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Research Article
Activity of Ethanol Extract of Songga Wood (*Strychnos lucida*) as an Antimicrobial and Antibiofilm Agent Against *Bacillus* species, *Salmonella typhi* and *Candida albicans*

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Abstract

Background and Objective: The extensive use of antibiotics has created selective pressure leading to antimicrobial resistance, with biofilm formation serving as a major defense mechanism. Nearly 80% of infections involve biofilm-producing microorganisms that conventional antibiotics fail to eradicate. This study aimed to evaluate the antimicrobial and antibiofilm properties of ethanol extract from songga wood (*Strychnos lucida*) against *Bacillus* sp., *Salmonella typhi* and *Candida albicans*. **Materials and Methods:** Antimicrobial and antibiofilm activities of *S. lucida* ethanol extract were assessed using the microdilution assay at graded concentrations (0.0625-1%). Biofilm inhibition was examined during both intermediate and maturation phases. Statistical analysis was performed to determine significance ($p<0.05$). **Results:** At the highest tested concentration (1%), the extract demonstrated strong antimicrobial activity, inhibiting 83.89% of *Bacillus* sp., 80.03% of *S. typhi* and 82.43% of *C. albicans*. Pronounced antibiofilm activity was also observed, with inhibition rates of 75.36, 75.58 and 71.82% during the intermediate phase and 61.16, 61.76 and 61.14% during the maturation phase, respectively ($p<0.05$). **Conclusion:** *Strychnos lucida* ethanol extract exhibited significant antimicrobial and antibiofilm effects, indicating its potential as a natural candidate for developing alternative therapeutic agents. Further studies on its active compounds and mechanisms are warranted to support clinical applications.

Key words: Biofilm, *Strychnos lucida*, *Bacillus* sp., *Salmonella typhi*, *Candida albicans*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious diseases are conditions caused by microorganisms such as viruses, bacteria, fungi, parasites or by infectious proteins known as prions. Terminologically, infection refers to the process by which microorganisms enter the human body and subsequently grow or reproduce[1,2]. Antibiotic resistance arises due to the excessive and inappropriate use of antibiotics. It is broadly defined as a bacterial adaptation that reduces or nullifies the effectiveness of antibiotics. The contributing factors to this resistance involve both microbial mechanisms and human-related behaviors[3]. One of the key characteristics that enhances bacterial survival against antimicrobial agents is biofilm formation. A biofilm is a structured microbial community encapsulated within a self-produced extracellular polysaccharide matrix, which adheres to biotic or abiotic surfaces[4]. Microorganisms tend to attach to surfaces, proliferate and develop into biofilm structures resembling mucous-like layers. This process facilitates environmental interactions among different microbial species[5]. Biofilm development is now recognized as a major mediator of infection, with an estimated 80% of all infections being associated with microbial biofilm formation[6].

Medicinal plants can serve as alternative sources of resistance-modifying agents due to their diverse content of secondary metabolites. Several indigenous plant species from Indonesia remain under-investigated for their potential to inhibit bacterial growth. One such plant is *Strychnos lucida*, commonly known as Kayu Songga. This plant has long been used by local communities as a traditional remedy for infections. Songga Wood is an endemic species found in the Bima region of Indonesia, where it is locally referred to as "songga" in the native language[7,8]. Empirically, the Dompu community believes that the stem of *S. lucida* has medicinal properties that can treat various illnesses. The bark is traditionally used to treat toothaches, wounds and malaria; the seeds are used to alleviate malaria, diarrhea and muscle pain; and the roots are used to treat abdominal pain or diarrhea[7,8].

Several previous studies have reported the bioactivity of *Strychnos lucida* (kayu songga). The methanolic extract of *S. lucida* wood has demonstrated antioxidant activity based on the DPPH assay, as well as anti-inflammatory effects through the reduction of Interleukin-8 (IL-8) levels[9]. Another study also revealed that the methanolic extract exhibited antioxidant activity in the FRAP assay and was able to decrease the level of the pro-inflammatory cytokine IL-1β[10].

