *L. inermis* belongs to the family Compositae and Lythraceae respectively. *V. amygdalina* is particularly abundant in tropical grasslands and has a bitter taste which makes it to be locally called "ewe ewuro"; an English translation of Yoruba language meaning bitter as expatiated by Ibrahim et al. [7]. *L. inermis* is also known as "hinna" in Arabic. The name hinna refers to the dye prepared from the plant which is used in the art of temporary body art (staining). Hinna has being of use since ancient times most especially in the Eastern world and among the Muslims to dye different parts of the body. *L. inermis* is commonly found in the Northern part of Nigeria known as "ewe laali" in Yoruba ethnic group.

V. amygdalina has been reported to provide various medicinal properties which exert a killing and inhibitory upshot on some aqueous-borne microbes as opined by Effraim et al. [8]. Antibacterial properties of *V. amygdalina* have also been reported by Ibrahim et al. [7]. Antibacterial properties of *V. amygdalina* have also been detailed by Ghamba et al. [9]. Multifarious investigations conducted on *V. amygdalina* had reported that it possess diverse natural ingredients such as flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, glycosides as demonstrated by Adedapo et al. [10]; Quasie et al. [11]; Luo et al. [12]. Antifungal properties of *L. inermis* have been reported by Rahman et al. [13] and antibacterial activity of *L. inermis* has also been stated by Sarma [14] and Al-Daamy et al. [15]. Wassim et al. [16] also observed the presence of glycosides, phystosterol, steroidal compounds, tannins and flavonoids in methanol extracts of *L. inermis.*

Therapeutic plants are loaded with copious assortment of derived metabolites of antimicrobial repertoire including; saponins, tannins, alkaloids, phenols, flavonoids, terpenoids as opined by Tiwari and Singh [17], Lewis and Ausubel [18]. This has largely led to the advent of multiple drug resistance in bacteria which has thereby consequently led to a surge in mortality emanating from relapsing bacterial maladies.

Therefore, we carried out a comparative study on the antibacterial and antifungal properties of crude extracts of *V. amygdalina* and *L. inermis* on some reported multiple antibiotic-resistant bacteria and fungi isolated from Onyearugbulem stream, Nigeria. The synergistic effect of combination of the two crude extracts of the plants was also evaluated.

2. MATERIALS AND METHODS

2.1. Study vicinity

Onyearugbulem stream is located in Akure, Ondo State, South-West Nigeria. The stream is a receiving water body for a major city abattoir which releases its effluent directly into the stream after poor treatment of its wastewater. The stream flows across densely populated community.

2.2. Preparation and extraction of plant leaves

The plants samples were collected, authenticated and grinded into powdered form. A 100 g of the plants' powder was added into 100 ml of distilled water and 100% ethanol to attain aqueous and ethanol

extracts respectively. The filtrate was collected and concentrated using rotary evaporator (RE-52A Union Laboratories, England) at 40°C as accomplished by Atata et al. [19]. Before use, the extracts were subjected to a sterility test by the introduction of 2 ml of the reconstituted extract using dimethyl sulfoxide (DMSO) (Delson Pascal Laboratories, Nigeria) into 10 ml of sterile nutrient broth and incubated at 37°C for 24 hours. A sterile extract was designated by an appearance of clearness of the broth [20].

2.3. Phytochemical analysis of the leaves extracts

Phytochemical tests were carried out in order to identify the existence of phytochemical ingredients in *V. aymgdalina* and *L. inermis* via customary methods illustrated by Odebiyi and Sofowora [21].

2.4. Bacterial source

Biochemically and molecularly-confirmed bacterial isolates (previous study) Olusola-Makinde et al. [22] were used for this study. They were isolated from Onyearugbulem stream and stored in the culture collection bank of the Department of Microbiology, Federal University of Technology, Akure (FUTA) which include: *Stenotrophomonas acidaminiphilis, Proteus mirabilis, Alcaligenes faecalis, Proteus penneri,* and *Bacillus cereus.*

2.5. Presumptive identification of fungal isolates

Description of fungal isolates such as texture of colony, spore or conidia-producing structures and spore shapes were documented. The features were observed from fungal tissues grown on Potato Dextrose agar after 72 hours, spore and mycelium characteristics were studied using visible observation and microscope at low power magnification (x40).

