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Nature's Answer to Biofilm Resistance: Investigating the Therapeutic Power of Vernonia Amygdalina, Ocimum Gratissimum, and Kalanchoe Pinnata

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ABSTRACT

Biofilm-associated infections remain a major clinical challenge due to the heightened resistance of biofilm-forming pathogens to conventional antibiotics. This study evaluated the antimicrobial and anti-biofilm activities of Vernonia amygdalina, Ocimum gratissimum, and Kalanchoe pinnata plants widely used in African traditional medicine against clinically isolated multi-drug-resistant organisms. Methanolic, ethanolic, and aqueous leaf extracts were prepared and tested against Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans using agar well diffusion, minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC), and synergy assays (FICI). Results showed that methanolic extracts produced the highest zones of inhibition (up to 30.0 mm), followed by ethanolic and aqueous extracts. Synergistic interaction was observed in the combined extracts (ΣFICI = 0.19), particularly effective against E. coli and C. albicans. The observed antimicrobial effects are attributed to phytochemicals including sesquiterpene lactones (vernodalin, vernomygdin), flavonoids (luteolin), saponins, and alkaloids, which are known for disrupting microbial membranes and inhibiting biofilm formation. This study highlights the therapeutic potential of these ethnobotanical species as sources of multi-targeted anti-biofilm agents. Further studies are recommended to isolate specific bioactives, quantify biofilm disruption, and evaluate cytotoxicity to support clinical application.

Keywords: *Biofilm, Antimicrobial Resistance, Vernonia amygdalina, Ocimum gratissimum, Kalanchoe pinnata, Phytochemicals, Vernodalin, Luteolin, Synergism.*

Introduction

Biofilms are organized assemblies of microorganisms that embed themselves within a self-generated protective matrix, allowing them to firmly attach to a variety of surfaces, including both living tissues and inert materials [1]. Biofilm-forming microorganisms represent a significant hurdle in modern clinical practice due to their remarkable ability to withstand antibiotics and standard disinfection protocols. In contrast to their free-floating (planktonic) counterparts, microbes residing within biofilms exhibit up to a thousand-fold increase in resistance to antimicrobial agents. This heightened resilience contributes to the persistence and recurrence of infections, often rendering conventional treatments ineffective. Biofilms are central to the pathogenesis of numerous chronic and device-associated infections, including those involving urinary catheters, prosthetic implants, chronic non-healing wounds, and respiratory complications such as those seen in cystic fibrosis. Their presence not only complicates patient management but also prolongs hospital stays, increases healthcare costs, and elevates morbidity rates [2].

Antimicrobial resistance (AMR) has emerged as a pressing global health concern, with biofilm-associated resistance representing a critical and often overlooked contributor to the persistence and

complexity of this growing threat [3]. The World Health Organization (WHO) has emphasized the critical need for the discovery and development of new antimicrobial agents that can effectively combat both multidrug-resistant organisms and biofilm-associated infections. Of particular concern are the ESKAPE pathogens Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—which are well-recognized for their capacity to resist multiple antibiotics and form highly resilient biofilms that complicate treatment outcomes and prolong patient recovery [4].

Given this situation, the need for alternative therapeutic strategies, especially those derived from natural sources, has gained significant attention. Plants with ethnomedicinal relevance offer a promising reservoir of bioactive compounds capable of disrupting biofilms and restoring antimicrobial efficacy.

Despite substantial advancements in antimicrobial research, prevailing therapeutic regimens exhibit limited efficacy against biofilm-associated infections. This therapeutic challenge is primarily attributed to the intricate three-dimensional architecture and physicochemical robustness of the extracellular polymeric substance (EPS) matrix, which confers enhanced protection to resident microbial communities by impeding antimicrobial penetration, facilitating horizontal gene transfer, and promoting metabolic heterogeneity and phenotypic resistance within the biofilm microenvironment [5]. Synthetic antibiotics frequently exhibit suboptimal penetration into biofilm structures or become functionally compromised due to the distinct physicochemical conditions prevailing within these microbial consortia. Additionally, the widespread and indiscriminate application of conventional antimicrobial agents has markedly contributed to the selection pressure driving the emergence and proliferation of multidrug-resistant (MDR) phenotypes, a phenomenon particularly pronounced among biofilm-producing pathogenic organisms [6].

Despite the well-documented antimicrobial activities of various plant species, there remains a scarcity of research focused on their effectiveness against microbes embedded within biofilms. Specifically, Vernonia amygdalina, Ocimum gratissimum, and Kalanchoe pinnata plants commonly utilized in traditional medicine have not been thoroughly investigated for their potential to inhibit or disrupt biofilm-associated infections [7]. This knowledge gap highlights the pressing necessity for comprehensive scientific research to identify and characterize the bioactive constituents and elucidate their mechanisms of action against drug-resistant and biofilm-forming microorganisms.

