Current Trends in Biotechnology and Pharmacy
Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

Antibiofilm activity of ethanolic root extract of *Vetiveria zizanioides* against dental pathogens

N. Premjanu[1]*, Rizwana Khatun M R[2], S. Srividya[3], B.R. Malathy[4], and R. Revathy[5]

[1]Department of Biochemistry, Sathyabama Dental College and Hospital
[2]CRRI, Sathyabama Dental College and Hospital, Chennai 600119
[3]Department of Biochemistry, Sathyabama Dental College and Hospital
[4]Department of Microbiology, Sathyabama Dental College and Hospital
[5]Department of Pharmacology, Sathyabama Dental College and Hospital
*Corresponding author: janu6kishore@gmail.com

Abstract

Biofilm infections, shielded from antibiotics and immune defences, pose a major health threat. Plants offer potential solutions due to their diverse bioactive compounds. This study investigated the root extract of *Vetiveria zizanioides* for its composition and tested for its ability to inhibit biofilms, fight bacteria and fungi, reduce inflammation, and combat oxidative stress in pathogens. The anti-biofilm properties were assessed using test tube method and a crystal violet assay. Phytochemical composition was analysed via Gas Chromatography-Mass Spectrometry (GC-MS). Antimicrobial activity was tested against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Candida albicans* using both agar well plate and micro broth dilution methods. Antioxidant and anti-inflammatory effects were determined by DPPH and protein denaturation assays, respectively. The ethanolic extract contained 17 identified compounds, primarily sesquiterpenes and fatty acids. Major compounds were identified as 17-hydroxy-3,20-dioxopregna-1,4,ol, z,z-8,10-hexadecadien-1-ol, formic acid, campesterol, cedren-13-ol, It inhibited biofilm formation in various pathogens, with the strongest effect against *Staphylococcus aureus* (75%) and the weakest against *Pseudomonas aeruginosa* (25%). The extract also displayed antimicrobial activity against all tested bacteria and fungi, with the best activity against *Staphylococcus aureus* (23mm inhibition zone). Additionally, it exhibited dose-dependent antioxidant and anti-inflammatory properties. These findings suggest *Vetiveria zizanioides* extract has significant potential for combating biofilm-related infections. Future research should focus on isolating and purifying active compounds, assessing their safety and long-term effects, and exploring their potential as therapeutic agents.

Keywords: *Vetiveria zizanioides,* anti-biofilm, antimicrobial, antioxidant and anti-inflammatory

Introduction

The oral microbiome consists of inter-kingdom microorganisms, which includes a few viruses, fungal species and roughly 700 different bacterial species belonging to 13 independent phyla (1). These interactions collectively determine the composition of the oral microbiome. Microorganisms in the oral microbiomei interact synergistically, mutualistically, and antagonistically, collectively shaping its composition (2). The oral cavity supports the growth of a diverse microbiome, due to its, humid, warm and nutrient-rich surroundings. The composition of the oral microbiota is influenced by factors such as saliva composition, diet, hygiene habits, and the anatomical structure of the oral cavity. This results in a vast diversity of microorganisms

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

within the oral microbiome, which occupy distinct ecological niches including the cheek, palate, tongue surface, teeth, gingiva, and periodontal pocket (3). Alterations in factors like saliva composition, diet, and hygiene habits can result in an imbalance of the oral microbiota, known as dysbiosis (3). Moreover, the complex interaction among the oral microbiome, the body's immune response, and dietary patterns plays a pivotal role in the creation of harmful biofilms within theoral cavity (4). These biofilms, characterized by their ability to adhere to dental surfaces, are implicated in the pathogenesis of various oral infections, significantly impacting dental health and well-being (4). Both Gram-positive and Gram-negative bacteria have the capability to form biofilms on various surfaces, including the hard surfaces of teeth and the soft tissues of oral mucosa, like epithelial cells, dental surfaces as well as medical devices such as dental implants, orthodontic prostheses(5). Among the most prevalent bacterial species involved in biofilm formation are *Enterococcus faecalis*, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridans, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa* (6).

The process of pathogenic oral biofilm development follows a sequential pattern. Initially, a salivary pellicle forms, originating from salivary glycoproteins that adhere to the tooth surface. Subsequently, initial adhesion occurs as early colonizer bacteria in saliva recognize binding proteins within the acquired pellicle and attach to them. As the biofilm grows, various bacterial species become incorporated, leading to biofilm maturation. Finally, the bacteria disperse from the biofilm and spread to colonize new surfaces (7). These Bacterial biofilms secrete a complex mixture of polysaccharides, proteins, fatty acids, extracellular DNA and proteins mainly composed of D-amino acids collectively referred to as extracellular polymeric substance (EPS) (8). EPS plays several crucial roles in biofilm development and function, including stabilizing the biofilm structure, mediating adhesion and resistance, preventing water loss, acting as a resin, retaining and digesting nutrients, balancing production and degradation, enabling communication and quorum sensing, and even serving as a carbon and energy source (9). These resilient communities of bacteria safe guard themselves from conventional antibiotics and immune defences, leading to chronic, challenging infections.

The National Institutes of Health (NIH) reports that a staggering 80% of microbial infections in the human body are caused by biofilm-associated microorganisms (10). The primary challenge with biofilm-related infections lies in the detection of causative species, as biofilm samples often yield culture-negative results. This issue, attributed to the robust integration or uncultivability of bacteria within biofilms, extends to implant and catheter-related infections, where bacterial identification is notably difficult. Historically, biofilm bacteria have been deemed unculturable. Moreover, certain pathogenic bacteria, unable to thrive in culture media, are thought to become virulent within the host or environmental conditions, leading to infection (11). The pervasive role of bacterial biofilms in chronic infections is well-documented, with a wide array of conditions such as otitis, diabetic foot ulcers, rhinosinusitis, chronic pneumonia in cystic fibrosis, osteomyelitis, and infective endocarditis being attributed to these microbial structures (12). Approximately two-thirds of hospital-acquired infections are attributed to *S. aureus Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. These infections often involve biofilms on medical devices like dental implants, which can result from contact with infected individuals or during surgeries, leading to severe health outcomes. Chronic conditions such as periodontitis and urinary tract infections are also linked to biofilms, which are difficult to treat due to their resistance to antibiotics and the immune system. The onset of biofilm-related infections involves microbial contamination, a compromised

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

immune response near the device, and a generally weakened immune function (13). *Candida* infections, including invasive candidiasis, have increased in severity and are a major cause of morbidity and mortality in healthcare settings, particularly among older adults and newborns. *Candida* is a natural inhabitant of oral cavity and a part of oral microbiota. The interaction between *Candida* and its host relies on yeast virulence and host-related elements, including comorbidities, immunity, and age. *Candida* frequently contributes to biofilm-related infections, particularly in the context of medical devices. The liberation of yeast cells from biofilms can result in widespread infection or fungemia (14). These infections collectively exert a significant health toll, are not only prevalent but also annually contribute to considerable mortality and morbidity worldwide. In recognition of this growing threat, a significant increase in research efforts is imperative to develop effective strategies for preventing and managing these complex infections(15). Plants provide promising avenues owing to the production of varied novel bioactive secondary metabolites. In this context, the current study is intended to explore one such plant namely *Vetiveria zizanioides.*

Vetiveria zizanioides commonly known as vetiver, is a perennial long tufted grass belonging to the family Poaceae. It is native to India and well-recognized in southern India, where it goes by various names such as khus, vetiver, and vala in different Indian languages Vetiver is an aromatic and ornamental plant, widely used for bathing and perfumery purposes which has been acknowledged since ancient times. Numerous studies have explored its chemical composition, with sesquiterpenes being the primary components found in the roots and phenolic compounds in the leaves(16). *Vetiveria zizanioides* contains a variety of chemical constituents, including ß-vetivone, vetivone, vetivene, vetiverol, vetivenate, vetivenyl, vetivazulene, khusimol, iso-khusimol, khositone, khusimone, tripene-4-ol, terpenes, benzoic acid, ß-humulene, and epizizianalThe predominant component in the roots is valencene, constituting 30.36%, while the shoots and leaves primarily contain 9-octadecenamide, 1,2-benzenedicarboxylic acid, and diisooctyl ester. Analysis reveals a rich presence of terpenoids in the plant's volatile oil, with three monoterpenes, two sesquiterpenes, and one triterpene identified in the shoot volatiles, and a predominance of sesquiterpenes in the root volatiles (17). Vetiver serves multiple purposes, including being aromatic, antifungal, cooling, antiemetic, diaphoretic, haemostatic, expectorant, diuretic, and stimulant. It has also been used traditionally for managing conditions such as hysteria, insomnia, skin diseases, asthma, amentia, amenorrhea, antispasmodic effects, kidney problems, gallstones, and as a mosquito repellent. Additionally, vetiver exhibits antioxidant and anti-inflammatory properties (18). Laboratory studies have demonstrated vetiver's antimicrobial activity, particularly against pathogens like *Staphylococcus aureus* and *Escherichia coli*. It exerts bacteriostatic and fungistatic effects, inhibiting bacterial growth (19). Furthermore, Vetiver oil's inherent aroma characteristics contribute to its pleasant odour, is known for its sedative properties. Traditionally, *Vetiveria zizanioides* has been employed in aromatherapy to mitigate stress, anxiety, nervous tension, and insomnia (20).

