International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Special Issue 4, 2025

Original Article

ANTIMICROBIAL AND ANTI-BIOFILM EFFECTIVENESS OF *SAURAUIA VULCANI* KORTH. ETHANOLIC EXTRACT: AN *IN VITRO* STUDY OF *STAPHYLOCOCCUS AUREUS* ATCC®25923™ AND *CANDIDA ALBICANS* ATCC®10231™

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Received: 19 Apr 2025, Revised and Accepted: 18 Sep 2025

ABSTRACT

Objective: Removable and Fixed orthodontic appliances can increase the risk of bacterial and fungal infections when there is poor plaque control. The study aims to assess the antimicrobial and anti-biofilm effectiveness of *Saurauia vulcani* Korth. Ethanol Extract of Leaves (SVEL) against microbes, i. e, *Staphylococcus aureus (S. aureus)* and *Candida albicans (C. albicans).*

Methods: The phytochemical screening and Total Phenolic Content (TPC) of SVEL were determined and simultaneously analyzed using qualitative analysis, as per the Folin-Ciocalteu method. The antimicrobial test consists of the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentration (MBC/MFC), and analysis of membrane leakage from Nucleic Acids, proteins, Calcium (Ca^{2+}), and Potassium (K^{+}) in the microbes. The effectiveness of the anti-biofilm test was also analyzed by using the crystal violet solution.

Results: The SVEL presented flavonoids, glycosides, tannins, saponins, and steroids/triterpenoids. TPC was 150,61±0,53 mg (GAE/g). The MIC value of SVEL against *S. aureus* was obtained at 12.5 mg/ml, and against *C. albicans* at 3.125 mg/ml. The MBC was obtained at 100 mg/ml, while the MFC of C. *albicans* was obtained at 50 mg/ml. DNA and protein, as well as Ca^{2+} and K^{+} leakage tests, revealed that the leakage value increased with the SVEL concentration, but remained below that of the positive controls (Listerine Green Tea and CHX/Ketoconazole). In anti-biofilm testing, the Minimal Biofilm Inhibition Concentration (MBIC) was determined at 100 mg/ml.

Conclusion: SVEL is effective as an antimicrobial and anti-biofilm agent against *S. aureus* and *C. albicans.*

Keywords: *Saurauia vulcani*, *Staphylococcus aureus*, *Candida albicans*, Minimum inhibitory concentration, Membrane leakage, Antibiofilm

DOI: https://dx.doi.org/10.22159/ijap.2025.v17s4.07 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Acrylic removable orthodontic appliances for young patients and fixed orthodontic appliances for adult patients are susceptible to attachment by microorganisms, including bacteria and fungal infections. In fixed orthodontics, plaque is quickly trapped between the wire, the elastomeric material, and the bracket. *S. aureus* (43.2%) and *C. albicans* (35%) were found in higher percentages than other microorganisms in the oral environment, followed by *Streptococcus* species (5.8%), and Fungi (*C. tropicalis* (23.7%) [1]. The higher colonizers of *Staphylococcus spp.* and *Candida spp.,* found in gingival periodontitis disease, are considered normal flora microbes in the oral environment [2]. Decreased salivary flow rate, acidic pH levels during sleep, and poor oral and dental hygiene in orthodontic patients can lead to infections in the oral cavity, such as oral candidiasis. Somehow, mouthwash or antiseptic gel is required as an additional plaque control, in place of toothbrushing.

Using natural ingredients, aloe vera, which had a comparable result to medicine, was considered safer and had fewer side effects than chemical drugs invented in mouthwash or toothpaste [3-5]. *Saurauia vulcani* Korth. Ethanolic Leaves Extract (SVEL) is one of the 239 medicinal herbs from the Simalungun Bataknese ethnic [6]. The results of the phytochemical analysis revealed that the SVEL secondary metabolites are flavonoids, tannins, glycosides, saponins, and steroids/triterpenoids, which function as antimicrobials and other medicinal agents [7].

The study's purpose is to observe the antimicrobial and anti-biofilm effectiveness of the SVEL as a natural product against *S. aureus* and *C. albicans,* which are considered to be used as a mouthwash to control the oral pathogens which were found in removable or fixed orthodontic patient oral health.