2.6. Antimicrobial susceptibility testing of isolates

The surface of sterile MHA plates was streaked with the chaste culture of the uniform bacterial cell suspension. A sterile cork borer, 4 holes were bored on already solidified sterile Mueller Hilton agar (MHA) (Oxoid, Basingstokes, UK) plates. Different concentrations of the crude extract were filter-sterilized into respective holes using a sterile millipore membrane filter with pore sizes of 0.22 µm (Delson Pascal Laboratories, Nigeria) unto the freshly prepared MHA plates already seeded with the test organisms as conducted by Esimone et al. [23]. Four different concentrations (25, 50, 75 and 100 mg/ml) of each extract indicating the four holes were bored and labeled on the MHA (Oxoid, Basingstokes, UK) plates. The antimicrobials present in the plant extract are allowed to disperse out into the medium. The width of region of inhibition was measured in millimeters using a vernier caliper (Delson Pascal Laboratories, Nigeria).

2.6.1. Evaluation of minimum inhibitory concentration of plant extracts

The plate method of Willey et al. [5] was used for the determination of the minimum inhibitory concentration (MIC). The tested organisms were streaked on Muller Hilton agar plates and incubated for 24 hours. After which four wells (6 mm diameter each) were bored onto the agar plate using sterile cork-borer. 1ml of the aqueous and ethanol extracts of *V. amygdalina* and *L. inermis* was introduced into the four wells with concentrations of 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l and then inoculated into the wells. The plates were then incubated at 37°C for 24 hours after which they were observed for growth as measured in millimetre. The lowest concentration of the *V. amygdalina* and *L. inermis* extracts that inhibited the growth of test organisms was taken as the MIC.

2.6.2. Determination of Minimum Bactericidal Concentration of plant extracts

Isolates from the plates used in the MIC assays which showed lowest growth after incubation were streaked out on solidified nutrient agar plates using sterile inoculating loop and incubated at 37°C. The lowest concentration that showed the lowest growth on plates after 24 hours of incubation indicates bactericidal effect and was taken as Minimum Bactericidal Concentration (MBC).

2.7. Antifungal activity of plant extracts

An inoculum of identified fungal species was added swabbed on the entire surface of the potato dextrose agar medium. Sterile 5 mm disc in diameter dipped in solutions of the solvents ethanol and aqueous plants extract were aseptically placed on the already solidified agar. The petri-plates were left for 1 hour at ambivalent temperature as a time of pre-incubation dispersion to ease the upshots of variant in occasion between the usages of the diverse solutions. Then the petri-plates were incubated at 24°C for 2-3 days and examined for antimicrobial activity. The width of region of inhibition was recorded and compared with the standards (National Committee on Clinical Laboratory Standard – NCCL) [24].

2.8. Statistical analysis

Analysis of Variance (ANOVA) was used to compare the significance of different concentrations of aqueous and ethanol extracts of *Lawsonia inermis* and *Vernonia amygdalina* with their respective zones of inhibition using SPSS (Statistical Packages for Social Sciences).

3. RESULTS

3.1. Percentage recovery of crude plant extracts

This study showed the percentage recovery of *Vernonia amygdalina* to be 11.78% while that of *Lawsonia inermis* to be 16.13% (Table 1).

Table 1. Percentage recovery of plant extracts.

Plant extracts	Weight before extraction (g)	Weight after extraction (g)	Percentage recovery (%)
Aqueous *L. inermis*	25.00	0.30	1.20
Ethanol *L. inermis*	6.82	1.10	16.13
Aqueous *V. amygdalina*	41.43	1.40	3.40
Ethanol *V. amygdalina*	23.20	2.50	10.78

3.2. Phytochemical profile of crude plant extracts

This study revealed the presence of phytochemicals such as tannin, saponins, alkaloids, oxalate, phylate, and flavonoids, steroids and phenols in both aqueous and ethanol leaf extracts of *V. amygdalina*. Phytochemicals of ethanol and aqueous leaf extracts of *L. inermis* revealed the presence of carbohydrate, saponins, and sterols and tannins (ethanol leaf extract) while the aqueous leaf extract revealed only the presence of flavonoids, while, alkaloids, glycoside and renins are absent as juxtaposed in Table 2.