Given its rich chemical composition and diverse bioactive properties, *Vetiveria zizanioides* offers a multifaceted approach to combating biofilm infections by combining antibacterial, antifungal, antioxidant, and anti-inflammatory activities. This comprehensive action can help address the diverse challenges posed by biofilms, including antimicrobial resistance and inflammatory responses. Studying its efficacy against biofilms, forming pathogens, can provide valuable insights into its therapeutic potential in combating biofilm-related infections.

This research endeavours to explore the antibiofilm potential of *Vetiveria*

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

zizanioides root extract against dental pathogens. Objectives encompass elucidating its phytochemical profile, assessing antimicrobial efficacy against prevalent oral pathogens, and evaluating anti-inflammatory and antioxidant properties. Furthermore, this research seeks to investigate the extract's ability to inhibit biofilm formation, offering valuable insights into natural agents for managing oral biofilm-related diseases.

Materials and Methods

Preparation of ethanolic extracts of *Vetiveria zizanioides*

Dried roots of *Vetiveria zizanioides* were powdered and then 40 g of the powder was soaked in 400 mL of ethanol. For extraction, the extract was continuously stirred at room temperature for 48 hours using a magnetic stirrer. Then the ethanol extract was filtered using Whatman filter paper (No.1). The filtrates were concentrated under reduced pressure at 45-50°C using a rotary evaporator and stored at 4°C until analysis.

Phytochemical analysis by Gas chromatography–mass spectrometry (GC-MS)

1 µl volume of ethanol extract from *Vetiveria zizanioides* was utilized for GC/MS analysis. The analysis was conducted using a GC QP 2010 instrument by SHIMADZU, which consisted of an AOC-20i autosampler and a gas chromatograph connected to a mass spectrometer (GC-MS). The GC-MS mass spectrum interpretation relied on the National Institute Standard and Technology (NIST) database

Antimicrobial activity in Vetiver Root Extract

Media and inoculum

For antimicrobial activity, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 67120 and *Candida albicans* (ATCC 10231) were used in this investigation. The bacteria used were cultivated on Müller-Hinton agar (MHA) at 35°C for 24 h. The fungi were cultivated on Sabouraud dextrose agar (SDA).

Antimicrobial activity by agar diffusion test

Sterile petri dish plates were prepared, each containing 20 ml of sterilized Müller-Hinton agar for antibacterial testing and 20 ml of sterilized Sabouraud dextrose agar (SDA) for antifungal testing. The media in these plates was allowed to solidify. Fresh culture suspensions of bacteria and fungi (100 µl each) were swabbed onto their respective plates. Using a sterile gel puncher, wells of 10 mm diameter were created approximately 2 cm apart on the agar surface. A stock solution of plant extract at a concentration of 1 mg/ml was added to each well, allowing it to diffuse at room temperature. The plates were then incubated for 24 hours, and afterward, the inhibitory zones' diameters around each well were measured and recorded (21).

Minimum inhibitory concentration (MIC) by microbroth dilution method

The minimum inhibitory concentration (MIC) was assessed using the microbroth dilution method on slants (1 ml). Plant extracts were serially diluted by factors of two, resulting in concentrations of 50, 25, 12.5, and 6.25 mg/ml. To these dilutions, 100 µl of microorganism suspension was added. The tubes were then incubated at 37°C for 24 hours. After incubation, sub-cultures were prepared on Mueller Hinton agar for bacteria and Sabouraud dextrose agar (SDA) for fungi. Bacterial and fungal growth was observed the following day. The MIC was defined as the lowest concentration of plant extract that inhibited bacterial or fungal growth in the subcultures. (21).

DPPH Radical Scavenging Assay

The DPPH Radical Scavenging Assay is a widely used method for assessing the antioxidant activity of plant extracts. This assay relies on the capacity of antioxidants to reduce

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10.5530/ctbp.2024.4s.8

the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) to its corresponding hydrazine form. The reduction of DPPH is accompanied by a colour change from purple to yellow, which can be quantified spectrophotometrically at a wavelength of 517 nm. For the assay ascorbic acid is taken as standard. Dissolve 250 mg of ascorbic acid in 50 mL of distilled water to create a 5 mg/mL stock solution. Then, dilute 10 mL of the stock solution with 100 mL of distilled water to obtain a 500 µg/mL working standard of ascorbic acid. Various concentrations of working standard of ascorbic acid and the ethanolic extract of *Vetiveria zizanioides* (50-500µg/ml) were prepared. Then mixed 100 µl of plant extract and ascorbic acid of each concentration with 300 µl of the DPPH solution followed by incubation of the mixture at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using a spectrophotometer. The Inhibition Percentage was calculated using the following formula (22):

Inhibition% = ((Ablank - Asample) / Ablank) × 100
where:
A blank is the absorbance of the blank (DPPH solution without plant extract).
A sample is the absorbance of the sample (DPPH solution with plant extract).

Anti-inflammatory activity by Protein Denaturation Assay

The albumin denaturation assay followed the method outlined by Williams et al. with minor adjustments. (23) During the preparation of the reaction mixture, various concentrations of *Vetiveria zizanioides* ethanolic extract (ranging from 50 to 500 µg/ml) were used. 0.05 ml of the plant extract was combined with 0.45 ml of 5% bovine serum albumin and incubated at 37°C for 20 minutes. Subsequently, the reaction mixture was heated to 57°C for 3 minutes, followed by the addition of 2.5 ml of 0.5 M phosphate buffer (pH = 6.3) to each sample. Copper-alkaline reagent and 1% (v/v) Folin-Ciocalteu's reagent were then introduced and incubated for 10 minutes. The absorbance was measured at 650 nm. The results were compared to those obtained using diclofenac sodium (at a concentration of 200 µg/ml) as a reference drug.

% Inhibition of denaturation = (1-D/C) X100.
Where D is the absorbance of test sample and C is the absorbance of negative control (without the test sample or reference drug).

Determination of Biofilm Formation by test tube method and crystal violet assay

Sterile test tubes were prepared, with 100 µl of Mueller Hinton agar for bacterial testing and Sabouraud dextrose agar (SDA) for fungal testing. Fresh bacterial and fungal suspensions (at a concentration of 1.0 McFarland) were added (100 µl each) to the tubes and incubated for 48 hours at 37°C. To assess biofilm formation, the tube contents were washed with 200 µl of normal saline, followed by the addition of 200 µl of 0.1% crystal violet stain and another 20 minute incubation. Subsequently, each tube was thoroughly washed with deionized water, and 96% ethanol (200 µl) was added. The optical density (OD) of the adherent microorganisms was measured using a spectrometer at 630 nm. Biofilm formation was quantified using the following formula (21).