MATERIALS AND METHODS

Preparation, identification, and ethanolic extract preparation of *Saurauia vulcani* Korth. leave (SVEL)

SVEL was collected at a wildscape from Semangat Gunung Village, Karo, Tiga Panah, North Sumatera, Indonesia, with *Global Positioning System (GPS)* 3°13'28.6" N 98°31'05.9" E (3.224604, 98.518310). It was identified at The Herbarium Medanense (MEDA), Universitas Sumatera Utara, with batched No. 6647/MEDA/2021. This study was approved by the Ethical Committee of Health Research, Universitas Sumatera Utara, which announced the study approval with letter No.1017/KEPK/USU/2022.

The 1 kg SVEL powder were dissolved in 7.5 liters of 96% ethanol solvent for three days. The maceration results were filtered twice and evaporated until a concentrated extract was obtained. Extract dilutions were prepared with dimethylsulfoxide (DMSO) to obtain various concentrations, i. e., 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml, and 300 mg/ml.

Preparation of *S. aureus* and *C. albicans*

Staphylococcus aureus ATCC® 25923™ was inoculated into a tube containing 10 ml of 0.75% NaCl in 9 ml Mueller-Hinton Broth (MHB). The *Candida albicans* ATCC® 10231™ from Potato Dextrose Agar (PDA) was suspended in a tube containing 10 ml Potato Dextrose Broth (PDB). Both suspensions homogenized and equalized to a turbidity of 0.5 Mc. Farland. (1.5 x 106 CFU/ml). Each analysis was carried out in triplicates observation.

Total phenol content (TPC)

The TPC of the sample was evaluated using a combined mixture of 100 ml of SVEL (500 µg/ml) with 7.9 ml of distilled water and 0.5 ml of Folin-

Reagent Ciocalteu's (1:10 v/v). The mixture was then vortexed for 1 min. After mixing, 1.5 ml of 20% sodium bicarbonate solution in aqueous form was added. The resulting solution was then subjected to intermittent shaking and allowed to stand for 90 min. A Uv/V spectrophotometer at 775 nm was used to measure the absorbance. The total phenolic content was determined by calculating the amount of gallic acid equivalent in milligrams per g of extract. The methanol solution was utilized as a control in the experiment.

The phenol content was expressed in milligrams per g of extract, standardized to Gallic Acid Equivalents (GAE). The experimental procedures were conducted in triplicate. The following formula is used to calculate the total phenol content (TPC):

$$C(GAE)=\frac{c\times V}{M}\times F$$

Abbreviations:

C: (GAE) Phenolic content as a gallic acid equivalent

c: Concentration measured from a standard curve (µg/ml)

V: The volume utilized in the assay (ml)

M: Mass of the sample used in the experiment (g)

F: Dilution factor

Antimicrobial activity test

a) Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC)

An antimicrobial MIC test was carried out using the Kirby-Bauer disc diffusion test for six of the SVEL concentrations, DMSO (negative control), Listerine Green Tea, Ketoconazole, and Chlorhexidine 0,2% (CHX) (positive control) samples. The inhibition zone obtained will be measured with a caliper after the *S. aureus plates are* incubated at 37 °C and the *C. albicans* is incubated at 20 °C for 24 h.

Further analysis for MIC included a bactericidal/fungicidal test to determine the MBC and MFC using the agar dilution method. Each microbe was swabbed from the Kirby Bauer plates and immersed in 2 ml of Mueller-Hinton Broth (MHB) for *S. aureus* and Potato Dextrose Broth (PDB) for *C. albicans* for 10 min. One milliliter of MHB/PDB was added to 15 ml of Plate Count Agar (PCA) and incubated at 37 °C for 24 h. The number of colonies formed was measured using the colony counter machine afterward [8, 9].

