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Antibiofilm activity of plant lectins: Evaluating the anti-adhesion properties of *Treculia africana*, *Moringa oleifera*, and *Allium sativum* on clinical bacterial isolates

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Abstract:

Background: The increasing prevalence of biofilm-associated infections caused by multidrug-resistant organisms has intensified the search for alternative antimicrobial agents. This study investigated the antibiofilm activity of lectins isolated from *Treculia africana* leaves (TAL), *Moringa oleifera* leaves (MOL), and *Allium sativum* bulbs (ASL) against selected clinical bacterial isolates.
Methodology: Lectins were extracted from the three plant parts using modified conventional crude extraction methods. The antibiofilm activities against clinical *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* isolates were evaluated at extract concentrations of 500µg/ml, 100µg/ml and 10µg/ml in a 96-well U-bottom microtitre plates for 4 hours and 24 hours, by biofilm inhibition and quantification assays. The antibiofilm activity, performed in triplicates, was expressed as percentage inhibition, and classified as weak (<40%), moderate (40–60%), or strong (>60%).
Results: The result showed that 500µg/ml TAL lectin extract at 4-hour incubation showed moderate anti-biofilm activity against *S. aureus* (41% inhibition), but minimal or no activity against other isolates. At 24-hour incubation, TAL extract produced moderate anti-biofilm activity against *K. pneumoniae* (43%) and *B. subtilis* (43.3%), with weak activity against *S. aureus* (19%) and *P. aeruginosa* (17.3%). MOL lectin extract produced stronger activity than TAL extract, reducing biofilm adhesion at 500µg/ml by 35–66% across all isolates after 4 hours and up to 57% after 24 hours, particularly against *K. pneumoniae* and *B. subtilis*. ASL extract produced selective antibi-biofilm activity, mainly against *S. aureus* (37% at 4 h; 22.3% at 24 h) and *P. aeruginosa* (42.3% at 24 h), with little or no effect on other isolates. Across all lectins, antibiofilm activity decreased with lower concentrations, and none completely inhibited bacterial adhesion.
Conclusion: The result indicates the promise of these plant-based lectins as a natural agent for controlling biofilm-associated infections.

Keywords: Plant lectins; Antibiofilm; Multidrug-resistant; *Moringa oleifera*; *Treculia africana*; *Allium sativum*

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Activité antibiofilm des lectines végétales: Évaluation des propriétés anti-adhésives de *Treculia africana, Moringa oleifera* et *Allium*

sativum sur des isolats bactériens cliniques

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Résumé:

Contexte: La prévalence croissante des infections associées aux biofilms causées par des organismes multirésistants a intensifié la recherche d'agents antimicrobiens alternatifs. Cette étude a examiné l'activité antibiofilm de lectines isolées de feuilles de *Treculia africana* (TAL), de feuilles de *Moringa oleifera* (MOL) et de bulbes d'*Allium sativum* (ASL) contre des souches bactériennes cliniques sélectionnées.
Méthodologie: Les lectines ont été extraites des trois parties de la plante par une méthode d'extraction brute conventionnelle modifiée. L'activité antibiofilm contre des souches cliniques de *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* et *Bacillus subtilis* a été évaluée à des concentrations d'extrait de 500µg/ml, 100µg/ml et 10µg/ml dans des plaques de microtitration à 96 puits à fond en U, pendant 4 heures et 24 heures, par des tests d'inhibition et de quantification du biofilm. L'activité antibiofilm, réalisée en triplicata, a été exprimée en pourcentage d'inhibition et classée comme faible (<40%), modérée (40–60%) ou forte (> 60%).
Résultats: Après 4 heures d'incubation, l'extrait de lectine TAL (500µg/ml) a présenté une activité anti-biofilm modérée contre *S. aureus* (41% d'inhibition), mais une activité minimale, voire nulle, contre les autres souches. Après 24 heures d'incubation, l'extrait TAL a induit une activité anti-biofilm modérée contre *K. pneumoniae* (43%) et *B. subtilis* (43,3%), et une faible activité contre *S. aureus* (19%) et *P. aeruginosa* (17,3%). L'extrait de lectine MOL a présenté une activité supérieure à celle de l'extrait TAL, réduisant l'adhérence du biofilm de 35 à 66% pour toutes les souches après 4 heures et jusqu'à 57% après 24 heures à la concentration de 500µg/ml, notamment contre *K. pneumoniae* et *B. subtilis*. L'extrait d'ASL a présenté une activité antibiofilm sélective, principalement contre *S. aureus* (37% à 4 h; 22,3% à 24 h) et *P. aeruginosa* (42,3% à 24 h), avec peu ou pas d'effet sur les autres isolats. Pour toutes les lectines testées, l'activité antibiofilm a diminué avec la concentration, et aucune n'a inhibé complètement l'adhésion bactérienne.
Conclusion: Ces résultats suggèrent le potentiel de ces lectines d'origine végétale comme agent naturel pour lutter contre les infections associées aux biofilms.