With the escalating threat of antimicrobial resistance compromising the efficacy of conventional antibiotics, there is an increasing scientific impetus toward the exploration of alternative and sustainable therapeutic strategies. Natural products, especially those derived from medicinal plants, have long constituted a cornerstone in pharmaceutical development, owing to their rich reservoir of structurally diverse and biologically active constituents. These phytochemicals possess a wide spectrum of pharmacological activities, including antimicrobial, anti-inflammatory, and immunomodulatory effects. Notably, unlike many synthetic antimicrobials that often act on a single molecular target, plant-derived compounds frequently exert multi-targeted mechanisms of action, thereby potentially mitigating the emergence and propagation of resistant microbial strains [8].

Vernonia amygdalina, Ocimum gratissimum, and Kalanchoe pinnata are prominent ethnopharmacological species extensively utilized in African traditional medicine for the management of infectious diseases, wound healing, and inflammatory disorders. Empirical evidence has established their antimicrobial, antioxidant, and tissue-regenerative properties, indicating potential therapeutic relevance against biofilm-associated infections. Nonetheless, the precise mechanisms by which these phytochemicals modulate microbial biofilm architecture, potentiate antibiotic efficacy, or inhibit biofilm initiation remain inadequately characterized and warrant further investigation [9].

Investigating these plants not only offers the potential for novel anti-biofilm agents but also aligns with the global movement toward phytomedicine and the reintegration of traditional knowledge into modern therapeutic development.

Materials and Methods

Biochemical Methods

Microscopy and biochemical tests were performed according to the protocols described by Cheesbrough [10]. These included Gram staining for classification of bacterial isolates into Gram-positive and Gram-negative groups based on their cell wall properties; spore staining and motility tests for the differentiation of spore-forming and motile bacteria; catalase and oxidase tests to detect the presence of specific metabolic enzymes; and Kligler Iron Agar (KIA) and indole tests to assess sugar fermentation capabilities and the ability to degrade tryptophan, respectively.

Antimicrobial and Anti-biofilm Assays

Antimicrobial susceptibility testing was performed using the agar well diffusion method [11]. Mueller-Hinton Agar was inoculated with standardized microbial suspensions (0.5 McFarland). Wells were filled with plant extracts at concentrations ranging from 100–500 mg/mL. Chlorhexidine (1.25 mg/mL) served as the control. Zones of inhibition were measured after 24 h incubation at 37 °C and reported as mean ± SD.

Phytochemical Screening of Plant Extracts

Qualitative phytochemical screening of the aqueous, ethanolic, and methanolic extracts of Vernonia amygdalina, Ocimum gratissimum, and Kalanchoe pinnata was conducted to identify the presence of bioactive secondary metabolites associated with antimicrobial and anti-biofilm activities. Standard procedures described by Harborne [12] and Sofowora [13] were employed.

Results

Table 1.0: Identification and characterization of isolated organisms.

Isolate	Morphology	Gram Reaction	Spore Stain	Catalase Test	Oxidase Test	Citrate Test	Coagulase Test	Gas Butt	Slope	Urease Test	Indole Test	Motility Test	Organisms
A	Golden yellow raised colonies on Nutrient Agar	+ Cocci in clusters	-	+	-	-	+	+ Y	R	+	-	-	*Staphylococcus aureus*
B	White to grey flat colonies on Nutrient Agar	- Rod shaped in chains	-	+	+	-	-	+ Y	R	+	-	-	*Klebsielia pneumoniae*
C	Smooth, translucent colonies with irregular spreadingedge.	- Rod shaped in pairs	-	+	+	-	-	+ Y	R	-	-	+	*Pseudomonas aeruginosa*
D	Large, circular, grayish-white to white, moist, smooth, and opaque colonies	- Rod shaped in chains	-	-	+	-	-	+ Y	R	-	+		*Escherichia coli*

Kligler Iron Agar Test Results Table.

Organism	Glucose	Lactose	Gas	H_2S	KIA Reaction	Remarks
Staphylococcus aureus	Ferments	Ferments	+/-	-	A/A (Yellow/ Yellow)	Can ferment both glucose and lactose; KIA not commonly used for Gram-positive cocci
Klebsiella pneumoniae	Ferments	Ferments	+	-/+	A/A, Gas, (± H_2S)	Typical Enterobacteriaceae pattern; do not produce H_2S
Pseudomonas aeruginosa	Non-fermenter	Non-fermenter	-	-	K/K (Red/Red)	Oxidative metabolism only; does not ferment glucose or lactose
Escherichia coli	Ferments	Ferments	+	-	A/A, Gas	Strong lactose and glucose fermenter; gas commonly produced

KIA Interpretation Key

Symbol Meaning

- A: Acid (yellow) – fermentation
- K: Alkaline (red) – no fermentation
- Gas: Bubbles, cracks, or lifting agar
- H_2S: Black precipitate in the butt
- + : Positive
- - : Negative

Table 2.0: Identification and characterization of fungi.