b) Nucleic acid (NA) and protein leakage test

MHB medium was used for *S. aureus C. albicans,* and PDB was used for *C. albicans,* and both were filled with 1 ml in each labeled tube. Concentrations of SVEL of 50 mg/ml and 100 mg/ml, DMSO, Listerine Green Tea, CHX, and ketoconazole were analyzed, respectively. *S. aureus* and *C. albicans* were incubated using the same method as before. The suspension was centrifuged at 3500 rpm (20 min) to extract the supernatant liquid to be analyzed for the leakage of NA and protein from the microbes with a UV-Vis Spectrophotometer at a wavelength of 260 nm and 280 nm. The release of nucleic acids (DNA and RNA) and proteins indicates the occurrence of cell damage due to membrane leakage or a disturbance in cell wall permeability [10].

c) Calcium (Ca^{2+}) and potassium (K^{+}) leakage test

The supernatant liquids of *S. aureus and C. albicans* were obtained by the previous NA and protein leakage test, and were continuously added with Cerium trichloride ($CeCl_3$) solution for Ca^{2+}ion and Lanthanum oxide (La_2O_3) solution for K^{+}ion leakage test. Analysis used the Atomic Absorption Spectrophotometer (AAS, Z-2000 Hitachi) with wavelengths of 422.7 nm and 766.5 nm at the Industrial Chemical Technology Polytechnic Medan, Indonesia [11].

Anti-biofilm activity test

An anti-biofilm test was conducted of using 24-well polystyrene plates. Each 0.1 ml suspension of *S. aureus* or *C. albicans* was added to well plates containing MHB liquid medium for *S. aureus* and PDB for *C. albicans*. Then, 2 ml of SVEL with a concentration of 50 mg/ml, 100 mg/ml, DMSO, Listerine Green Tea, CHX for *S. aureus*, and ketoconazole for *C. albicans* were added. *S. aureus* was incubated at 37 °C while *C. albicans* were incubated at 20 °C for 24 h. The well plates were rinsed with aquabidest, and the crystal violet solution was added. After 15 min, the well plates were washed with aquabidest, and 96% alcohol was added to the well plates. Absorbance/Optical density (OD) was measured using a UV-Vis Spectrophotometer with a wavelength of 600 nm [12]. The percentage of biofilm eradication (%) was then calculated:

$$\text{Biofilm eradication }\% = \frac{(\text{Control OD} - \text{Test OD})}{\text{Control OD}} \times 10$$

Statistical analysis

The data were carried out in triplicate, and the statistics were analyzed using SPSS version 26.0. The Kruskal-Wallis test assessed the calcium, potassium leakage, and biofilm eradication. One-way ANOVA tests were used to evaluate the nucleic acid and the protein leakage.

RESULTS

The front color of the *Saurauia vulcani* Korth's leaves, which were extracted from the Semangat Gunung village, was dark green, and the back was hairy with a light brown color (fig. 1). The mean width of the leaves was 31,96±4,87 cm, and the length was 16,96±1,99 cm. It has a white flower and brown fruit with a kid-friendly taste. It is sweet and fragrant but slightly slimy (fig. 1).

The SVEL's phytochemical screening of this study showed the presence of flavonoids, glycosides, tannins, saponins, and steroids/triterpenoids. The TPC of the SVEL was 150,61±0,53 mg (GAE/g).

Antimicrobial activity test

a) Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC)

The antimicrobial activity test for MIC, MBC/MFC of SVEL was presented in fig. 2 and table 1. The SVELs MIC from the Kirby-Bauer disc diffusion test started at 12.5 mg/ml for *S. aureus* and 3.125 mg/ml for *C. albicans*. The MBC and MFC values of *S. aureus* and *C. albicans* with a reduction above 98% were at a concentration of 100 mg/ml and 50 mg/ml, respectively.