Mots-clés: Lectines végétales; Antibiofilm; Multirésistance; *Moringa oleifera*; *Treculia africana*; *Allium sativum*

Introduction:

Biofilms are common in nature because they represent a survival strategy used by bacteria (1). Microbial biofilms represent a major challenge in both clinical and industrial settings due to their exceptional resistance to antimicrobial agents and disinfectants (2,3). Biofilms are structured communities of microbial cells enclosed in a self-produced extracellular polymeric matrix that adheres to biotic or abiotic surfaces (4). Clusters of bacteria embedded in a self-produced matrix and affixed to a surface or one another are known as bacterial biofilms (1). This sessile mode of growth provides microorganisms with enhanced tolerance to hostile environmental conditions, antibiotics, and host immune responses, contributing to persistent infections and increased morbidity and mortality rates (5). In clinical environments, biofilm-associated infections are implicated in up to 80% of chronic microbial diseases, including those involving indwelling medical devices such as catheters, prostheses, and implants (6).

The expression levels of many genes linked to the maturation and generation of exopolysaccharide (EPS), commonly referred to as "slime" or bacterial EPS, rapidly alter when bacteria adhere to the surface (7). The existence of a self-produced EPS and limited motility make

the adhesion stable, irreversible, and permanent if cells stay on the substrate surface for a long time without any physical or chemical interference (8,9). Therefore, preventing bacterial attachment has emerged as a promising strategy for controlling biofilm development, offering an advantage over conventional antimicrobial therapies that primarily target planktonic cells. This has spurred interest in identifying natural anti-adhesion agents capable of disrupting bacterial colonization without exerting selective pressure for resistance.

Compounds and extracts derived from this plant exhibit a wide range of biological activities, including oxytocic effects, modulation of the central nervous system, anti-inflammatory action, anti-tubercular properties, anti-ulcer activity, hepato-renal protective effects, as well as anti-trypanosomal, anticancer, and antidiabetic potentials (10,11). Plant-derived lectins, a diverse group of carbohydrate-binding proteins, have attracted attention as potential antibiofilm and anti-adhesion agents due to their ability to recognize and bind specific sugar moieties on microbial cell surfaces (12). These interactions can block key receptors involved in adhesion, interfere with biofilm matrix synthesis, and inhibit cell-to-cell communication (quorum sensing) (13,14).

Several studies have demonstrated that lectins from various plant sources exhibit inhibitory effects on pathogenic bacteria by preventing their attachment to epithelial cells or abiotic surfaces (15,16). Widely prevalent throughout West and Central Africa, *Treculia africana* (African breadfruit) yields 20–30 pods of edible seeds annually and is a delicacy but underutilized food security crop among the Igbo people of southeast Nigeria (17). *Moringa oleifera* (drumstick or horseradish tree) is native to India but grows well in tropical and subtropical climates because it can withstand drought and moderate winter. All parts of the plant have nutritional and commercial value (18). *Allium sativum* is a highly prized culinary and therapeutic herb with a variety of pharmacological qualities, such as anti-inflammatory, anti-cancer, antioxidant, and antibacterial activities. This study investigates the anti-adhesion activities of lectins extracted from *T. africana*, *M. oleifera*, and *A. sativum* against selected bacterial pathogens.