Isolate	Morphology	Microscopy	Germ tube test	Organism
A	White to grey flat colonies on potato dextrose agar	Round to oval yeast cells, with filamentous hyphae	+	Candida albicans

Table 3.0: Zones of inhibition of Vernonia amygdalina, Ocimum gratissimum and Kalanchoe pinnata combined on isolated organisms

Conc. (mg/ml)	Zones of inhibitions (mm±SD)		
	Water	Ethanol	Methanol
500	24.0±0.45	26.0±0.45	30.0±0.60
400	23.5±0.38	25.5±0.35	29.5±0.58
300	22.0±0.28	24..0±0.30	28.0±0.50
200	21.0±0.25	23.0±0.28	27.0±0.48
100	20.0±0.20	22.0±0.25	26.0±0.45
Control	19.00		

(Diameter of well used = 5.00mm. Values were taken in triplicates with standard deviation)

- ≤17.0mm= Resistant
- 17.0-19.0mm= moderate
- ≥19.00mm= susceptible
- mg/ml= milligram per mil
- mm= millimeter
- SD= Standard difference

Aqueous extracts showed zones of inhibition range (20.0-24.0mm) When compared with zone of inhibition of control (19.00mm) was found to exhibit a high susceptibility to the isolated Concomitant bacteria, Ethanolic extracts with zones of inhibitions range (22-26.0mm) showed higher susceptibility while methanolic extracts with 26.0 to 30.0mm zones of inhibitions showed highest susceptibility to the isolated concomitant bacteria; Likewise, the zones of inhibitions followed a concentration based trend.

Table 4.0: Synergistic/Antagonistic/Additive interaction of the extracts.

Av. Mic of Extract A in combination (mg/ml)	Av. Mic of Extract A alone (mg/ml)	Average MIC of extract B in combination (mg/ml)	Av. Mic of extract B alone (mg/ml)	Av. Mic of extract C in combination	Av. Mic of extract C alone
24.20	193.7	24.20	531.2	24.20	937.5

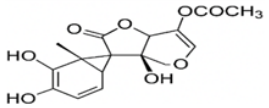
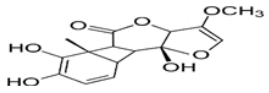
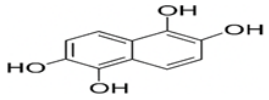
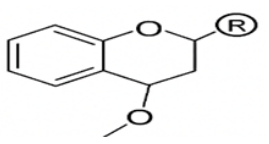
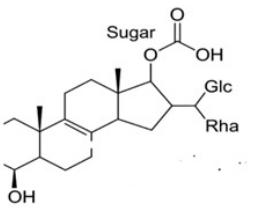
ΣFIC O.19<0.5
Effect Synergistic

$$FICI = \frac{\text{MIC of Extract A in combination}}{\text{MIC of Extract A alone.}} + \frac{\text{MIC of Extract B in combination}}{\text{MIC of Extract B alone}} + \frac{\text{MIC of Extract C in combination}}{\text{MIC of Extract C alone.}}$$

Legend
- MIC: Minimum inhibitory concentration
- mg/ml: Milligram per milliliter

Interpretation of ΣFIC values:
- ≤ 0.5: Synergistic
- > 0.5 – 1.0: Additive
- > 1.0 – 4.0: Antagonistic

Bioactive Compounds Identified in the Studied Medicinal Plants, Their Structure Classes, and Reported Activities

Compound	Plant Source	Chemical Class	Representative Structure Features	Bioactivities
Vernodalin	Vernonia amygdalina	Sesquiterpene lactone		Antibacterial, cytotoxic, antiparasitic
Vernomygdin	Vernonia amygdalina	Sesquiterpene lactone		Antiplasmodial, antimicrobial
Luteolin	All three plants	Flavonoid (flavone subclass)		Antioxidant, antimicrobial, biofilm inhibition
Flavonoids	All three plants	Polyphenolic compounds		Anti-inflammatory, antioxidant, anti-quorum sensing
Saponins	Kalanchoe pinnata (mainly)	Glycosylated triterpenoids		Antifungal, immunomodulatory, hemolytic
Alkaloids	V. amygdalina, O. gratissimum	Basic nitrogen-containing		Antimicrobial, enzyme inhibition, anti-biofilm

Discussion

The current study explored the antimicrobial potential of Vernonia amygdalina, Ocimum gratissimum, and Bryophyllum pinnatum, individually and in combination, against clinically relevant biofilm-forming pathogens. The isolates Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans were selected due to their documented involvement in multidrug-resistant infections and their association with the ESKAPE group, known for evading conventional therapies [14]. Biochemical and microscopic characterizations affirmed the identity of these pathogens, and their susceptibility patterns were assessed using well diffusion and MIC/MBC/MFC assays following CLSI standards [15].