Table 1: Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of SVEL against *S. aureus* and *C. albicans*

Concentration (mg/ml)	Inhibitory zone (mm)		Colony number (CFU/ml)	
	S. aureus	*C. albicans*	*S. aureus*	*C. albicans*
DMSO	0,00±0,00	0,00±0,00	4300	2660
3,125	0,00±0,00	8,40±0,26	3970	2420
6,25	0,00±0,00	8,80±0,26	2880	1170
12,5	7,27±0,12	9,47±0,06	2390	89,8
25	7,93±0,12	9,53±0,26	1150	68
50	9,13±±0,49	9,50±0,26	113	53,3
100	9,90±0,17	10,63±0,06	52	25,7
200	10,60±0,10	12,20±0,20	0	12,8
300	11,63±0,38	13,53±0,29	0	6,44
Listerine	10,53±0,32	13,33±0,15	0	3,85
CHX	14,43±0,06	-	0	0
Ketoconazole	-	28,63±3,35	0	0

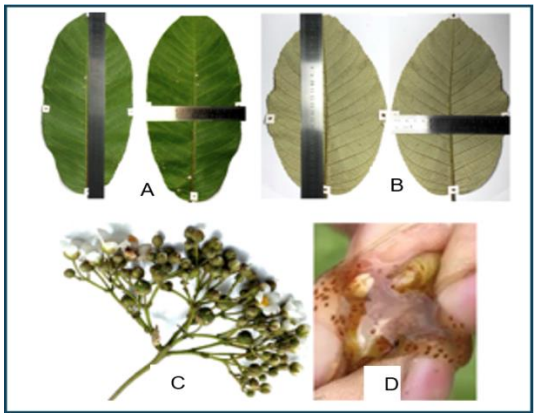

Fig. 1: *Saurauia vulcani* Korth. A and B. Front and back surfaces of a leaf. C. Flower of SVEL. D. Inside SVEL's fruit and seed

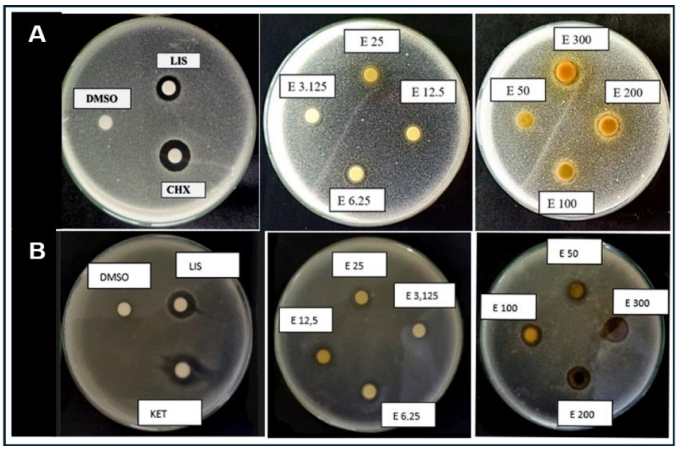

Fig. 2: Minimum inhibitory concentration (MIC) SVEL for six concentrations, DMSO, listerine green tea, ketoconazole (Ket), and chlorhexidine 0,2% (CHX), A. *S. aureus. B. C. albicans*

Table 2: Nucleic acid and protein leakage of SVEL against *Staphylococcus aureus* and *Candida albicans*

Microorganism	Concentration (mg/ml)	NA leakage (abs)	Protein leakage (abs)
S. aureus	DMSO	0,002±0,002	0,005±0,002
	50	0,105±0,003	0,162±0,002
	100	0,131±0,001	0,190±0,003
	Listerine	0,053±0,003	0,080±0,002
	CHX	0,088±0,001	0,123±0,002
p-value		0,009*	0,001*
C. albicans	DMSO	0,000±0,000	0,000±0,000
	50	0,085±0,006	0,114±0,006
	100	0,096±0,003	0,142±0,002
	Listerine	0,114±0,003	0,161±0,001
	Ketoconazole	0,125±0,003	0,174±0,003
p-value		0,009*	0,001*

Abbreviation: Abs: Absorbance. *p<0.05: significant difference according to the Kruskal-Wallis test for NA leakage and One-way ANOVA for Protein leakage.