OD $\leq$ ODc= No biofilm producer
ODc< OD $\leq$ 2 × ODc= Weak biofilm producer
2 × ODc< OD $\leq$ 4 × ODc= Moderate biofilm producer
4 × ODc< OD= Strong biofilm producer.
ODc: Optical density of growth control

Determination of Anti Biofilm Activity using modified crystal violet assay

Sterile test tubes were prepared, with 100 µl of Mueller Hinton agar for bacterial testing and Sabouraud dextrose agar (SDA) for fungal testing. To each test tube, 100 µl of *Vetiveria zizanioides* ethanolic extract was added. Fresh bacterial and fungal suspensions (at a concentration of 1.0 McFarland) were introduced (100 µl each) and incubated for 48

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

hours at 37°C. To assess biofilm inhibition, the tube contents were washed with 200 µl of normal saline, followed by the addition of 200 µl of 0.1% crystal violet stain and another 20-minute incubation. Subsequently, each tube was thoroughly washed with deionized water, and 96% ethanol (200 µl) was added. The optical density (OD) of the adherent microorganisms was measured using a spectrometer at 630 nm. Biofilm inhibition was calculated using the following formula (21).

OD of bacteria= [(OD growth control – OD sample) / OD growth control] × 100.

Results and Discussion

For the phytochemical study of *Vetiveria zizanioides* roots, ethanol was used as an extraction solvent The phytochemicals present in the ethanolic extract were characterized and identified by GC-MS analysis. A total of 17 compounds were identified. The major identified compounds are presented in (Table 1 & Figure 1). The identified

Table 1:GC-MS analytical report of ethanolic extract of *Vetiveriazizanioides*

Retention time	Area	Area %	Name of the compound	Chemical formula	Molecular weight (g/mol)	Chemical nature
30.848	159902	1.72	Gamma.-Gurjunenepoxide-(2)	$C_{15}H_{24}O$	220.3505	Sesquiterpene Epoxide.
31.587	345155	3.72	Cedren-13-Ol,8-	$C_{15}H_{24}O$	220.3505	Sesquiterpene Alcohol
32.913	281695	3.03	Trans-ValerenylAcetate	$C_{15}H_{24}O_2$	236.35	Sesquiterpene Ester
33.3	199040	2.14	Pentacyclo(3.3.0.02,4.03,7.06,8)Octane	C_8H_8	104.15	Hydrocarbon
33.579	989073	10.66	FormicAcid	HCOOH	46.03	CarboxylicAcid
35.724	134330	1.45	Acetamide,2-(Diethylamino)-N-(2,	C_2H_5NO	59.07	Amides
37.428	135866	1.46	DibutylPhthalate	$C_{16}H_{22}O_4$	278.35	Phthalate
38.411	153308	1.65	HexadecanoicAcid,EthylEster	$C_{18}H_{36}O_2$	284.4772	Fatty Acid Ester
41.408	1185808	12.78	Z,Z-8,10-Hexadecadien-1-Ol	$C_{16}H_{30}O$	238.41	Fatty Alcohol
41.484	555876	5.99	OctadecanoicAcid	$C_{18}H_{36}O_2$	284.48	Saturated Fatty Acid
41.6	217456	2.34	2-AminoethanethiolHydrogenS	$C_2H_7NS_2$	77.15	Thiol
41.91	136229	1.47	EthylOleate	$C_{20}H_{38}O_2$	310.51	Fatty Acid Ester
48.124	169715	1.83	Bis(2-Ethylhexyl)Phthalate	$C_{24}H_{38}O_4$	390.56	Phthalate
48.865	3922276	42.26	17-Hydroxy-3,20-Dioxopregna-1,4,Ol	$C_{21}H_{32}O_5$	372.47	Sterol.
51.25	184809	1.99	(2E)-6Acetoxy-2-Methylhexenal	$C_9H_{14}O_3$	170.09	Sesquiterpene Epoxide
53.1	327350	3.53	Campesterol	$C_{29}H_{50}O$	426.72	Sterol
54.162	184094	1.98	Stigmasterol	$C_{29}H_{48}O$	412.69	Sterol

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

compounds represent a mix of steroids, fatty alcohols, carboxylic acids, sterols, and terpenes.The major ones being 17-hydroxy-3,20-dioxopregna-1,4,ol, (42.26), Z,z-8,10-hexadecadien-1-ol,(12.78), Formic acid(10.66), Octadecanoic Acid (5.99), Cedren-13-ol (3.72) and Campesterol, (3.53). These compounds could disrupt microbial membranes, inhibit essential enzymatic processes, or interfere with cell signalling pathways. Cedren-13-ol disrupts bacterial
cell membranes, increasing membrane permeability. This interference affects vital cellular processes, making bacterial cells more vulnerable to damage and death. Formic acid triggers a rapid intracellular burst of reactive oxygen species (ROS), resulting in oxidative damage and cell death. This process affects both metabolic activity and membrane integrity (24). Moreover in *Candida* species formic acid triggers programmed cell death by activating caspase, resulting in apoptosis-like cell demise(25).Octadecanoic acid due to its amphiphilic nature disrupts bacterial cell membranes and also interferes with fatty acid metabolism, energy metabolism pathways, including the citrate cycle(26). Similar to our findings a study conducted by Kannappan and colleagues discovered that *Vetiveria zizanioides* root extract contains sesquiterpenes as a prominent constituent (27). Yet in another study Krishnaveni revealed the presence of Saponins, Flavanoids and Phenols in *Vetiveria zizanioides* root extract (19).

Antimicrobial activity of *Vetiveria zizanioides* ethanolic root extract

The Vetiver ethanolic extract exhibited a significant antimicrobial activity as shown in Figures 1 & 2. The observed variations in the zone of inhibition highlight the differential sensitivity of the tested microorganisms to the Vetiver ethanolic extract. *Staphylococcus aureus* exhibited the highest sensitivity, followed by *Candida albicans, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa. Staphylococcus aureus* displayed the largest zone of inhibition at 23 mm, suggesting a robust and effective inhibition of growth by the Vetiver extract against this Gram-positive

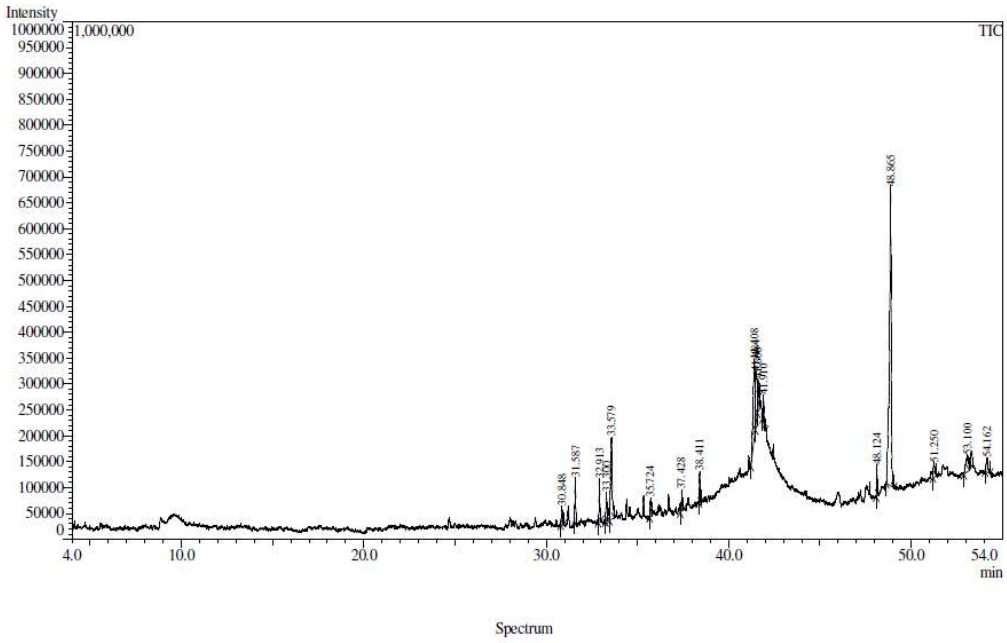

Figure1: Chromatogram of ethanolic extract of *Vetiveriazizanioides*

Antibiofilm Activity of *Vetiveria zizanioides*

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

bacterium. The Vetiver ethanolic extract exhibited antimicrobial effect against *E. Coli and Klebisilla pneumoniae*, resulting in a zone of inhibition measuring 16 mm. The Vetiver ethanolic extract exhibited a moderate antimicrobial effect against *Pseudomonas aeruginosa*, resulting in a zone of inhibition measuring 11mm. Against the fungal pathogen *Candida albicans*, the Vetiver extract demonstrated a significant zone of inhibition measuring 19 mm, suggesting notable antifungal activity. The findings of this study corroborate the observational evidence thatGram-positive bacteria exhibit greater susceptibility to plant Extracts compared to Gram-negative bacteria. These disparities can be attributed to the structural differences in cell walls Gram-positive bacteria possess a single-layered cell wall, while Gram-negative bacteria have a multilayered structure. Consequently, the passage of active compounds through the Gram-negative cell wall may be hindered (28). Gram-Negative Bacteria possess an effective permeability barrier in the form of a thin lipopolysaccharide exterior membrane, which restricts the penetration of plant extracts. While Gram-positive bacteria possess a mesh-like peptidoglycan layer, this structure renders it more susceptible to permeation by the extracts (29). The outer membrane of Gram-negative bacteria has narrow porin channels within this membrane which limits the penetration of hydrophobic molecules. Moreover, the limited fluidity of the lipopolysaccharide leaflet impedes the inward diffusion of lipophilic products. Additionally, Gram-negative bacteria exhibit high intrinsic resistance due to the presence of efflux systems in their outer membrane, which limits antimicrobial diffusion into the cells (30).