Despite its traditional medicinal use, the antimicrobial and antibiofilm activities of *Strychnos lucida* ethanol extract against *Bacillus* sp., *Salmonella typhi* and *Candida albicans* have not yet been scientifically investigated.

Therefore, this research needs to be conducted to evaluate the antimicrobial and antibiofilm properties of ethanol extract from songga wood (*Strychnos lucida*) against *Bacillus* sp., *Salmonella typhi* and *Candida albicans*.

MATERIALS AND METHODS

Study area: The experimental work was carried out in the Microbiology Laboratory of the Faculty of Medicine and the Microbiology Research Laboratory of the Faculty of Pharmacy, Halu Oleo University, from May to July, 2025.

Materials: The materials used in this study included ethanol extract of *Strychnos lucida* wood collected from Dompu, West Nusa Tenggara; distilled water (WaterOne®); Potato Dextrose Broth (PDB) (HIMEDIA®); Mueller Hinton Broth (MHB) (HIMEDIA®); microbial strains *Bacillus* sp., *Salmonella typhi* and *Candida albicans*; McFarland standard solution (HIMEDIA®); Gentian Violet (OneMed®); ethanol; dimethyl sulfoxide (DMSO) solution (Meteora Pelangi); Eppendorf tubes; yellow and blue pipette tips; 96-well microplates (NEST®); 96% ethanol; chloramphenicol (Nova®) and nystatin (Phapros®).

Methods

Sample preparation and extraction: Dried *Strychnos lucida* wood (simplicia) was prepared for extraction using the maceration method. A total of 500 g of powdered simplicia was immersed in 96% ethanol, ensuring complete coverage of the plant material. The maceration was carried out over 72 hrs with occasional agitation. The extract was then separated from the plant residue using filter paper, followed by solvent evaporation under reduced pressure at 60°C using a rotary evaporator to obtain a concentrated crude extract[11-14].

Preparation of positive controls: Positive control solutions were prepared using 1% (w/v) chloramphenicol for bacterial assays and 1% (w/v) nystatin for fungal assays. Each compound (1 g) was accurately weighed and dissolved in 100 mL of DMSO[5,11].

Preparation of microbial cultures for antimicrobial assay: *Bacillus* sp. and *Salmonella typhi* were cultured on Nutrient Agar (NA) at 37°C for 24 hrs, whereas *Candida albicans* was grown on potato dextrose agar (PDA) at 37°C for 72 hrs. The optical densities of microbial suspensions were adjusted to 0.1 at 595 nm, corresponding to the 0.5 McFarland standard (approximately 1.5×10 CFU/mL). Subsequently, the suspensions were diluted in fresh broth to an OD of 0.01 for bacterial strains and an OD of 0.38 for *C. albicans*[11].

Antimicrobial assay: The antimicrobial activity was evaluated using a microdilution method in 96-well flat-bottom microplates. The test sample (ethanolic extract of *Strychnos lucida* stem) was prepared in serial concentrations of 1, 0.5, 0.25, 0.125 and 0.0625% (w/v). The positive controls used were 1% chloramphenicol (as an antibacterial agent) and 1% nystatin (as an antifungal agent). Bacterial suspension served as the growth control, while solvent control was prepared using the same solvent as in the sample preparation. A volume of 180 µL of microbial suspension containing either bacteria in Mueller-Hinton Broth (MHB) or fungi in potato dextrose broth (PDB) was dispensed into each well of the microplate. Subsequently, 20 µL of the test sample at the respective concentrations was added to the designated wells. The microplates were then incubated at 37°C for 24 hrs for bacterial strains and 72 hrs for *Candida albicans*. After incubation, absorbance readings were measured using a microplate reader at 595 nm for bacteria and 520 nm for yeast. Optical density (OD) was used to estimate cell density in the liquid culture and OD values were employed to calculate the percentage of microbial growth inhibition in the test samples. The percentage of inhibition was calculated using the following formula[6]:

$$\text{Inhibition (\%)} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Where:

$OD_{control}$ = Mean optical density of the growth control group

OD_{sample} = Average optical density of the test sample group

Anti-biofilm assay: A volume of 180 µL of bacterial suspension was dispensed into each well of a 96-well microtiter plate. The suspension consisted of 160 µL of *Bacillus* sp. or *Salmonella typhi* culture and 20 µL of Mueller-Hinton Broth (MHB). Subsequently, 20 µL of *Strychnos lucida* ethanol extract at a concentration of 1% (w/v) was added to the first well containing the bacterial suspension and mixed thoroughly. For *Candida albicans*, the procedure was slightly modified. A total of 180 µL of fungal suspension, comprising 160 µL of *C. albicans* culture and 20 µL of potato dextrose broth (PDB), was added to each well and incubated at approximately 37°C for 90 min to allow for biofilm attachment. After the initial incubation, wells were gently washed three times with 150 µL of sterile distilled water to remove non-adherent cells[5,6].

The plates were then incubated at 37°C for 24 hrs to allow biofilm development at the intermediate stage and for 48 hrs to achieve mature biofilm formation. Following incubation, the wells were washed three times with distilled water and air-dried at room temperature for 5 min to remove any residual moisture. To stain the biofilm, 125 µL of 1% gentian violet solution was added to each well and incubated at room temperature for 15 min. The wells were then rinsed three times with running water to remove excess stain. Subsequently, 200 µL of 96% ethanol was added to each well to solubilize the stained biofilm[15].

Optical density (OD) was measured at 595 nm using a microplate reader. All assays were performed in triplicate. The OD values were used to calculate the percentage of biofilm inhibition using the formula as in antimicrobial assay[6,16,17].

Media without microbial inoculum served as a negative control. Microbial suspensions without extract served as growth controls. The DMSO was used as the solvent control and a 1% (w/v) chloramphenicol solution served as the positive control for bacterial biofilms.

Statistical analysis: The data were statistically analyzed using a one-way ANOVA, followed by a *post hoc* test to assess significant differences between groups. A p-value below 0.05 ($p<0.05$) was interpreted as statistically significant, while p-values above 0.05 ($p>0.05$) indicated a lack of significance. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) with a 95% confidence interval.

RESULTS AND DISCUSSION

Antimicrobial activity of ethanol extract of songga wood (*Strychnos lucida*): At the highest tested concentration (1% b/v), the ethanol extract of songga wood (*Strychnos lucida*) exhibited considerable antimicrobial activity, with inhibition percentages of 83.89% against *Bacillus* sp. (Fig. 1a), 80.03% against *Salmonella typhi* (Fig. 1b) and 82.43% against *Candida albicans* (Fig. 1c). All assays were conducted in triplicate to ensure reproducibility.