3.3. Fungal profile of collected Onyearugbulem river water samples

Table 3 showed *Aspergillus niger*, *Aspergillus fumigatus* and *Mucor mucedo* are fungi organisms enumerated from the stream water samples analysed in this study.

Table 2. Phytochemical components of ethanol and aqueous extracts of *V. amygdalina* and *L. inermis.*

Phytochemicals	*V. amygdalina*		*L. inermis*	
	Ethanol extract	**Aqueous extract**	**Ethanol extract**	**Aqueous extract**
Oxalate	+	+	NA	NA
Phylate	+	+	NA	NA
Tannins	+	+	+	-
Saponins	+	+	+	+
Flavonoids	+	+	-	+
Cyanogenic glycoside	+	+	-	-
Alkaloids	+	+	-	-
Anthraquinone	+	+	NA	NA
Steroids	+	+	+	+
Phenol	+	+	NA	NA
Phlobatannins	-	-	NA	NA
Carbohydrate	NA	NA	+	+
Resins	NA	NA	-	-

Key: + = present, - = absent, NA = not applicable.

Table 3. Presumptive macro-morphological characteristics of fungal isolates.

Colony description	Morphological characteristics	Probable organism
Black colonies	Septate branched mycelium, brownish conidia, ascospores produced	*Aspergillus niger*
Grayish brown colonies	Broad hyphae, non-septate sporangiophore	*Mucor mucedo*
Greyish blue	Versicular shape with rough uniseptate sporangiosphore	*Aspergillus fumigatus*

Table 4. Antibacterial activity of aqueous extracts of *L. inermis and V. amygdalina* leaf extracts mixture (mm).

Organisms	Zones of inhibition (mean ± SD)				
	25 mg/ml	**50 mg/ml**	**75 mg/ml**	**100 mg/ml**	**Perfloxacin (10 µg)**
Stenotrophomonas acidaminiphilis	6.22±1.01^{a}	8.5±0.06^{b}	8.0±0.06 ^{b}	1.00±0.06^{a}	13.0±1.16 ^{b}
Proteus mirabilis	6.41±0.11^{a}	9.5±0.10^{c}	9.0±0.10^{b}	12.0±0.06^{c}	12.0±0.14^{a}
Alcaligenes faecalis	6.15±0.20^{a}	7.6±0.06^{a}	9.4±0.10^{c}	13.5±0.06^{b}	9.0±0.11^{b}
Proteus penneri	6.11±031^{a}	6.21±0.10^{a}	8.40±0.10^{a}	12.4±0.06^{b}	14.0±1.13^{c}
Bacillus cereus	6.30±0.10^{a}	4.20±0.06^{b}	1.90±0.10^{a}	9.0±0.05^{c}	15.0±0.13^{b}

Means with different superscripts along similar column are extensively dissimilar.

Table 5. Antibacterial activity of ethanol extract of *L. inermis and V. amygdalina* extracts mixture (mm).

Organisms	Zones of inhibition (mean ± SD)				
	25 mg/ml	**50 mg/ml**	**75 mg/ml**	**100 mg/ml**	**Perfloxacin (10 µg)**
Stenotrophomonas acidaminiphilis	7.30±0.06^{b}	7.20±0.06^{a}	15.5±0.06^{c}	19.0±0.06^{b}	11.0±1.12 ^{b}
Proteus mirabilis	6.77±0.06^{c}	16.5±0.06^{b}	11.0±0.06^{b}	18.0±0.06^{b}	14.0±0.10^{a}
Alcaligenes faecalis	6.37±0.06^{a}	8.30±0.17^{b}	22.0±0.10^{b}	29.0±0.10^{c}	10.0±0.09^{b}
Proteus penneri	7.07±0.12^{a}	8.03±0.15^{a}	13.3±0.15^{a}	20.0±0.06^{c}	8.03±1.14^{c}
Bacillus cereus	6.67±0.12^{c}	7.83±0.15^{c}	13.0±0.06^{b}	15.4±0.12^{a}	12.0±1.11^{b}

Means with different superscripts along similar column are extensively dissimila.