Our results are in accordance with Krishnaveni who exhibited that the *Vetiveria zizanioides* extract, at a concentration of 50%, exhibits strong antibacterial activity against the positive pathogenic microorganism *Staphylococcus aureus*, surpassing its effect on the negative pathogenic microorganism *Escherichia coli* (19). But in another study by Subhadra Devi et al, contrary to our findings, exhibited that ethanolic extract of *Vetiveria zizanioides* inhibited gram negative bacteria than gram positive bacteria (20). Yet in another study by Muthukrishnan et al the antimicrobial activity

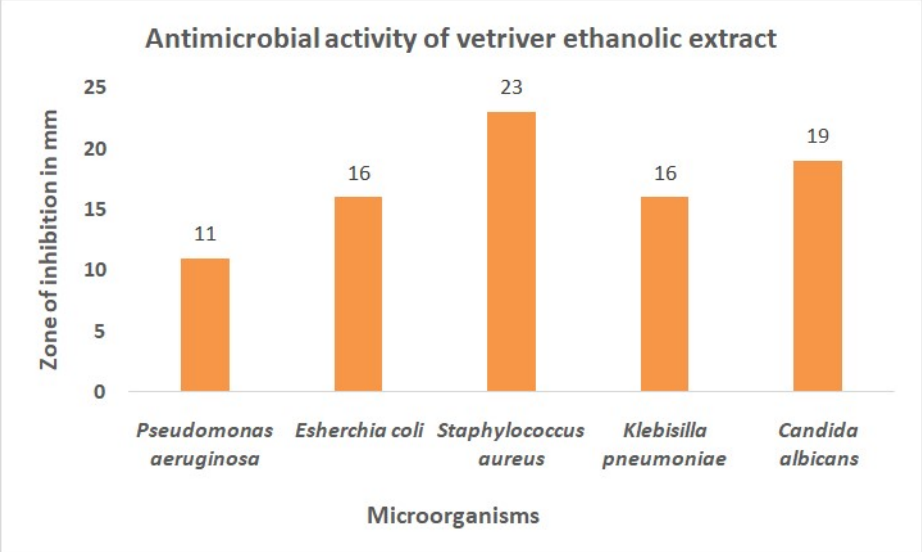

Figure 2: Antimicrobial activity of *Vetiveriazizanioides*root extract

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

of *Vetiveria zizanioides* chloroform extract showed the maximum zone of inhibition for *E. coli, S. aureus*, and *P. aeruginosa* was found to be 20 mm, 24 mm and 25 mm respectively. Whereasf or *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus faecalis,* and *Enterococcus faecalis*, the zone of inhibition measured 21 mm. *Candida albicans* exhibited a 28 mm zone of inhibition, while against *Cryptococcus neoformans*, it was 22 mm (31). These results were similar to our research outcomes.

Minimum Inhibitory Concentration (MIC) of *Vetiveria zizanioides* ethanolic extract

The Minimum Inhibitory Concentration (MIC) of *Vetiveria zizanioides* ethanolic extract is depicted in Table 2. The results indicate variations in the sensitivity of different microorganisms to the *Vetiver* ethanolic extract. *Escherichia coli* and *Staphylococcus aureus* are common Gram-negative and Gram-positive bacteria, respectively, associated with various infections. A MIC of 12.5 mg/mL for both indicates that the extract is more potent against these bacteria suggesting higher susceptibility compared to the other tested pathogens. It suggests a higher efficacy, as lower concentrations (12.5 mg/mL) are sufficient to inhibit their growth. *Pseudomonas aeruginosa* is a notoriously resilient Gram-negative bacterium often associated with nosocomial infections and antibiotic resistance. *Klebsiella pneumoniae* is another Gram-negative bacterium frequently implicated in hospital-acquired infections. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed a MIC of 50mg/ml, indicating a moderate inhibitory effect. The extract has the ability to inhibit both gram positive bacteria and gram-negative bacteria. For *Candida albicans*, the MIC is 25 mg/mL. This suggests that this fungus is moderately sensitive to the extract, requiring a slightly higherconcentration (25 mg/mL) compared to *E. coli* and *S. aureus*, but lower than *P. aeruginosa and K. pneumoniae.*The extract's ability to inhibit both bacteria *(P. aeruginosa, E. coli, S. aureus, K. pneumoniae*) and a fungus (*C. albicans*) suggests a broad-spectrum antimicrobial potential, making it versatile for different types of infections.

Mahajan et al. reported MIC values against *Staphylococcus aureus* and *Pseudomonas aeruginosa* ranging from 6.25 to 25 mg/ml. In our study, the MIC was 12.5 mg/ml and 50 mg/ml, respectively, against all biofilm-producing microorganisms (32). Meanwhile, Priya et al. found a minimum inhibitory concentration of 12.5 mg/ml for *Staphylococcus aureus*, whereas for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, the MIC was 25 mg/ml (21), which is similar to our findings. In our study *Staphylococcus aureus* exhibited the same MICbut for *Pseudomonas aeruginosa*, the MIC was 50mg/ml which is slightly higher compared to their result. Similarily, yet in another study by Alireza et al., the reported minimum inhibitory concentration (MIC) was 25mg/mL for *Staphylococcus aureus* and 50mg/mL for *Pseudomonas aeruginosa* (33), findings that align with our own research outcomes. In our study *Pseudomonas aeruginosa*, the MIC was 50 mg/ml which is same but for *Staphylococcus aureus* it is slightly lower compared to their result. It suggests a higher

Table2: Minimum Inhibitory Concentration (MIC) of *Vetiveriazizanioides*ethanolic extract

Microorganism	Minimum Inhibitory Concentration (MIC)
Pseudomonasaeruginosa	50mg/mL
Escherichia coli	12.5mg/mL
Staphylococcus aureus	12.5mg/mL
Klebsiellapneumoniae	50mg/mL
Candida albicans	25 mg/mL

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

efficacy, as lower concentrations (12.5 mg/mL) are sufficient to inhibit their growth.