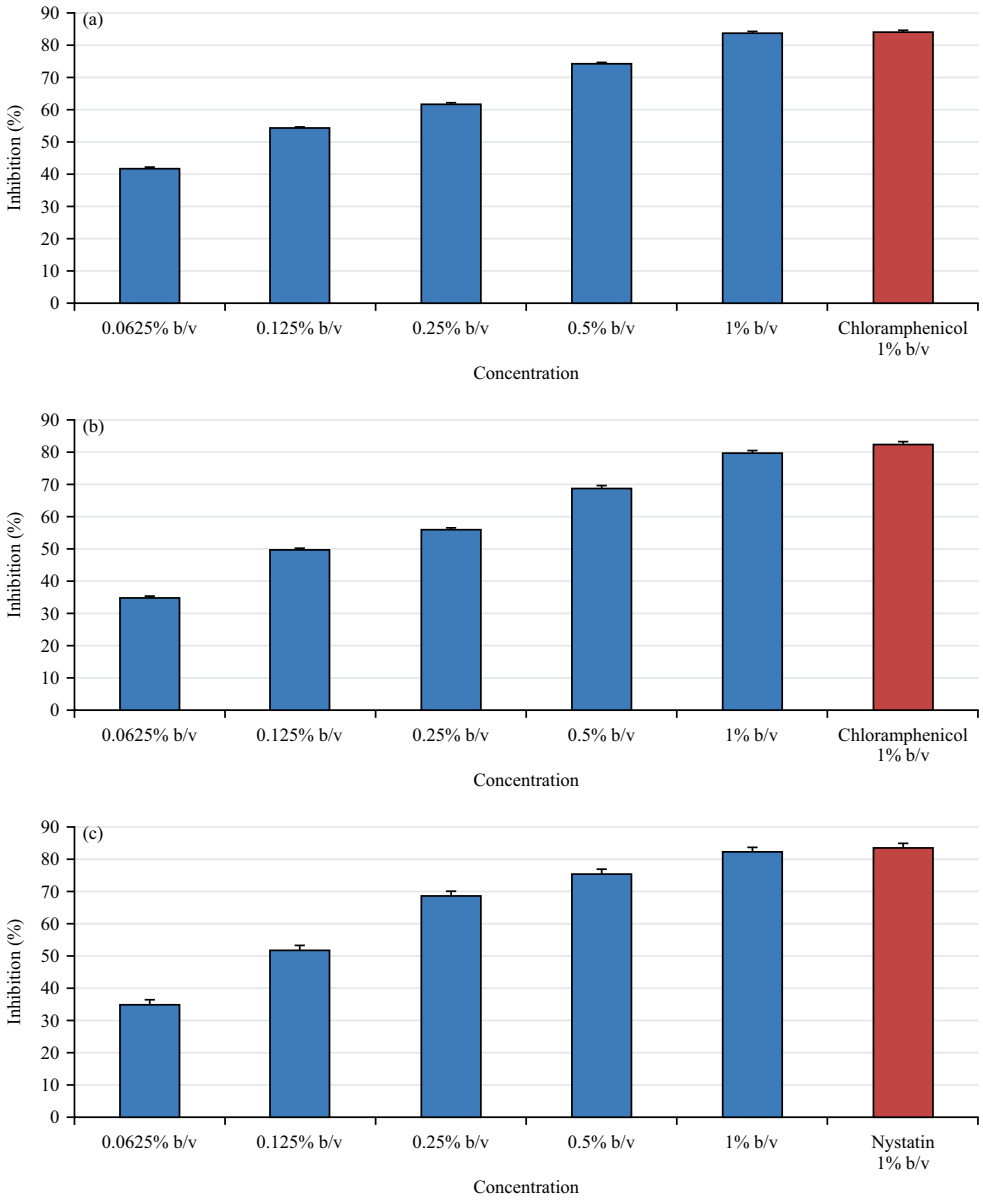

Fig. 1(a-c): Inhibition of microbial, (a) *Bacillus* sp., (b) *Salmonella typhi* and (c) *Candida albicans*

The ANOVA analysis revealed a statistically significant difference in inhibition across the extract concentrations (0.0625-1%), with a p-value <0.05, as further confirmed by the LSD *post hoc* test. The antimicrobial activity increased proportionally with the extract concentration.

This inhibitory effect is presumed to be associated with the presence of bioactive compounds such as alkaloids, phenolics, flavonoids and tannins, which act through various mechanisms, including disruption of microbial protein synthesis, damage to the cell membrane and interference with microbial metabolism. These findings support the potential use of *Strychnos lucida* wood as a promising source of natural antimicrobial agents[18].

Anti-biofilm assay

***Bacillus* sp.:** At the highest tested concentration (1%), the ethanol extract of songga wood (*Strychnos lucida*) demonstrated considerable inhibition of monomicrobial biofilm formation during the intermediate phase (24 hrs), with an average inhibition of 75.36±1.13%. In comparison, chloramphenicol (1% w/v), used as a reference drug, exhibited 76.88±0.10% inhibition (Fig. 2a). Statistical analysis revealed no significant difference between the extract and the positive control ($p>0.05$), although a slight reduction in inhibition was observed with the extract. This reduced efficacy compared to antimicrobial activity may be attributed to the rapid capability of bacteria to initiate biofilm formation within 24 hrs.