Materials and method:

Culture media and antibiotics:

Culture media used were Trypton Soy broth (Oxoid, UK), Mueller-Hinton (MH) agar (Oxoid, UK), nutrient agar, and Trypton soy agar powder (Oxoid, UK). All culture media were prepared aseptically in line with the manufacturers' instructions. The antibiotic powder used in this work was ciprofloxacin which was a gift from Juhel Nigeria ltd.

Microorganisms:

The bacterial strains used in this study comprised two Gram-positive species (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative species (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). These clinical isolates were sourced from the laboratory of the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria. Each isolate was initially grown in Tryptone Soy broth and subsequently sub-cultured onto Tryptone Soy agar chosen for its suitability in supporting biofilm formation. Stock cultures were maintained on Tryptone Soy agar slants and refrigerated at 4–6 °C.

Plant materials:

Fresh, mature *Moringa oleifera* leaves were sourced from the Abakpa Nike community in Enugu State, and *Allium sativum* (garlic) bulbs together with *Treculia africana* (African breadfruit) seeds were purchased at Ogbete Main Market, Enugu State, Nigeria. All plant specimens were taxonomically identified by Dr. C. S. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria.

Extraction of lectins from plant materials:

Using modified conventional methods, crude lectin proteins were isolated from *T. africana*, *A. sativum*, and *M. oleifera*. With modifications, the extraction procedure for *M. oleifera* was carried out in accordance with Khatun et al., (19). A 0.2 M NaCl solution (pH 6.5) containing polyvinylpyrrolidone (PVP) was combined with dried leaf powder, left overnight at 4°C, centrifuged, and the supernatant precipitated with 100% saturated ammonium sulfate. After dialyzing the precipitate against Tris-HCl buffer (pH 8.4) and distilled water, it was lyophilized.

The extraction process for *T. africana* was carried out using the modified approach proposed by Laija et al., (20). Seed flour that had been defatted was extracted at 4°C in phosphate-buffered saline (PBS, pH 7.2). Proteins were dialyzed, lyophilized, and separated using ammonium sulfate precipitation (40% and 60% saturation). Ground bulbs of *A. sativum* were mixed with NaCl in PBS (pH 7.4), centrifuged, and the resulting supernatant precipitated with 70% ammonium sulfate. The protein pellet was dialyzed and lyophilized.

Preparation of stock solution and standardization of test bacteria:

For antibiofilm and anti-adhesion assay, stock solution of the lectin extracts were prepared by dissolving 1000mg of each extract in 5ml of sterile Tryptone Soy broth to obtain a concentration of 200 mg/ml. A 0.5 McFarland turbidity standard, equivalent to 1.5×10^8 CFU/ml, was prepared from barium chloride and sulfuric acid solutions and used to standardize bacterial inocula. Test bacteria were adjusted to match the 0.5 McFarland standard by suspending overnight cultures in sterile saline to achieve a final population of approximately 10^8 CFU/ml before use in antimicrobial assays.

Determination of antibiofilm activity of the plant lectin extracts:

For the determination of anti-adhesion properties, different concentrations of the three plant lectin extracts were prepared from the stock solution at 50mg/ml, 10mg/ml, and 1mg/ml. Following the addition of 100µl of each extract to the 96-well U-bottom microtitre plates, each well received an equivalent volume of 100 µl of standardized culture (1.0×10^6 CFU/ml) of every test bacterium, resulting in a final volume of 200µl and final concentrations of 500µg/ml, 100µg/ml and 10µg/ml. Approximately 2.5µg/ml of ciprofloxacin served as the positive control, while 2.5µg/ml of TSB and the test bacteria served as the negative control. Three duplicates of each procedure were performed.

To promote cell attachment and biofilm formation, the plates were sealed with sterile sealant and incubated at 37^{o}C for 4 and 24 hrs, respectively, in a humidified container without shaking. The quantification of biofilm biomass was evaluated using the crystal violet staining method after incubation.