Antioxidant activity using DPPH radical scavenging activity

The results demonstrate a concentration-dependent increase in DPPH inhibition percentage with escalating concentrations of Vetiver ethanolic extract as shown in Table 3. This suggests a positive correlation between the extract's concentration and its antioxidant activity. At the lowest concentration of 50 µg, the DPPH inhibition percentage was 56.25%, indicating a substantial ability to scavenge free radicals. As the concentration increased, reaching 80.25 % at 500 µg, the radical scavenging potential increased significantly.The antioxidant activity of standard Ascorbic acid also demonstrates a concentration-dependent increase in DPPH inhibition percentage. However, the antioxidant activity of the extract is lower than standard Ascorbic acid. The antioxidant activity of the extract may be due to the presence of phytochemicals such as 17-hydroxy-3,20-dioxopregna-1,4,ol, Z,z-8,10-hexadecadien-1-ol,Formicacid, Octadecanoic Acid,Cedren-13-ol and, Campesterol. It is thought to do this by scavenging free radicals and by protecting cells from oxidative damage. The antioxidant potency of Vetiver oil is attributed to cedr-8-en-13-ol, its most abundant compound. This compound restores cellular levels of SOD (superoxide dismutase), GPX (glutathione peroxidase), and CAT (catalase) activities (34). Campesterol functions as an antioxidant by elevating phenolic and flavonoid levels, neutralizing free radicals through electron-proton transfer processes, enhancing DPPH scavenging, chelating metals, and activating enzymes like catalase, superoxide dismutase, and glutathione peroxidase. Additionally, it inhibits oxidases (35). Researcher Muthukrishnan & Manogaran has demonstrated that *Vetiveria zizanioides* is a source of various phytochemicals with antioxidant capabilities, including alkaloids, flavonoids, tannins, saponins, and phenols. The presence of these compounds enhances the plant's ability to neutralize free radicals, thereby under scoring its significant antioxidant properties (31). The findings of Subhadra devi study align with the observational evidence, similar to our own results. They have shown that the ethanolic extract of *Vetiveria zizanioides* roots exhibits potent antioxidant activity across various in vitro assays, including reducing power, superoxide anion radical scavenging, deoxyribose degradation, total antioxidant capacity, total phenolics, and total flavonoid composition. Moreover, the extract effectively scavenges free radicals such as O_2, H_2O_2, OH, and NO in a dose-dependent manner (36). Similarly, in a study Luqman et al compared two *Vetiveria zizanioides* genotypes, KS1 exhibited superior antioxidant properties, including higher ferric reducing antioxidant power (FRAP), DPPH inhibition, total phenolic content (TPC), and reducing power (RP) potential compared to the gulabi genotype. As the extract

Table3: Antioxidant activity using DPPH radical scavenging activity

Concentrationinmicrogram	DPPHinhibitionpercentageofAscorbicacid	DPPHinhibitionpercentageofVetriverextract
50	60.25±0.23	56.25±0.34
100	68.16±0.43	64±0.23
200	72.16±0.13	69.16±0.43
300	76.85±0.33	72.85±0.25
400	80.63±0.25	75.625±0.13
500	85.97±0.25	80.25±0.25
IC_{50}	117.80	125.07

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

concentration increased, antioxidant activity also increased (37), which were similar to our findings. Antioxidants play a crucial role in mitigating oxidative stress, which is implicated in various diseases. The observed antioxidant potential of Vetiver ethanolic extract suggests its possible role in promoting health by counteracting oxidative damage.

Anti-inflammatory activity by Protein Denaturation Assay

The denaturation of proteins is one of the causes of inflammation. The Vetiver ethanolic extract exhibited a significant anti-inflammatory activity as shown in Figure 3.

The results demonstrate a clear dose-response relationship between the concentration of Vetiver ethanolic extract and the inhibition percentage. As the concentration increased, there was a corresponding increase in inhibitory activity. At the lowest concentration of 50 µg, the inhibition was 39.14%, indicating a moderate inhibitory effect. However, as the concentration increased to 500 µg, the inhibition reached 82.61%,demonstrating a substantial enhancement in inhibitory activity. The concentration of 500 µg resulted in an inhibition percentage of 82.61%, approaching the positive control's inhibition of 86.96%. These results were comparable to those of diclofenac sodium (200 µg/ml) as mentioned in Figure 3. This suggests that the extract's inhibitory potential may reach a plateau beyond a certain concentration. Campesterol, regulates interleukins and immune responses, by effectively modulating both pro-inflammatory and anti-inflammatory cytokines (38) The phytochemical present in the extract stimulates the antioxidant enzyme system, reduces the oxidative stress and maintains a balance between oxidants and antioxidants, which is crucial in combating inflammation (18). A study conducted by Su-Tze Chou et al on the essential oil of *Vetiveria zizanioides* (VZ-EO) exhibited anti-inflammatory properties by modulating the expression of inflammation-related enzymes (HO-1, iNOS, and COX-2) and cytokines (TNF-α, IL-1β, and IFN-β) in LPS-stimulated RAW 264.7 macrophages. Additionally, VZ-EO's antioxidant activity contributes to its anti-inflammatory effects, as it reduces LPS-induced superoxide anion production and

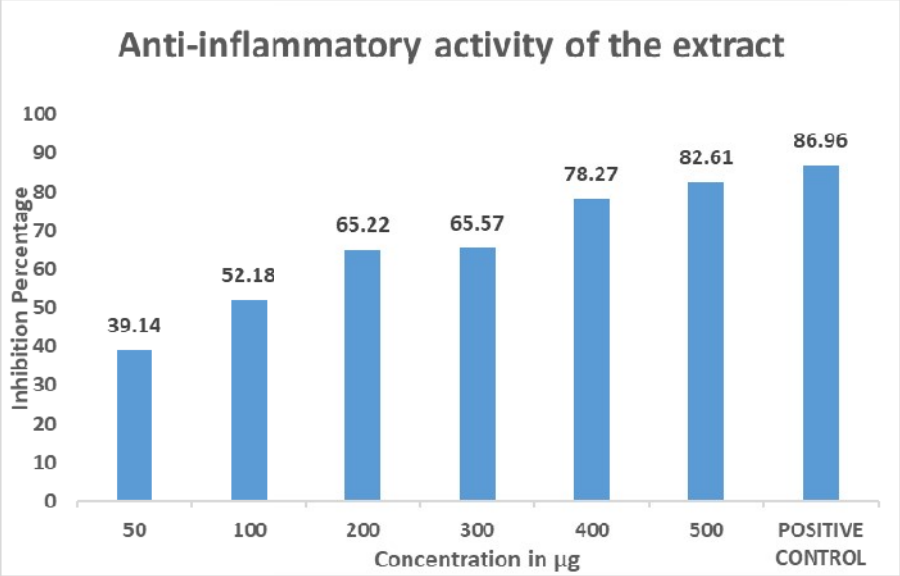

Figure3: Anti-inflammatory activity of *Vetiveriazizanioides*ethanolic extract

Antibiofilm Activity of *Vetiveria zizanioides*

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

malondialdehyde(MDA)levels (39).In another study by Mohammad et al the ethanol extracts of *H. procumbens* and *U. dioica*, both individually and in combination, were assessed for their anti-denaturation effects using an albumin denaturation assay. The results demonstrated concentration-dependent inhibition of protein (albumin) denaturation by these extracts (40), which were similar to our findings. In a study the essential oil of *Vetiveria zizanioides* was evaluated using standard inflammation models, including acetic acid-induced writhing, carrageenan-induced paw edema, and the formalin test. The oil demonstrated effects similar to aspirin, reducing abdominal writhing, formalin-induced pain, and carrageenan-induced inflammation. This suggests its analgesic and anti-inflammatory potential. The proposed mechanism, based on the study's results, involves the inhibition of leukocyte migration to the inflammation site, preventing the progression and chronicity of inflammation, indicating its broader anti-inflammatory applications(41). While the ethanolic vetiver extract may exhibit anti-inflammatory properties, it is essential to recognize and address its limitations, which includes potency, mechanism of action, safety profile, bioavailability, and clinical evidence. These considerations are crucial for assessing its suitability and potential in therapeutic applications for inflammation.

Biofilm Formation

Adhesion to a biotic or abiotic surface is considered to be the initial step in the formation of biofilm by microorganisms. The results indicate varying levels of biofilm formation among the tested microorganisms as shown in Figure 4.

Staphylococcus aureus exhibited the highest biofilm formation rate, suggesting a higher risk of persistent infections associated with this pathogen. The high biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa* underscores the clinical challenges posed by these pathogens in terms of treatment resistance and recurrence. *Klebsiella pneumoniae* and *Candida albicans* exhibited a moderate biofilm formation rate indicating an intermediate ability to form biofilms compared to other microorganisms. *Escherichia coli* demonstrated a lower ability to form biofilm, aligning with its usual presentation as an acute, rather than chronic, infection.