3.4. Antimicrobial susceptibility profile of bacterial isolates

All bacterial isolates were highly resistible to all concentrations of mixed aqueous extracts of *L. inermis* and *V. amgdalina* leaf extracts (Table 4). *Alcaligenes faecalis* was highly susceptible to 100 mg/ml concentration of mixed ethanol extracts of *L. inermis* and *V. amygdalina* at 29.0±0.10 mm (Table 5).

3.5. Minimum inhibitory concentration and minimum bacteriocidal concentration of crude plant extracts against bacterial isolates

This study revealed that both 50 mg/ml and 75 mg/ml concentrations varied constancy as the MIC while 50 mg/ml concentration was constant as MBC of aqueous and ethanol leaf extracts of *V. amygdalina* and *L. inermis* against all bacterial isolates (Table 6 and 7).

Table 6. Minimum inhibitory and minimum bactericidal concentrations of aqueous and ethanol leaf extracts of *V. amygdalina.*

Organisms	MIC (mg/ml)		MBC (mg/ml)	
	Aqueous	Ethanol	Aqueous	Ethanol
Stenotrophomonas acidaminiphilis	75	75	50	50
Proteus penneri	50	50	50	50
Proteus mirabilis	75	75	50	50
Alcaligenes faecalis	75	50	50	50
Bacillus cereus	50	75	50	50

Table 7. Minimum inhibitory and minimum bactericidal concentrations of and ethanol leaf extracts of *L. inermis.*

Organisms	MIC (mg/ml)		MBC (mg/ml)	
	Aqueous	Ethanol	Aqueous	Ethanol
Stenotrophomonas acidaminiphilis	75	75	50	50
Proteus penneri	50	50	50	50
Proteus mirabilis	50	75	50	50
Alcaligenes faecalis	75	50	50	50
Bacillus cereus	75	75	50	50

3.6. Antifungal susceptibility profile crude plant extracts

Aspergillus fumigatus and *A. niger* showed appreciative susceptibility to 150 mg/ml concentration of ketoconazole (control) at 16.00 mm and 15.33 mm respectively compared to all concentrations of ethanol and aqueous leaf extracts *V. amygdalina* and *L. inermis* (Table 8 and 9).

Table 8. Antifungal activity of ethanol leaf extracts of *V. amygdalina* and *L. inermis* (mm).

Fungal isolates	Zone of inhibition (mean ± SD)				
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml	Ketoconazole (150 mg/ml)
Aspergilllus fumigatus	6.2±0.05^{a}	7.66±0.05^{b}	11.05±0.05^{c}	12.67±0.05^{c}	16.00
Aspergillus niger	6.0±0.15^{a}	4.07±0.06^{c}	4.67±0.12^{b}	4.33±0.10^{b}	15.33
Mucor mucedo	6.45±0.14^{b}	7.13±0.45^{a}	3.33±1.11^{a}	5.45±1.23^{b}	7.67

Means with different superscripts along similar column are extensively dissimilar.

Table 9. Antifungal activities of aqueous leaf extract of *V. amygdalina* and *L. inermis* (mm).

Fungal isolates	Zone of inhibition (mean ± SD)				
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml	Ketoconazole (150 mg/ml)
Aspergillus fumigatus	6.40±0.01[a]	6.59±0.09[b]	8.97±0.05[c]	9.33±0.05[b]	16.00
Aspergillus niger	7.33±0.03[a]	5.00±0.05[b]	5.67±0.05[b]	6.67±0.05[c]	15.33
Mucor mucedo	6.08±0.23[a]	3.04±0.15[a]	6.71±0.23[c]	8.23±0.05c	7.67

Means with different superscripts along similar column are extensively dissimilar.

4. DISCUSSION

Administration of antibiotics in chemotherapy has been associated with a number of shortcomings especially growing microbial antibiotic resistance, therefore, replacements such as the use of medicinal plants are considered. In this study, plant extract recovery in this research finding revealed ethanol extract of *L. inermis* had higher percentage recovery yield (16.13%) when compared with that of *V. amygdalina* (10.78%). This observation was analogous with Odey et al. [25] who recovered 11.96% from stem barks of *V. amygdalina* using ethanol as extraction solvent in line with extraction method used in this study. The discrepancy in the percentage recovery of studied plants might be due to the phytochemical constituents present in the leaves extracts.