Table 3: Calcium (Ca^{2+}) and potassium (K^{+}) leakage of SVEL against *Staphylococcus aureus* and *Candida albicans*

Microorganism	Concentration (mg/ml)	Calcium leakage (abs)	Potassium leakage (abs)
S. aureus	DMSO	0,068±0,007	0,060±0,001
	50	1,319±0,000	1,220 ±0,000
	100	2,112±0,000	1,954±0,000
	Listerine	14,052±0,000	0,774±0,000
	CHX	14,131±0,000	0,774±0,000
p-value		0,008*	0,008*
C. albicans	DMSO	0,086±0,021	0,000±0,000
	50	0,958±0,000	0,114±0,000
	100	1,610±0,000	0,142±0,000
	Listerine	2,980±0,000	0,161±0,000
	Ketoconazole	3,209±0,000	0,174±0,000
p-value		0,008*	0,008*

Abbreviation: Abs: Absorbance. *p<0.05: significant difference according to the Kruskal-Wallis test.

Table 4: Biofilm activity assay of SVEL against *S. aureus* and *C. albicans*

Microorganism	Concentration (mg/ml)	Biofilm eradication (%)
S. aureus	DMSO	0,00±0,00
	50	42,46±0,54
	100	56,44±0,41
	Listerine	52,35±0,43
	CHX	55,61±0,42
p-value		0,008*
C. albicans	DMSO	0,00±0,00
	50	23,65±0,14
	100	50,12±0,00
	Listerine	64,89±0,00
	Ketoconazole	75,54±0,00
p-value		0,008*

*p<0.05: significant difference according to the Kruskal-Wallis test.

b) Nucleic acid (NA) and protein leakage test

The membrane leakage analysis is presented in table 2, where it is described that the NA and protein started to leak at a concentration of 50 mg/ml both for *S. aureus* and *C. albicans.*

c) Calcium (Ca^{2+}) and potassium (K^{+}) leakage test

The membrane leakage analysis for Ca^{2+} and K^{+} also started to leak at a concentration of 50 mg/ml both for *S. aureus* and C. albicans, but the concentration of 100 mg/ml could not leak the Ca^{2+} and K^{+} as well as the positive control groups (table 3).

d) Anti-biofilm activity test

The antibiofilm activity test for SVEL is presented in table 4. It shows that the minimal biofilm inhibition concentration (MBIC) above 50% was at 100 mg/ml for both *S. aureus* and *C. albicans.*

DISCUSSION

The Bataknese herbal has recently been favoured to be analysed, knowledge of local wisdom should be maintained by residents and improved by researchers. *Saurauia vulcani* Korth, *Allium chinense* G. Don, and many others should be analysed further to find other medicinal purposes [13]. The SVEL's phytochemical qualitative screening contained flavonoids, glycosides, tannins, saponins, and steroids/triterpenoids by each reagent. One of the quantitative analyses used to predict the phytochemicals was the Total Phenolic Content (TPC). Flavonoids are from the phenolic phytochemicals content, which aims to prevent bacterial cell division and cause damage to bacterial cell wall permeability, microsomes, and lysosomes due to the interaction between flavonoids and bacterial nucleic acid (NA). These compounds can damage the permeability of the bacterial cell wall and cytoplasm, and also inhibit membrane function by penetrating the lipid layer to disrupt the outer membrane's barrier function. This mechanism induced leakage of the microbe's cellular. Inhibiting fungal growth, flavonoids can disrupt cell membrane permeability, inhibit mitochondrial electron transport, and decrease ATP production, ultimately leading to fungal cell death.

Tannins function as antibacterials by inhibiting the reverse transcriptase and DNA topoisomerase enzymes, preventing bacterial cells from forming. In fungi, tannins work by inhibiting the biosynthesis of ergosterol, which forms fungal cell membranes. Tannins can also inhibit chitin synthesis, which forms fungal cell membranes [14, 15]. Saponins also exhibit antimicrobial properties by reducing surface tension, which results in increased permeability and cell leakage, leading to the release of intracellular compounds [15, 16].

The triterpenoids in SVEL are pentacyclic compounds with the molecular formula of $C_{30}H_{48}O_3$ and 3-hydroxy,12(13)-en,28-oleanolic acid, also known as oleanolic acid [17]. Triterpenoids/steroids in some natural resources have an antibacterial effect by inhibiting protein synthesis and causing leakage of liposomes [18, 19]. Steroids and triterpenoids interact with phospholipid cell membranes, which can be permeated by lipophilic compounds, resulting in decreased membrane integrity and changes in cell membrane morphology, leading to cell brittleness and lysis [19].