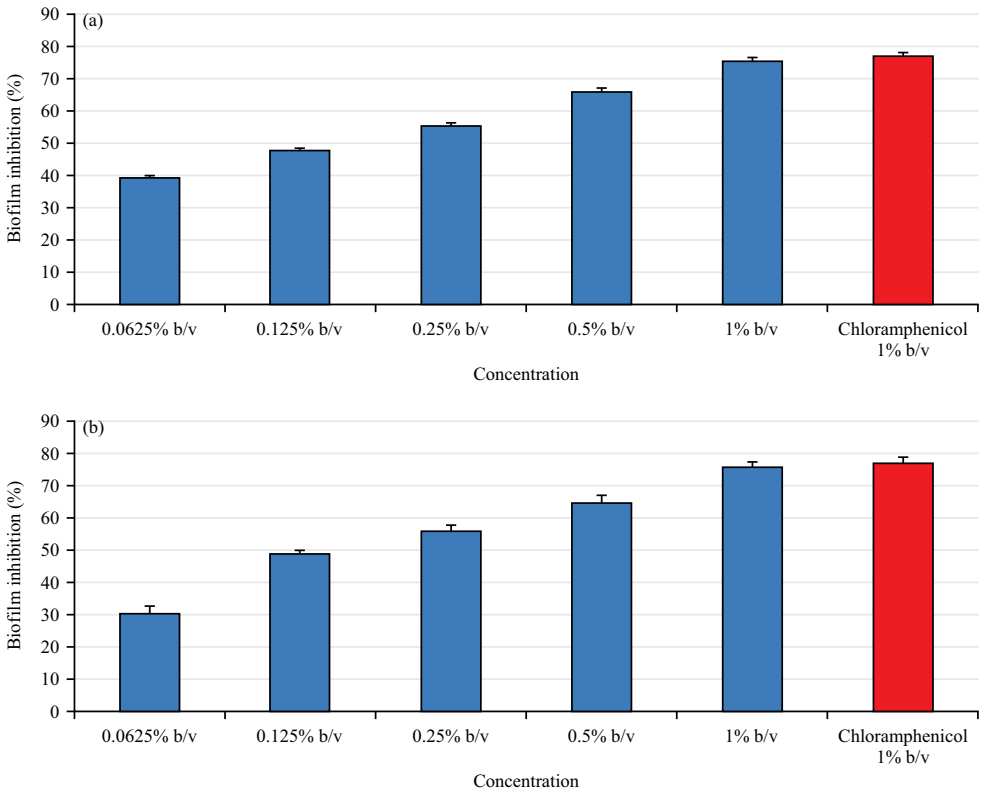

Fig. 2(a-b): Inhibition of *Bacillus* sp., biofilm, (a) Intermediate phase at 24 hrs and (b) Maturation phase at 48 hrs

During this intermediate phase, the extracellular polymeric substances (EPS) matrix is still forming and remains relatively unstructured. Therefore, the extract may exert its antibiofilm effect by disrupting early EPS production and hindering microbial adhesion, suggesting a potential mechanism of action in interfering with early-stage biofilm development[19].

During the maturation phase, the ethanol extract of songga wood (*Strychnos lucida*) exhibited a biofilm inhibition activity of 61.16±0.59%, which was comparable to that of chloramphenicol (62.11±0.76%) used as the positive control (Fig. 2b). Compared to the intermediate phase, the inhibitory activity observed at this stage was lower. This reduction may be attributed to the prolonged duration of biofilm development during the maturation phase, in which the biofilm's defense system becomes more robust and structurally complex. The formation of a denser and more cohesive mucus layer, which is characteristic of mature biofilms, was clearly observed, particularly as the biofilm became more firmly adhered to the well surfaces. These findings suggest that the mature biofilm matrix presents greater resistance to antimicrobial penetration and disruption, thereby limiting the bioactivity of the extract at later stages of biofilm development.

Statistical analysis using *post hoc* testing was performed to identify which extract concentrations exhibited significant differences in biofilm inhibition activity. The results revealed that all tested concentrations (0.0625, 0.125, 0.25, 0.5 and 1%) showed statistically significant differences from each other ($p<0.05$). However, at the highest concentration (1%), there was no statistically significant difference compared to the positive control ($p>0.05$) during the intermediate phase. The biofilm inhibition activity of *Bacillus* sp., at various concentrations of the ethanol extract is illustrated in Fig. 2.