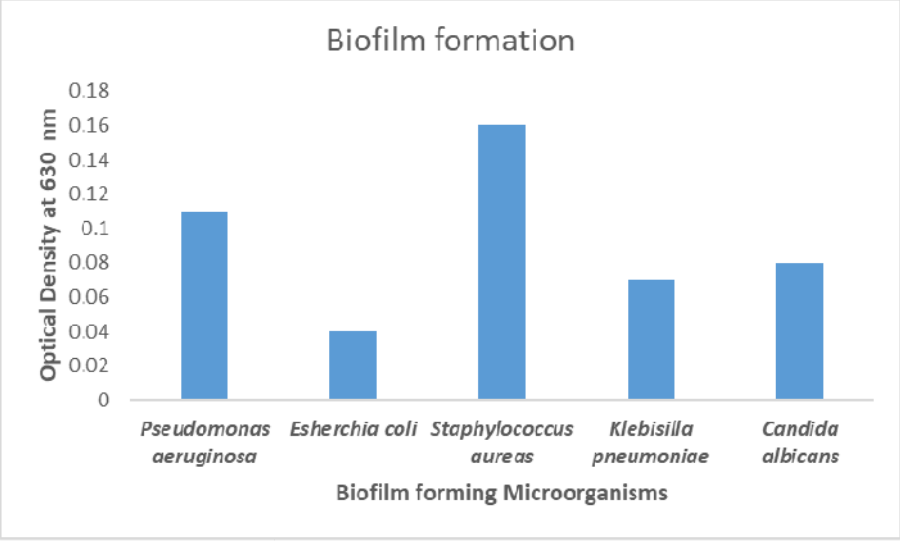

Figure4: Biofilm formation by test microorganisms

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

Nair and his colleagues selected 3 species frequently associated with denture stomatitis, namely *Candida albicans, Staphylococcus aureus* and *Streptococcus mutans* for their biofilm forming capability for the experiment. The study demonstrated that both fungal and bacterial species can coexist and form biofilms in the same environment (42). The result coincides with our outcome. Similar to our findings, in a separate investigation, *Candida albicans*, in conjunction with *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, demonstrated the ability to form biofilms. *C. albicans* engages in physical attachment, extracellular signaling, and metabolic cross-feeding interactions with co-existing oral bacteria. These multidimentional interactions contribute to the complex biofilm architecture (43).

The equilibrium between the host and the microbiome relies on multiple factors, including the lifestyle, host's genotype, physiology and immune system efficiency (44). Additionally, the preferences of the inhabited niche, such as variations in pH, temperature, and nutrient availability, play a significant role. These factors collectively influence the biofilm formation capabilities of these microorganisms and composition of the microflora (45).

Anti-Biofilm activity of *Vetiveria zizanioides* ethanolic root extract

The study shows that the *Vetiveria zizanioides* ethanolic root extract is capable of inhibiting the biofilm formation as depicted in Figure 5. The varying degrees of biofilm inhibition highlight the microorganism-specific response to the Vetiver ethanolic extract. The highest inhibition observed against *Staphylococcus aureus* and *Escherichia coli* suggests a potent impact on both Gram-positive and Gram-negative bacteria. The substantial biofilm inhibition against *Staphylococcus aureus* is particularly relevant due to the role of this pathogen in chronic infections. The extract's ability to inhibit biofilm formation may contribute to mitigating the challenges posed by persistent infections *Candida albicans*, a fungal pathogen causing oral and genital infections,

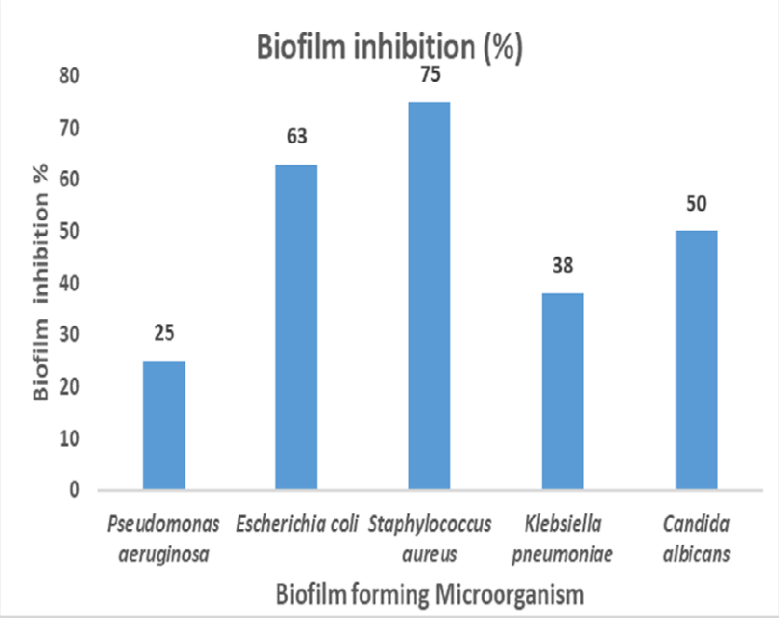

Figure5: Anti-Biofilm activity of *Vetiveriazizanioides*ethanolic root extract

Antibiofilm Activity of *Vetiveria zizanioides*

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

displayed a moderate biofilm inhibition of 50%. The observed biofilm inhibition activity against *Candida albicans* suggests the extract's antifungal potential. This is significant, considering the increasing prevalence of fungal infections and the role of biofilms in their pathogenesis.

Many crude plant extracts were capable of inhibiting biofilm formation by *Staphylococcal sp*. such as *Quercus cerris, Alnus japonica, Rubus ulmifolius, Coriandrum sativum, Mentha piperita and Pimpinella anisum* (46-49). In another study, by Muhammad Shahid et. al. the methanolic extract of *Elettaria Cardamomum* and *Cichorium Intybus* exhibited the highest inhibition of biofilm formation against *Staphylococcus aureus* (55.92%), while the lowest inhibition was observed with the aqueous extract of *Cichorium Intybus* (AqEC) against *Escherichia coli* (20%) (50), which were similar to our findings. Elmasri et al demonstrated that sesquiterpenes found in the plant *Teucrium polium* play a crucial role in inhibiting biofilm formation in *Staphylococcus aureus*. These sesquiterpenes achieve this effect by modulating the membrane fatty acid composition (51). Similarly, *V. zizanioides* root extract (VREX) prevents the biofilm formation of MRSA and its clinical strains by down regulating of the genes responsible for adhesion (27).

In our study the phytochemicals present in*Vetiveria zizanioides* roots extract represent a mix of steroids, fatty alcohols, carboxylic acids, sterols, and sesquiterpenes. To inhibit biofilm growth, various compounds utilize six primary mechanisms: substrate deprivation, membrane rupture, binding to adhesion complexes and cell walls, protein binding, interaction with eukaryotic DNA, and inhibition of viral fusion (52). Active secondary metabolites found in both aqueous and alcoholic extracts specifically target one or more stages of biofilm formation, thereby reducing bacterial virulence associated with biofilm production. The distinct modes of action of each compound contribute to the extract's antibacterial and antibiofilm properties. Phenolic compounds can damage cell structure and membranes, inhibiting bacterial protein synthesis. Flavonoids, with antibacterial properties disrupt cell wall formation by suppressing cytoplasm function, interrupt nutrient exchange, thereby inhibiting bacterial energy supply, inhibit enzymes that produce quorum-sensing signals, disrupting cell communication during biofilm formation. Steroids cause lysosome leakage and membrane phospholipid disruption, reducing cell membrane integrity and leading to cell lysis (53). Sesquiterpenes, natural compounds, exhibit versatile mechanisms in inhibiting biofilm formation by interfering with initial adhesion thereby disrupting bacterial attachment to surfaces. Secondly, they modulate the gene expression by, downregulating adhesion-related genes (*sdrD*, *spa*, *agr*, *hld*) and upregulating capsular polysaccharide genes (*cap5B*, *cap5C*). Thirdly they disrupt cell membrane, modulate metabolic process and interfere with cell signalling (54). Protein denaturation through the use of proteinase K resulted in decreased biofilm thickness and modified composition. This approach holds potential as an adjunctive strategy for biofilm management by specifically targeting biofilm proteins (55). VREX demonstrates a comprehensive range of antibiofilm effects: it inhibits biofilm formationinmethicillin-resistant *Staphylococcus aureus* (MRSA) and its clinical counterparts, preserves cellular viability, exerts microscopic impact on biofilm structure, suppresses the synthesis of key biofilm components (α-hemolysin toxin, exopolysaccharide, and slime), regulates adhesin genes (clfA,fnbA,and fnbB,) responsible for initial attachment, and contains sesquiterpenes as major constituents(27). Thereby, the antibiofilm activity of *Vetiveria zizanioides* may be attributed to the presence of these phytochemicals in the ethanolic extract.