The findings revealed a concentration-dependent antimicrobial effect across all plant extracts, with methanolic extracts consistently demonstrating the highest zones of inhibition, followed by ethanolic and aqueous extracts. Among the single plant extracts, B. pinnatum exhibited superior antimicrobial potency, especially at higher concentrations, consistent with prior studies suggesting its broad-spectrum phytochemical profile rich in alkaloids, flavonoids, and phenolic compounds [16]. Notably, the combination of the three extracts yielded a synergistic effect, evidenced by significantly enhanced zones of inhibition (up to 30 mm with methanol) and low MIC/MBC values, particularly against E. coli (MIC = 7.8 mg/ml, MBC = 15.6 mg/ml) and C. albicans (MIC = 3.90 mg/ml) [17]. The calculated Fractional Inhibitory Concentration Index (FICI $\leq$ 0.19) confirmed strong synergism between the plant constituents, highlighting the therapeutic potential of polyherbal formulations in overcoming resistance mechanisms [18].

The antimicrobial and potential anti-biofilm activities observed were attributed to the synergistic effects of several phytochemicals present in the extracts. Vernonia amygdalina contains sesquiterpene lactones such as vernodalin and vernomygdin, which have been shown to interfere with microbial membrane integrity [19]. Similarly, luteolin, a flavonoid identified in several of these plants, is known for its anti-inflammatory and antimicrobial properties [20]. Alkaloids, saponins, and phenolic compounds possess the ability to disrupt biofilm formation [21].

Despite the promising findings, this study has several limitations. First, microbial identification was based solely on phenotypic and biochemical methods without molecular confirmation (e.g., 16S rRNA or ITS sequencing), which could compromise taxonomic precision [22]. Secondly, the study employed in vitro assays only, and the observed antimicrobial effects may not translate directly to in vivo environments where host factors and bioavailability significantly influence outcomes [23]. Finally, the experimental design did not assess cytotoxicity or safety of the plant extracts, which is essential for considering therapeutic applications [24].

Nevertheless, this study contributes significantly to the growing body of evidence supporting the use of African ethnomedicinal plants in combating antimicrobial resistance. By demonstrating both individual and synergistic efficacy of V. amygdalina, O. gratissimum, and B. pinnatum against resistant pathogens, it opens a pathway for the development of phytotherapeutic agents that can serve as adjuvants or alternatives to existing antibiotics. Future studies should focus on molecular identification of pathogens, evaluation of anti-biofilm efficacy using standard quantification methods, phytochemical profiling, in vivo validation, and cytotoxicity assessment to fully ascertain clinical utility [25].

Conclusion

This study provides compelling evidence that Vernonia amygdalina, Ocimum gratissimum, and Kalanchoe pinnata possess potent antimicrobial properties against clinically relevant biofilm-forming pathogens, including Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans. The observed antimicrobial efficacy particularly the superior performance of methanolic extracts and the pronounced synergistic effect when the plant extracts were combined underscores the therapeutic potential of these ethnomedicinal plants as alternative or adjunct agents in the fight against multidrug-resistant and biofilm-associated infections.

The presence of bioactive phytochemicals such as vernodalin, vernomygdin, luteolin, saponins, flavonoids, and alkaloids likely contributed to the observed biological activities, either by disrupting microbial cell integrity or inhibiting biofilm formation. These findings support the traditional medicinal use of these plants and provide a scientific basis for further development of plant-derived anti-biofilm therapeutics.

However, to advance this promising line of inquiry, future research should incorporate molecular identification of microbial isolates, in-depth phytochemical profiling, standardized biofilm quantification assays, toxicity testing, and in vivo efficacy models. Such steps are essential to validate the clinical applicability of these natural products and facilitate their integration into modern antimicrobial therapy.

This study reinforces the relevance of traditional medicinal plants in addressing the global challenge of antimicrobial resistance and paves the way for the development of novel, plant-based interventions targeting biofilm-related infections.

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Ethics approval

Ethical approval was obtained from the appropriate institutional review board. Informed consent was also secured from all participating patients.

Competing interests

The author declares no competing interests.

Author's contributions

- Ebiloma Samuel is the sole contributor to this manuscript.

- Nkechi Chuks Nwachuckwu, proofread the manuscript.
- Happy Uchendu Ndom, vet the result.
- Hope Okereke, proofread the manuscript.

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