This study revealed the presence of phytochemicals such as tannins, saponins, alkaloids, oxalate, phylate, and flavonoids, steroids and phenols in both aqueous and ethanol leaf extracts of *V. amygdalina* as reported by Ghamba et al. [9]. The result of this study also corroborated that crude leaf extracts of *V. amygdalina* had some biologically-active ingredients that have been renowned to have antimicrobial assets as observed by Oluchi et al. [26]. These active ingredients include tannins, saponins, steroids, alkaloids and others.

Phytochemicals of ethanol and aqueous leaf extracts of *L. inermis* revealed the presence of carbohydrate, saponins, and sterols and tannins (ethanol leaf extract) while the aqueous leaf extract revealed only the presence of flavonoids. Carbohydrate, flavonoids, saponins, and steroids are phytochemical compounds presents in the ethanol extract of *L. inermis,* while, alkaloids, glycoside, renins, and tanins are absent as stated by Wassim et al. [16].

Phytochemicals of the *V. amygdalina* leaves extracts revealed the presence of phenols, oxalate, flavonoids, alkaloids, anthraquinones, saponins, tannins, cardiac glycosides, steroids and terpenoids which is similar to studies conducted by Oloyede and Boyo [27] on *V. amygdalina* which revealed that the plant leaf contained flavonoids, saponins, anthraquinones and alkaloids.

The mixture of aqueous extracts of *V. amygdalina* and *L. inermis* at 50 mg/ml concentration was only able to inhibit the growth of *S. acidiminiphilis*, *P. penneri*, *A. faecalis faecalis*, and *B. cereus* while, *P. mirabilis* and *A. faecalis* were resistant to the mixture of leaf extracts. 75 mg/ml of the mixed aqueous *V. amygdalina* and *L. inermis* extracts were able to inhibit all the bacterial organisms except *B. cereus*, while 100 mg/ml concentration inhibited all the organisms except *S. acidiminiphilis*. The mixed ethanol extracts of *V. amygdalina* and *L. inermis* revealed 50 mg/ml concentration was only able to inhibit the growth of *P. penneri*., while the other bacterial organisms were resistant. Both 75 mg/ml and 100 mg/ml concentrations were able to inhibit the growth of all selected bacterial isolates. This variation can be due to the differential sensitivity of bacteria corroborating cell walls of gram positive bacteria (*E. coli*), alluding to *B. cereus* and *L. macrolides*. The gram positive bacteria (*S. aureus*), alluding to *S. acidiminiphilis*, *P. penneri*, *P. mirabilis* and *A. faecalis* because they are sensitive to most extract according to Kitonde et al. [28].

All ethanol extracts of *V. amygdalina* and *L. inermis* extracts demonstrated more inhibitory activity against the bacterial isolates and fungal isolates than the aqueous extracts. This can be due to the ability of ethanol to

extract more of the essential oil and secondary plant metabolites which are believed to exert antibacterial activity on test organisms as supported by Udochukwu et al. [29]. This study revealed that minimum inhibitory concentration and minimum bactericidal concentration of *V. amygdalina* ethanol extract against *A. feacalis* was 50 mg/ml and 50 mg/ml respectively. The MIC of aqueous *V. amygdalina* and ethanol extracts for all organisms showed 50 mg/ml to have minimally inhibited the growth of *P. penneri* and *Bacillus cereus* for the aqueous extract of *V. amygdalina.* 75 mg/ml was shown to have been slightly susceptible against *Proteus mirabilis*, *S. acidiminiphilis*, *A. faecalis*. 75 mg/ml concentration of ethanol extract of *V. amygdalina* was also shown to have low susceptibility against *Proteus mirabilis*, *Bacillus cereus* and *S. acidiminiphilis*, while 50 mg/ml concentration of the ethanol *V. amygdalina* extract was shown to be minimally susceptible against *P. penneri*, and *A. faecalis*. This observation agrees with the investigation embarked upon by Okwu and Nnamdi [30]; Arekemase and Oyeyiola [31] as they revealed the higher efficacy of the crude extract of *V. amygdalina* on *S. aureus* (Gram-positive) than *E. coli* and *P. aeruginosa* (Gram-negative) may possibly be owing to soaring components of active ingredients of the extract and the configurational differentiation between the biocellularly-identified bacterial consortia in this study. The MBC of the aqueous extract of *V. amygdalina*, 50 mg/ml was shown to be constantly bacteriostatic for all organisms and for the ethanol extracts.