At the maturation phase, the microorganisms forming the biofilm have fully adhered to the substrate, making it more challenging for the ethanol extract of *Strychnos lucida* to inhibit or eliminate the biofilm compared to the intermediate phase. During this stage, the microbes develop a more complex biofilm defense system, including the production of a protective extracellular matrix and the activation of intercellular communication mechanisms known as quorum sensing. These findings are consistent with previous studies indicating that mature biofilms exhibit higher resistance to antimicrobial agents than those in the intermediate phase[20-22].

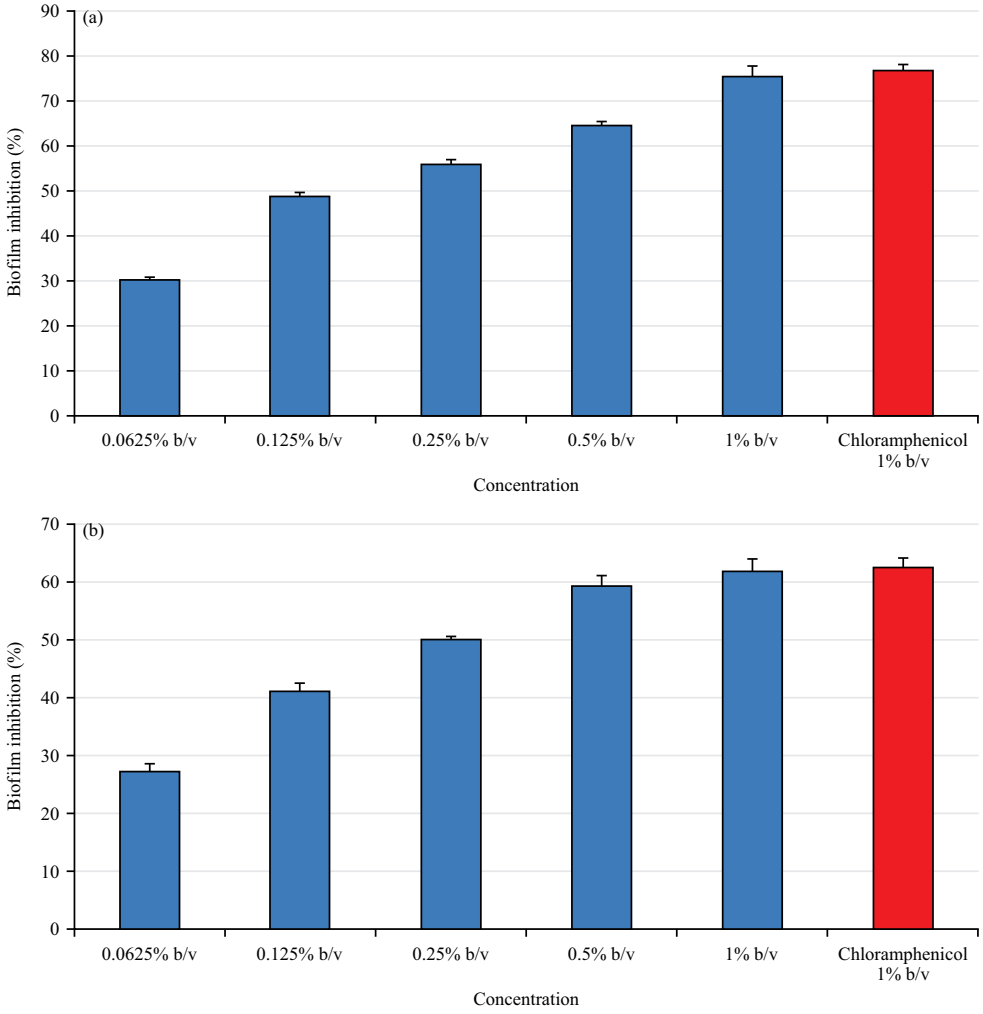

Fig. 3(a-b): Inhibition of *Salmonella typhi* biofilm, (a) Intermediate phase at 24 hrs and (b) Maturation phase at 48 hrs

***Salmonella typhi*:** The results of this study demonstrate that the ethanol extract of songga wood (*Strychnos lucida*) possesses antibiofilm potential against biofilm formation by the Gram-negative bacterium *Salmonella typhi*. At a concentration of 1%, the ethanol extract exhibited a biofilm inhibition activity of 75.58±2.09% during the intermediate phase (Fig. 3a) and 61.76±2.07% during the maturation phase (Fig. 3b).