Table 1 shows that the total phenolic content (TPC) of SVEL is 150.61±0.53 mg GAE/g. The TPC of SVEL was higher than that of the methanol extract (16.56 mg/g GAE), ethyl acetate extract (13.702 mg/g GAE), and hexane extract (7.155 mg/g GAE). The higher quantification of SVEL's TPC than the other extracts makes it possible to assume that SVEL has antioxidant effectiveness. TPC positively correlates with antioxidant activity and is considered an effective medicinal remedy [13].

The results of SVEL's MIC from the Kirby-Bauer disc diffusion test showed both groups started at 12.5 mg/ml for *S. aureus* and at the 3.125 mg/ml for *C. albicans*. The 300 mg/ml of SVEL has equal effectiveness to a positive control group (Listerine Green Tea) against both microbes, but is still below the CHX and Ketoconazole (The gold standard of each oral antibacterial and antifungal), notably that ketoconazole had a far higher inhibitory effect. Some literature reviews discussed *C. albicans* morphology, the pathogenesis and the medicine resistance, the biofilm formation and the it's co-adherent to *S. aureus* [20-22]. The basic form of *C. albicans* from the yeast to hypha and pseudohypha, and moreover become chlamydospores [22]. Ketoconazole is a generic drug considered equivalent to be potent drugs for candidiasis it is considered to have a lower dosage to have an inhibitory effect and less toxicity effect on mamalian membrane damage compared to miconazole and clotrimazole [20, 23].

The effectiveness of microbial inhibition increased in line with the increasing concentration of SVEL due to the more bioactive components of the extract in antimicrobial function to inhibit of *C. albicans* microbial growth [24]. The MIC for *S. aureus* from SVEL with the concentration of 300 mg/ml (300,000 ppm) was 11.63 mm; it was much lower than the chloroform extract of *Saurauia vulcani*, which was 19.9 mm at 1000 ppm or only 1 mg/ml [25]. The MIC for *S. aureus* is lower than that of *C. albicans* with the SVEL; on the contrary, for some other herbs*, Astragalus sarcocolla* Gum, which has a better MIC for *S. aureus* [26].

The MBC value of *S. aureus* was obtained at a concentration of 100 mg/ml with a reduction of 98.79%, while in *C. albicans,* the MFC value was obtained at a concentration of 50 mg/ml with a decrease of 98% (table 2). In theory, followed by Balouiri *et al.*, the MBC/MFC value was determined by looking at the minor concentration that could reduce the number of colonies by as much as 98%-99% [27]

The leakage of NA and protein is caused by damage or impaired cell membrane permeability, thus causing cell death due to SVEL's phytochemical constituent. The increase in absorbance value indicates an increase in the number of NA and protein molecules that leak out of the cell [28]. In table 3, the absorbance values for bacterial NA and protein leakage in ethanol solvents with a concentration of 50 mg/ml surpassed the results of the positive control for *S. aureus* but were found for *C. albicans.*

Calcium functions as a barrier to the cell wall used to form in Gram-positive bacteria. Potassium plays a role and unites the ribosomes. The release of Ca^{2+} and K^{+} from cells can cause depolarized bacteria's cytoplasmic membrane and fungal death [29, 30]. Increasing the intracellular calcium concentration and the presence of bacteria are