Assessment of biofilm biomass using crystal violet quantification assay:

Using modified crystal violet (CV) staining test as described by Stepanovic et al., (21), cell attachment was indirectly evaluated. To get rid of weakly attached cells, the plates were rinsed three times with sterile distilled water after incubation. After 30 minutes of air drying, 200µl of methanol was applied to each well to fix the remaining attached cells for 15 minutes.

The staining was done for five minutes using 200µl of 1% crystal violet after the plates had been emptied and allowed to air dry. Plates were oven-dried at 60^{O}C for 45 minutes after excess stains were removed with a running tap. Once the cells had dried, 200µl of 33% glacial acetic acid was used to resolubilize the dyes that had stuck to them.

The optical density of each destained well was then measured at 640 nm using an ELISA microtitre plate reader after 100µl of each well had been transferred to a fresh plate. The percentage inhibition was classified as weak (<40%), moderate (40–60%), or strong (>60 %) reduction, and calculated using the formula; % inhibition = (OD of experimental / OD of negative control) × 100, where OD is optical density, and % reduction = 100 – % inhibition.

Statistical analysis:

Data on antibiofilm activity were presented as percentage inhibition, and graphs were generated using Origin software.

Results

Antiadhesion property of *Treculia africana* leaf (TAL) lectins at 4 and 24 hours:

The result of the anti-adhesion activity of TAL lectin after 4 hours exposure is shown in Fig 1a. Complete inhibition of biofilm formation was not achieved by any treatment, including the positive control (ciprofloxacin, 2.5µg/ml), with some samples showing no detectable inhibition activity. At 500µg/ml, TAL produced percentage reductions in biofilm adhesion of *K. pneumoniae* (0%), *B. subtilis* (11%), *S. aureus* (41%), and *P. aeruginosa* (19%). At 100µg/ml, inhibition was absent (0%) in *K. pneumoniae* and *P. aeruginosa*, while *B. subtilis* and *S. aureus* showed reductions of 6% and 28%, respectively. At 10µg/ml, no inhibition (0%) was observed in any of the isolates except *S. aureus*, which exhibited 20% reduction. The positive control demonstrated weak anti-adhesion activity (<40% reduction).

The result of anti-adhesion activity of TAL after 24 hours exposure is shown in Fig 1b. None of the tested lectins completely inhibited bacterial attachment, including the positive control (ciprofloxacin), and some samples exhibited no detectable anti-adhesion effect. At concentration of 500µg/ml, TAL produced percentage reductions in biofilm adhesion as follows; *K. pneumoniae* (43%), *B. subtilis* (43.3%), *S. aureus* (19%), and *P. aeruginosa* (17.3%). When the concentration was reduced to 100µg/ml, inhibition levels were 8.3% for *K. pneumoniae*, 25% for *B. subtilis*, and 21% for *P. aeruginosa*, while *S. aureus* showed no measurable activity. At the lowest concentration (10µg/ml), reductions were observed in *K. pneumoniae* (14%) and *P. aeruginosa* (12%), with no anti-adhesion effect detected against *B. subtilis* or *S. aureus*.