Statistical analysis using a *post hoc* test revealed significant differences among the tested concentrations (0.0625, 0.125, 0.25, 0.5 and 1%) during the intermediate phase ($p<0.05$). However, during the maturation phase, no significant difference was observed between the 1% extract concentration and the positive control (chloramphenicol) ($p>0.05$). The detailed results of the antibiofilm activity against *Salmonella typhi* are presented in Fig. 3.

As illustrated in Fig. 3, the antibiofilm activity of the ethanol extract of songga wood (*Strychnos lucida*) decreased during the maturation phase compared to the intermediate phase. This reduction may be attributed to the denser and more complex extracellular polymeric substance (EPS) matrix formed during the maturation phase. The presence of a thick biofilm mucus layer adhered to the well surface was visibly more prominent, which likely hindered the penetration of the extract into the target microbial cells. Moreover, the prolonged duration of biofilm development during the 48 hrs maturation phase enabled the establishment of a more structured and well-organized microbial community, thereby increasing resistance to the antibiofilm agent.

***Candida albicans*:** This study evaluates the antibiofilm potential of ethanol extract of songga wood (*Strychnos lucida*) against biofilm formation by *Candida albicans*. The results showed that biofilm inhibition was more pronounced during the intermediate phase (24 hrs) compared to the maturation phase (48 hrs). The 1% ethanol extract of songga wood