The reported scientific data supports the antibacterial and antifungal effects of *Vetiveria zizanioides*, suggesting it could prevent biofilm development by pathogenic entities. Biofilms resist typical antimicrobials,

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

posing a significant healthcare challenge. Vetiver extract's properties may inhibit bacteria and fungi in biofilms, reducing their formation and negative impact on host tissues. Furthermore, the antioxidant and anti-inflammatory properties of vetiver extract can contribute to mitigating the inflammatory response associated with biofilm infections, promoting tissue healing, and reducing the risk of complications. Consequently, by targeting biofilm-forming microorganisms and modulating the host immune response, vetiver extract shows promise as a natural remedy with potential antibiofilm activity, offering a multifaceted approach to combating biofilm-related infections. The current research suggests that it could serve as mouth rinses, irrigation solutions, and intracanal medicaments. These applications are vital for the dental industry, particularly in developing effective plaque-control strategies (56).

Vetiveria zizanioides, while showing potential as an antibiofilm agent, faces several challenges. These include a lack of extensive clinical evidence, a narrower activity spectrum, difficulties in standardization and quality control, the potential for resistance development, absence of regulatory approval, and undefined dosing guidelines. Addressing these issues through further research and clinical trials is crucial for its potential use against biofilm-associated infections.

Conclusion

In this study ethanolic extract of*Vetiveria zizanioides* was studied for its phytochemical constituents by using GC MS analysis. 17 compounds were identified from *Vetiveria zizanioides* roots extract. They represent a mix of steroids, fatty alcohols, carboxylic acids, sterols, and sesquiterpenes. This diversity in chemical nature contributes to the extract's multifaceted biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antibiofilm properties. Each compound class may contribute to specific biological effects, and the synergistic interactions among these compounds enhance the overall efficacy of the *Vetiver* root extract. These compounds have been shown to interfere with the formation and structure of dental biofilms, and to inhibit the growth and metabolism of dental pathogens. The concentration-dependentresponses observed in antioxidant and inhibitory assays suggest the potential utility of *Vetiver* root extract in various applications, including pharmaceuticals, nutraceuticals, and functional foods. Hence it may be used in treating various biofilm-related infection. Despite the promise of *Vetiveria zizanioides* as an antibiofilm agent, it confronts obstacles such as insufficient clinical validation, limited antibacterial and antifungal range, standardizationchallenges, possible resistance emergence, lack of official sanction, and indeterminate dosage protocols. Future research is essential to ascertain its efficacy and safety for human use, which should encompass the isolation and refinement of active antibiofilm constituents, along with assessments of their ADMET profiles and long-term human impacts.

References

1. Kilian, M., Chapple, I. L. C., Hanning, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L., et al. (2016). The oral microbiome: An update for oral healthcare professionals. British Dental Journal, 221(10), 657–665.
2. Arzmi, M. H., Dashper, S., & McCullough, M. (2019). Polymicrobial interactions of *Candida albicans* and its role in oral carcinogenesis. Journal of Oral Pathology & Medicine, 48(7), 546-551.
3. Krzyściak, W., Jurczak, A., &Piątkowski, J. (2016). The role of human oral microbiome in dental biofilm formation. In Microbial Biofilms - Importance and Applications (pp. 229-382).
4. Su, Y., Yrastorza, J. T., Matis, M., Cusick, J., Zhao, S., Wang, G., & Xie, J. (2022). Biofilms: Formation, research models, potential targets, and methods for prevention and treatment. Advances in Science, 9(29), e22032912.
5. Deo, P. N., & Deshmukh, R. (2019). Oral microbiome: Unveiling the

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

fundamentals. Journal of Oral and Maxillofacial Pathology, 23, 122–128.
6. Chen, M., Yu, Q., & Sun, H. (2013). Novel strategies for the prevention and treatment of biofilm-related infections. International Journal of Molecular Sciences, 14(9), 18488–18501.
7. Milho, C., Silva, J., Guimarães, R., Ferreira, I. C. F. R., Barros, L., & Alves, M. J. (2021). Antimicrobials from medicinal plants: An emergent strategy to control oral biofilms. Applied Sciences, 11, 40205.
8. Gupta, P., Sarkar, S., Das, B., Bhattacharjee, S., &Tribedi, P. (2016). Biofilm, pathogenesis, and prevention – A journey to break the wall: A review. Archives of Microbiology, 198(1), 1–156.
9. Soria, S., Angulo-Bejarano, P. I., & Sharma, A. (2020). Biofilms: Development and molecular interaction of microbiome in the human oral cavity. Current Research and Future Trends in Microbial Biofilms, 61–75.
10. Jamal, M., et al. (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81, 7–118.
11. Brown, M. R. W., & Barker, J. (1999). Unexplored reservoirs of pathogenic bacteria: Protozoa and biofilms. Trends in Microbiology, 7(1), 46-50.
12. Burmølle, M., Thomsen, T. R., Fazli, M., Dige, I., Christensen, L., Homøe, P., et al. (2010). Biofilms in chronic infections - a matter of opportunity - monospecies biofilms in multispecies infections. FEMS Immunology and Medical Microbiology, 59(3), 324-336.
13. Stewart, P. S., & Bjarnsholt, T. (2020). Risk factors for chronic biofilm-related infection associated with implanted medical devices. Clinical Microbiology and Infection, 26(8), 1034-1038.
14. Fulgêncio, D. L. A., da Costa, R. A., Guilhelmelli, F., Silva, C. M. S., Ortega, D. B., de Araujo, T., et al. (2021). In vitro antifungal activity of pelgipeptins against human pathogenic fungi and *Candida albicans* biofilms. AIMS Microbiology, 7(1), 28-39.
15. Percival, S. L., Suleman, L., Vuotto, C., & Donelli, G. (2015). Healthcare-associated infections, medical devices, and biofilms: Risk, tolerance, and control. Journal of Medical Microbiology, 64, 323–334.
16. Dos Santos, D. S., et al. (2014). Seasonal phytochemical study and antimicrobial potential of *Vetiveria zizanioides* roots. Acta Pharmaceutica, 64, 495–501.
17. Huang, J., Li, H., Yang, J., Chen, Y., Liu, Y., Li, N., et al. (2004). Chemical components of *Vetiveria zizanioides* volatiles. Ying Yong Sheng Tai Xue Bao, 15(1), 170-172.
18. Grover, M., Behl, T., Bungau, S., & Aleya, L. (2021). Potential therapeutic effect of *Chrysopogon zizanioides* (Vetiver) as an anti-inflammatory agent. Environmental Science and Pollution Research, 28(28), 155.
19. Krishnaveni, V. (2016). Analysis of chemical components and antimicrobial activity on vetiver extract for home textile applications. Journal of Textile Science and Engineering, 6, 259.
20. Subhadra Devi V, K Asok Kumar, M. Uma Maheswari, A T Siva Shanmugam, & R Sankar Anand Devi et al. (2010). In vitro antibacterial activity of ethanolic extract of *Vetiveria zizanioides* root. International Journal of Pharmaceutical Sciences and Research, 1(9), 120-124.
21. Priya Mehrishi, Priti Agarwal, Shobha Broor, & Amisha Sharma (2020). Antibacterial and antibiofilm properties of medicinal plant extracts against multi-drug resistant Staphylococcus species and non-fermenter bacteria. Journal of Pure and Applied Microbiology, 14(1), 403-413.
22. Noor, N., Sarfraz, R. A., Ali, S., & Shahid, M. (2014). Antitumor and antioxidant potential of some selected Pakistani honeys. Food Chemistry, 143, 362–366.
23. Williams, L. A., O'Connor, A., Latore, L., Dennis, O., Ringer, S., Whittaker, J. A., et al. (2008). The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat-treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds,

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

without the use of animals, in the early stages of the drug discovery process. West Indian Medical Journal, 57, 327–3.

24. Du, L. (2012). Toxicity Mechanism of Formic Acid is Directly Linked to ROS Burst and Oxidative Damage in Yeast Saccharomyces cerevisiae. In Advanced Materials Research (Vols. 550–553, pp. 1060–1065).