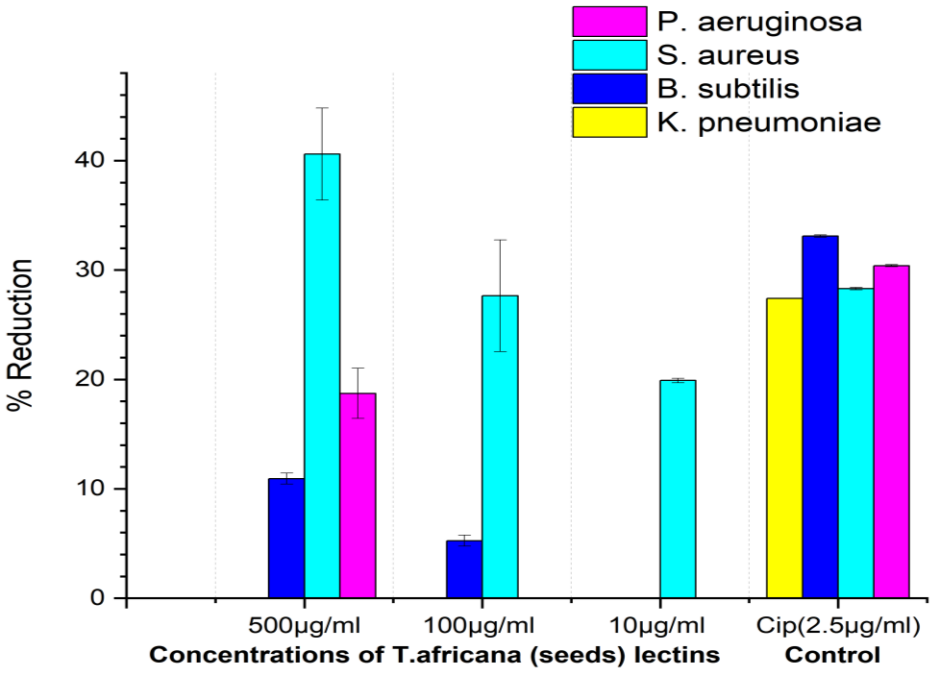

Fig 1a: Anti-adhesion property of *Treculia africana* (seeds) lectins at 4 hours exposure

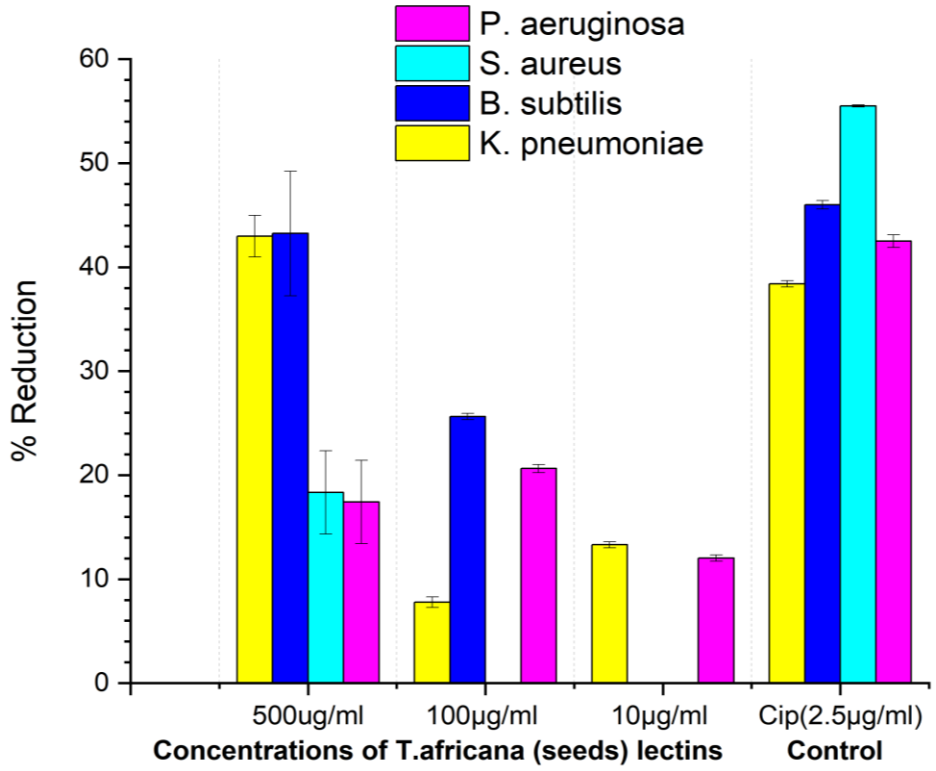

Fig 1b: Antiadhesion property of *Treculia africana* (seeds) lectins at 24 hours exposure

Anti-adhesion property of *Moringa oleifera* leaf (TAL) lectins at 4 and 24 hours:

The anti-adhesion potential of MOL lectin after 4 hours of exposure is shown in Fig 2a. Complete inhibition of biofilm attachment was not achieved by any of the treatments, including the positive control (ciprofloxacin, 2.5µg/ml), while certain samples exhibited no detectable anti-adhesion activity. At a concentration of 500µg/ml, MOL lectin reduced bacterial adhesion by 35% for *K. pneumoniae*, 36.4% for *B. subtilis*, 27% for *S. aureus*, and 66% for *P. aeruginosa*. When the concentration was reduced to 100µg/ml, *K. pneumoniae* and *S. aureus* showed no inhibition, whereas *B. subtilis* and *P. aeruginosa* exhibited 16% and 40% reductions, respectively. At the lowest concentration (10µg/ml), MOL lectin decreased adhesion by 27% in *K. pneumoniae*, 39% in *B. subtilis*, and 36% in *P. aeruginosa*, but had no observable effect on *S. aureus*.

The anti-adhesion effect of MOL lectin after 24 hours of treatment is as shown in Fig 2b. At a concentration of 500µg/ml, MOL lectin produced percentage reductions in biofilm adhesion as follows; *K. pneumoniae* (57%), *B. subtilis* (50%), *S. aureus* (39%), and *P. aeruginosa* (3%). At 100µg/ml, inhibition levels were *K. pneumoniae* (29%), *B. subtilis* (7.3%), *S. aureus* (19.3%), and *P. aeruginosa* (8.3%). At the lowest concentration of 10µg/ml, the reductions recorded were *K. pneumoniae* (35%), *B. subtilis* (15%), and *S. aureus* (16%), while *P. aeruginosa* showed no detectable anti-adhesion activity. None of the lectin extracts, including the positive control (ciprofloxacin, 2.5µg/ml), was able to completely prevent bacterial attachment, and in some cases, certain lectins appeared to promote cell adhesion rather than inhibit it.

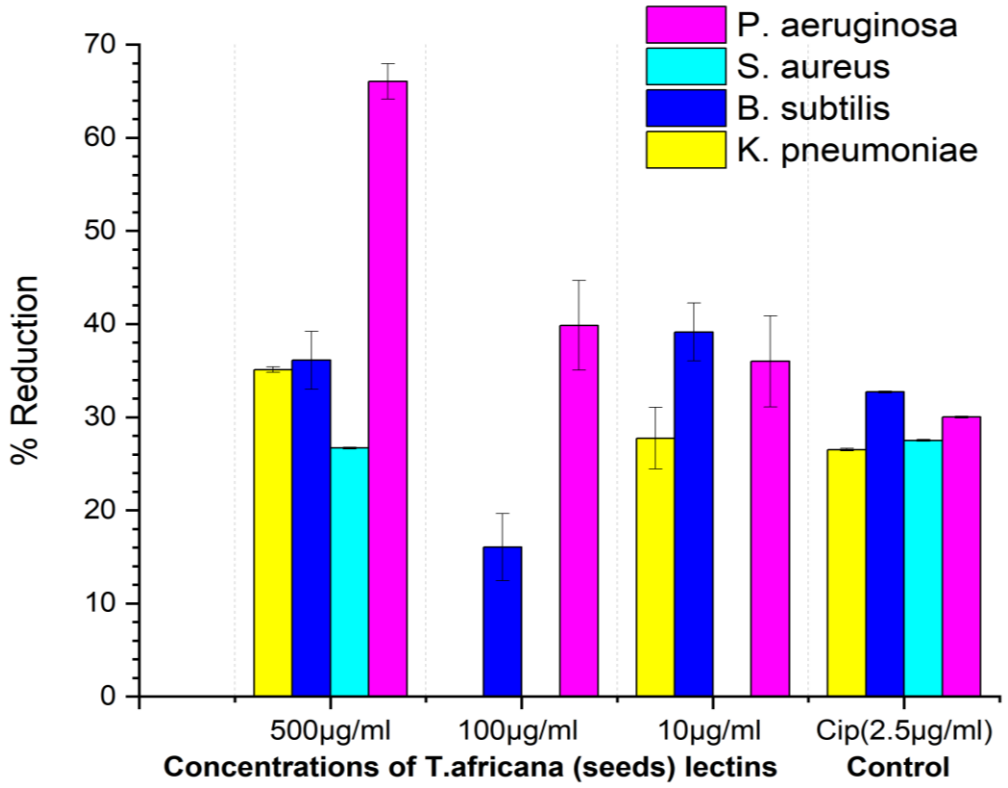

Figure 2a: Antiadhesion property of *Moringa oleifera* (leaves) lectins (in % reduction) at 4 hours exposure