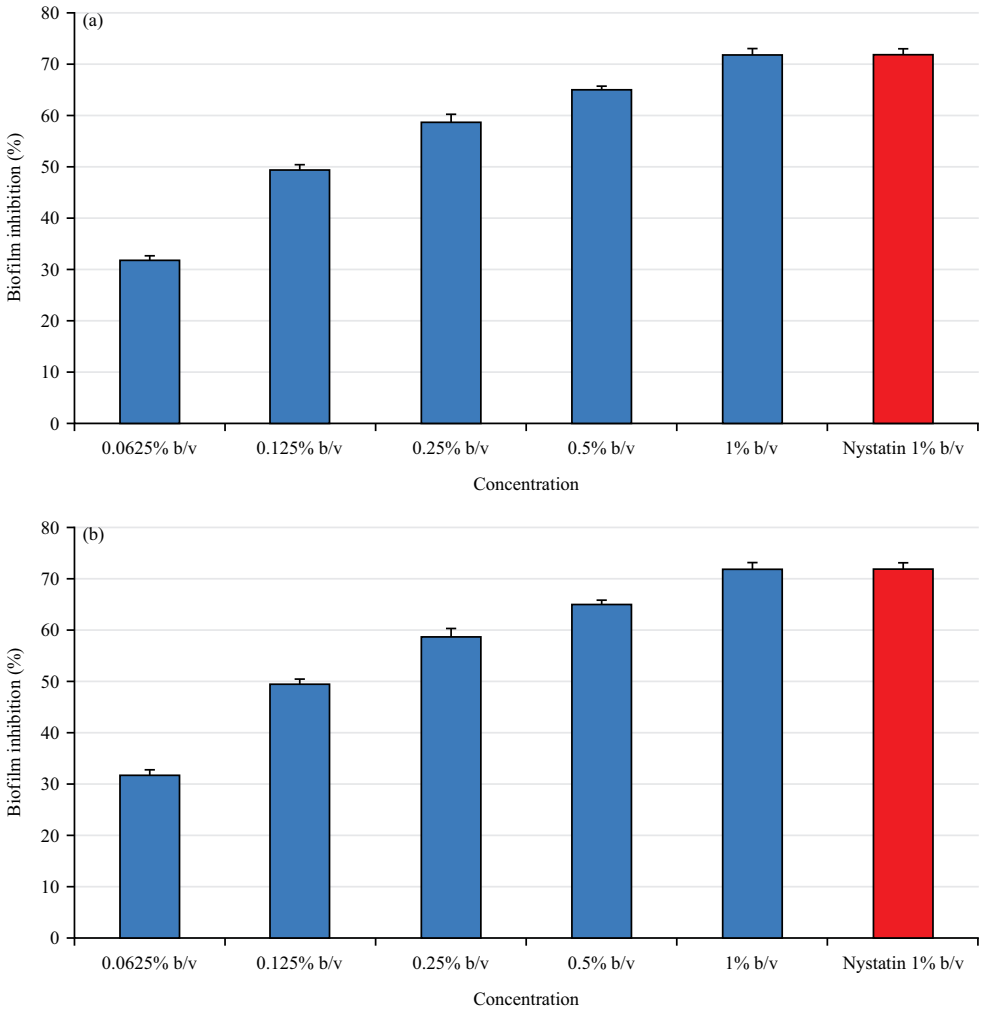

Fig. 4(a-b): Inhibition of *Candida albicans* biofilm, (a) Intermediate phase at 24 hrs and (b) Maturation phase at 48 hrs

(*Strychnos lucida*) exhibited the highest antibiofilm activity against *Candida albicans* compared to other tested concentrations. In the intermediate phase, the inhibition rate reached 71.82±1.15%, which was nearly equivalent to the inhibition produced by nystatin (71.85±0.11%) used as a positive control (Fig. 4a). In the maturation phase (48 hrs), the inhibition rate was 61.14±2.82% for the extract and 62.86±0.89% for nystatin (Fig. 4b).

The decrease in extract effectiveness during the maturation phase may be attributed to the complete and mature development of the *Candida albicans* biofilm at this stage. Mature biofilms possess a denser and more complex structure, with a thicker external mucus layer than those formed during the intermediate phase. Additionally, the amount of extracellular polymeric substances (EPS) produced in the maturation phase is significantly higher, serving as both a protective barrier and a nutrient source for the biofilm. This condition facilitates the continued formation of new microbial colonies and enhances their resistance to antimicrobial agents. These findings are consistent with previous studies, which reported that mature biofilms are more resistant to antimicrobial penetration. Mature biofilms are composed of yeast-based layers and hyphal elements that form a complex community encased in EPS[23-25].

Overall, antibiofilm activity testing against the three types of microorganisms demonstrated that the ethanol extract of songga wood (*Strychnos lucida*) is effective in inhibiting biofilm formation by Gram-positive *Bacillus* sp., Gram-negative *Salmonella typhi* and *Candida albicans*. This inhibitory activity is likely due to the presence of several bioactive compounds in the extract, including alkaloids, tannins, steroids/triterpenoids, phenols and flavonoids, along with strychnine derivatives known for their antimicrobial properties[26,27]. Phenols and tannins act as antibacterial agents,

while flavonoids exhibit antioxidant activity that also contributes to antimicrobial effects. Moreover, the steroid compounds in *Strychnos lucida*, particularly steroidal saponins, are recognized for their antibacterial activity, supporting the extract's overall effectiveness in biofilm inhibition[28].

CONCLUSION

The ethanol extract of songga wood (*Strychnos lucida*) demonstrated notable antimicrobial and antibiofilm activities. It showed strong inhibition against *Bacillus* sp., *Salmonella typhi* and *Candida albicans*, particularly during the intermediate phase of biofilm formation, though the effect declined at the maturation stage. These results suggest that *S. lucida* ethanol extract has significant potential as a natural source for biofilm-targeted antimicrobial agents.

SIGNIFICANCE STATEMENT

This study demonstrates that the stem of *Strychnos lucida* possesses strong antimicrobial and antibiofilm activities, supporting its potential as a natural source for developing biofilm-targeted therapies. The findings provide a scientific basis for its use in traditional medicine and contribute to the exploration of plant-derived antimicrobial agents.

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