25. Lastauskienė, E., Zinkevičienė, A., Girkontaitė, I., et al. Formic Acid and Acetic Acid Induce a Programmed Cell Death in Pathogenic *Candida* Species. Curr Microbiol 69, 303–310.

26. Masayuki Taniguchi, Akihito Ochiai, Kiyoshi Takahashi, Shunichi Nakamichi, Takafumi Nomoto, Eiichi Saitoh, et al. (2015). Antimicrobial activity and mechanism of action of a novel cationic α-helical octadecapeptide derived from α-amylase of rice. Biopolymers, 104(2), 73-83.

27. Kannappan, A., Gowrishankar, S., Srinivasan, R., Pandian, S. K., & Ravi, A. V. (2017). Antibiofilm activity of *Vetiveria zizanioides* root extract against methicillin-resistant *Staphylococcus aureus*. Microbial Pathogenesis, 110, 313-324.

28. Premjanu, N., & C. Jaynthy. (2017). Identification and characterization of antimicrobial metabolite from an endophytic fungus, Colletotrichum gloeosporioides, isolated from Lannea corammendalica. International Journal of ChemTech Research, 7(1), 369-374.

29. Rameshkumar, K. B., George, V., &Shiburaj, S. (2007). Chemical constituents and antibacterial activity of the leaf oil of cinnamomumchemungianum Mohan et Henry. Journal of Essential Oil Research, 19(1), 98–100.

30. Simões, M., Bennett, R. N., & Rosa, E. A. S. (2009). Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. Natural Product Reports, 26, 746–757.

31. Suriyavathana Muthukrishnan & Punithavathi Manogaran (2018). Phytochemical analysis and free radical scavenging potential activity of *Vetiveria zizanioides* Linn. Journal of Pharmacognosy and Phytochemistry, 7(2), 1955 -1960.

32. Maharjan, D., Singh, A., Lekhak, B., Basnyat, S., & Gautam, L. S. (2011). Study on antibacterial activity of common spices. Nepal Journal of Science and Technology, 12, 312-317.

33. Vasiee, A., Tabatabaei Yazdi, F., & Mortazavi, S. A. (2016). The antibacterial activity of Coriandrum (Coriandrum sativum) on pathogenic microorganisms “In vitro”. Iranian Journal of Infectious Diseases and Tropical Medicine, 20(71), 59-66.

34. Hsin-Yi, P., Lai, C.-C., Lin, C.-C., & Chou, S.-T. (2014). Effect of *Vetiveria zizanioides* essential oil on melanogenesis in melanoma cells, downregulation of tyrosinase expression, and suppression of oxidative stress. Scientific World Journal, 2014, 1–9.

35. Sundaram Sanjay, S., Shukla, A. K. (2021). Mechanism of Antioxidant Activity. In: Potential Therapeutic Applications of Nano-antioxidants. Springer, Singapore. Pp. 83-99.

36. Subhadradevi, V., Asokkumar, K., Umamaheswari, M., Sivashanmugam, A., &Sankaranand, R. (2010). In vitro antioxidant activity of *Vetiveria zizanioides* root extract. Tanzanian Journal of Health Research, 12(4), 274-279.

37. Luqman, S., Kumar, R., Kaushik, S., Srivastava, S., Darokar, M. P., & Khanuja, S. P. (2009). Antioxidant potential of the root of *Vetiveria zizanioides* (L.) Nash. Indian Journal of Biochemistry and Biophysics, 46(1), 122-125.

38. Nazir, S., Chaudhary, W. A., Mobashar, A., Anjum, I., Hameed, S., & Azhar, S. (2023). Campesterol: A Natural Phytochemical with Anti Inflammatory Properties as Potential Therapeutic Agent for Rheumatoid Arthritis: A Systematic Review. Pakistan Journal of Health Sciences, 4(05), 1-8.

39. Chou, S.-T., Lai, C.-P., Lin, C.-C., & Shih, Y. (2012). Study of the chemical composition, antioxidant activity, and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. Food Chemistry, 134, 262–268.

Vol. 18 (Supplementry Issue 4A) 88 - 105, November 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.4s.8

40. Haghparast Cheshmeh Ali, M. R., Khosroyar, S., & Mohammadi, A. (2018). Evaluation of in vitro anti-inflammatory activity of Harpagophytum procumbens and Urtica dioica against the denaturation of protein. Plant Archives, 18(1), 161-166.
41. Lima, G. M., Quintans-Júnior, L. J., Thomazzi, S. M., Almeida, E. M. S. A., Melo, M. S., Serafini, M. R.et al (2012). Phytochemical screening, antinociceptive and [sic]. Revista Brasileira de Farmacognosia, 22(2), 443-450.
42. Nair VV, Karibasappa GN, Dodamani A, Prashanth VK (2016) Microbial contamination of removable dental prosthesis at different interval of usage: An in vitro study. J Indian Prosthodont Soc 16: 346-351.
43. RapalaKozik, M., Surowiec, M., Juszczak, M., Wronowska, E., Kulig, K., Bednarek, A.. et al (2023). Living together: The role of *Candida albicans* in the formation of polymicrobial biofilms in the oral cavity. Yeast, 40, 303–317.
44. Rizzetto, L., De Filippo, C., & Cavalieri, D. (2014). Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease. European Journal of Immunology, 44(11), 3166–3181.
45. Zheng, D., Liwinski, T., &Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. Cell Research, 30(6), 492–506.
46. Bazargani, M. M., & Rohloff, J. (2016). Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control, 61, 156-164.
47. Hobby, G., Quave, C., Nelson, K., Compadre, C., Beenken, K., & Smeltze, M. (2012). Quercus cerris extracts limit *Staphylococcus aureus* biofilm formation. Journal of Ethnopharmacology, 144, 812–815.
48. Lee, J-H., Park, J-H., Cho, H., Joo, S., Cho, M., & Lee, J. (2013). Anti-biofilm activities of quercetin and tannic acid against *Staphylococcus aureus*. Biofouling, 29, 491–9.
49. Quave, C., Estévez-Carmona, M., Compadre, C., Hobby, G., Hendrickson, H., Beenken, K., et al. (2012). Ellagic acid derivatives from Rubus ulmifolius inhibit *Staphylococcus aureus* biofilm formation and improve response to antibiotics. PLoS ONE, 7(1), e28737.
50. Shahid, M., Kashif, M., Khan, K., Rehman, A. U., Anjum, F., Abbas, M., & Ali, M. (2022). Anti-Inflammatory and Biofilm Inhibition Potential of Methanolic and Aqueous Extract of Elettaria Cardamomum and Cichorium Intybus. Journal of Biological Regulators and Homeostatic Agents, 36(5), 1281-1289.
51. Elmasri, W. A., Hegazy, M. E., Aziz, M., Koksal, E., Amor, W., Mechref, Y., et al. (2014). Biofilm blocking sesquiterpenes from Teucrium polium. Phytochemistry, 103, 107–113.
52. Lu, L., Hu, W., Tian, Z., Yuan, D., Yi, G., Zhou, Y., et al. (2019). Developing natural products as potential anti-biofilm agents. Chinese Medicine, 14(1), 1-17.
53. Soesanto, S., Hepziba, E. R., Yasnill, &Widyarman, A. S. (2023). The Antibacterial and Antibiofilm Effect of Amoxicillin and *Mangifera indica* L. Leaves Extract on Oral Pathogens. Contemporary Clinical Dentistry, 14(2), 145-151.
54. Salinas, C., Florentín, G., Rodríguez, F., Alvarenga, N., & Guillén, R. (2022). Terpenes Combinations Inhibit Biofilm Formation in *Staphylococcus aureus* by Interfering with Initial Adhesion. Microorganisms, 10(8), 1527.
55. Karygianni, L., Paqué, P. N., Attin, T., & Thurnheer, T. (2021). Single DNase or Proteinase Treatment Induces Change in Composition and Structural Integrity of Multispecies Oral Biofilms. Antibiotics, 10(4), 400.
56. Karygianni, L., Cecere, M., Skaltsounis, A. L., Argyropoulou, A., Hellwig, E., Aligiannis, N et al. (2014). High-Level Antimicrobial Efficacy of Representative Mediterranean Natural Plant Extracts against Oral Microorganisms. BioMed Research International, 2014, 839019.