primary factors in bacterial death and the eradication of biofilms formed. *S. aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae* are some of the pathogenic bacteria that exhibit multidrug resistance and virulence [30]. According to table 4, the concentrations of Ca^{2+} and K^{+} leakage in *S. aureus* and *C. albicans* were still below those of the positive control (Listerine Green Tea and CHX/Ketoconazole). The SVEL had no significant effect on Ca^{2+} and K^{+}leakage from both *S. aureus and C. albicans* compared to positive controls. This finding also has the same result as *Clitoria ternatea* L. ethanolic extract [31]. The membrane disruption analysis of the microbes became speculative, with only the analysis of the leakage of nucleic acids, proteins, Ca^{2+}, and $K^{+}$by UV-Vis Spectrophotometry or atomic absorption spectroscopy and further analysis can be choosed with inductively coupled plasma spectrometry (ICP) [32]. Leakage Direct observation to the microbes and cells by fluorescence spectrophotometer, Fluorescence Microscopy (FM) or Transmission Electron Microscopy (TEM), was additionally suggested to the study due to our study's limitation [33]. Leakages is not linearly responsible directly to cell death but it is a consequents of damaged of a semipermeable membrane which induced the intracellular constituent leakage of the microbes, another analysis should be provided to measure the quality of the antimicrobial of the *S. aureus* and *C. albicans,* the anti-biofilm analysis was described below [34].

A community of microorganisms, embedded in a matrix of extracellular polymeric substances, polysaccharides, and proteins, will form a biofilm. The effectiveness potential as an antibiofilm from SVEL at a concentration of 100 mg/ml gave better results in inhibiting the formation of biofilms of *S. aureus* and *C. albicans*, where at this concentration, SVEL was able to inhibit at least 50% of biofilm formation and was determined as Minimal Biofilm Inhibition Concentration (MBIC) [35]. The inhibition of biofilm formation by SVEL is in line with research on the effect of Cladode (*Opuntia ficus-indica*) extract, which can inhibit the formation of *S. aureus* biofilm due to the presence of flavonoid compounds [12].

Some microbiota, such as *S. aureus*, *C. albicans,* and *Lactobacillus spp.,* are considered part of the microbial flora in the mucous membranes. *Lactobacillus spp.* is effective as an anti-infectious defense, which should not be eradicated completely, unlike *S. aureus* and *C. albicans* [36]. The 50% eradication of anti-biofilm from SVEL, as suggested by a later study including *Lactobacillus helveticus-13* and other microbes, which can form a biofilm, indicates its ability to colonize alongside other normal microbiota. This reason has become the limitation of the study, which further study should include the analysis of *Lactobacillus spp.*

This study found that SVEL has the effectiveness as an antimicrobial and antibiofilm to *S. aureus* and *C. albicans* as measured in MIC which has larger diameter compares to green tea mouthwash at the concentration of 300 mg/ml; 98% eradication to MBC/MFC analysis at a concentration of 100 mg/ml (*S. aureus)* and 50 mg/ml (*C. albicans);* The leakage of nucleic acid and cellular protein were above chlorhexidine and green tea mouthwash at a concentration of 100 mg/ml for *S. aureus*, and followed by *C. albicans* but not exceed the the controls; The leakage of potassium exceeded the control's leakagage and in the contrary to Calcium leakage was far below the control's for *S. aureus.*

Every microbe, i. e, bacteria, fungi, protozoa, viruses, has a different response to antiseptics and disinfectants due to their composition, cellular structure, and also their physiology. The advancement of antimicrobial examination analysis studies, i. e., intracellular constituents lysis and leakage, cell homeostasis, inhibition of enzymes, electron transport, oxidative phosphorylation, interaction between macromolecules biosynthetic process, and the last is microscopic examination of intracellular wall porosity, will provide the conclusion to examine the quality of the new antimicrobial agents. With the minimum of a few analysis from above mentioned were studied to the *Saurauia vulcani* Korth. Ethanol Extract of Leaves.

CONCLUSION

This study found that SVEL has the effectiveness as an antimicrobial and antibiofilm, as measured in MIC, MBC/MFC, leakage of NA, protein, potassium, and calcium, as well as antibiofilm activity test.

ACKNOWLEDGEMENT

We express the sincere grateful to our profession students Irvan Richardo, Steela Anggriani, Jesslyn Komala and Nur Haimisha Johaimi who helped the authors for the study. My honorable appreciation to the 4th International Dental Conference of Sumatera Utara (IDCSU) 2024 and the Research institution of Universitas Sumatera Utara, TALENTA which provide the funding, batched No.11119.1/UN5.1R/PPM/2022, for this study.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

There are no conflinct of interest between authors regarding this manuscript

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