This study also revealed 25 mg/ml was shown to have inhibited the growth of *A. niger* for the ethanol extract of *L. inermis* and *V. amygdalina* while 25 mg/ml of the mixed aqueous and ethanol extracts of *L. inermis* and *V. amygdalina* was shown not to have any inhibition against *A. fumigatus*. This can be due to the polarity of the two solvents (water and ethanol) used in this study such that extraction elicits the bioactive ingredients from the leaf extracts (bitter leaf and henna plant) in reference to their polarization, and also diminish the hostile nature of compounds in the extract in alliance with the study outcome of Jothiprakasam [32].

This study revealed 50, 75 and 100 mg/ml concentrations of ethanol leaf extracts of *L. inermis* and *V. amygdalina* show antifungal activity of 7.66±0.05 mm, 11.05±0.05 mm and 12.67±0.05 mm against *A. fumigatus* respectively while the same concentration showed a decreased antifungal activity of 4.07±0.06, 4.67±0.12 and 4.33±0.10 against *A. niger*. Antifungal activity of the combined leaf extracts shows low antifungal activity of 1.13±0.45 mm, 3.33±1.11 mm and 5.45±1.23 mm against *Mucor mucedo* at 50, 75 and 100 mg/ml concentration respectively.

The antifungal activity of the aqueous extracts at 50 mg/ml, 75 mg/ml and 100 mg/ml showed 6.59±0.09 mm, 8.97±0.05 mm and 9.33±0.05 mm respectively against *A. fumigatus*, while the same concentration showed a decreased antifungal activity of 5.00±0.05 mm, 5.67±0.05 mm and 6.67±0.0 5 mm against *A. niger.* Antifungal activity of the combined leaf extracts showed a decreased antifungal activity at 2.45±0.14 mm, 3.04±0.15 mm, 6.71±0.23 mm and 8.23±0.05 mm against *Mucor mucedo* at concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml respectively. The motive for this is that antimicrobial activity may be due to frequent liberated hydroxyl ions that have the potential to merge with the carbohydrates and proteins in the microbial cell wall as opined by Khalaphallah and Solman [33]. This study proved that ethanol extract was further proficient than water extract for *L. inermis* which is constant with Jung et al. [34]. This goes to indicate that *L. inermis* and *V. amygdalina* can have a synergistic proficiency to be used together albeit at higher concentration to increase the level of potency of the crude leaf extracts.

Ketoconazole (150 mg/ml), an antifungal agent/drug used as a positive control showed high antifungal activity of 7.67 mm, 15.33 mm, and 16.00 mm against *Mucor mucedo*, *A. niger* and *A. fumigatus* respectively which indicates the efficacy of ketoconazole in the treatment of fungal infections that can be possibly caused by the fungal organisms isolated from the contaminated surface water samples. This result is analogous to the

findings of Rahman [13] who reported amphotericin B fungi-noxious performance at the concentration of 150 µg/mL which were also parallel to lawsone against four fungal strains of *F. oxysporum*, *A. niger*, *A. flavus*, and *Penicillium* sp. that showed vulnerability for amphotericin B.

5. CONCLUSIONS

This study has shown that Onyearugbulem stream constitutes a serious public health concern, as pertaining to the hygienic quality and the risk barometer of the water being used for domestic purposes thereby necessitating urgent and effective intervention. It can also be deduced that the combined crude extracts inhibited the growth of the organisms as the antibacterial agent (perfloxacin) and antifungal agent (ketoconazole) used as positive control also exhibited a bacteriocidal and appreciative antifungal effects against the water-borne pathogens. It is thereby recommended that further exploration of *V. amygdalina* and *L. inermis* be carried out as they can confer a synergistic effect when combined and can also serve as a resource of innate artifact for prospective usage in the administration of some water-borne multiple chemotherapeutic recalcitrant fecal marker bacteria.

Authors' Contributions: OOO came up with the concept of the study and supervised the study. BMT conducted the literature search, methodology, analyze/interpreted the data. BMT wrote the first manuscript draft. OOO edited and reviewed the draft. Both authors approved the final manuscript.

Conflict of Interest: The authors has no conflict of interest to declare.

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