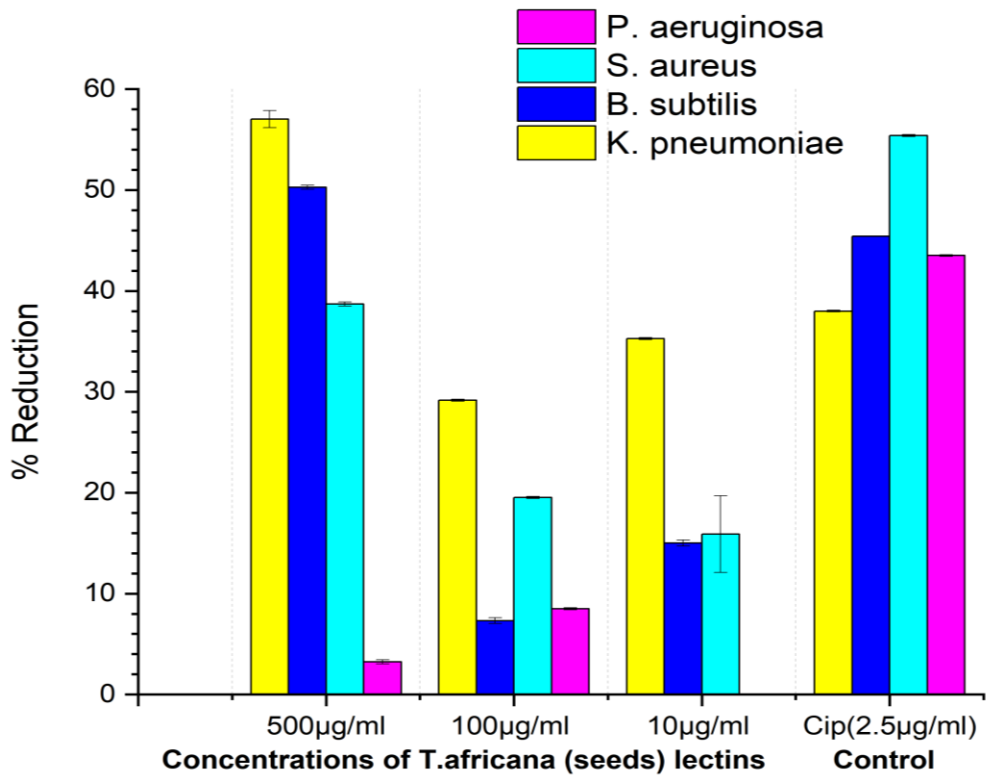

Figure 2b: Antiadhesion property of *Moringa oleifera* (leaves) lectins at 24 hours exposure

Anti-adhesion property of *Allium sativum* bulbs (ASL) lectins at 4 and 24 hours:

The effect of ASL lectin in inhibition of biofilm cell adhesion (at 4 hours) is shown in Fig 3**.** At a concentration of 500µg/ml and after 4 hours of exposure, the following anti-adhesion effects on the tested bacterial isolates were; *K. pneumoniae* (0%), *B. subtilis* (0%), *P. aeruginosa* (0%), and *S. aureus* (37%) (Fig. 3a). When the concentration was reduced to 100µg/ml, no inhibitory activity was observed against any of the isolates except *S. aureus*, which showed a 19% decrease in biofilm adhesion. At 10µg/ml, ASL lectin displayed no detectable anti-adhesion effect on any of the bacterial species. Notably, none of the plant lectins, including the positive control, ciprofloxacin (2.5µg/ml) completely prevented bacterial cell attachment.

The antibiofilm activity of ASL lectin after 24 hours of exposure is as shown in Fig 3b. At concentration of 500µg/ml, ASL lectin exhibited percentage reductions in biofilm adhesion as follows; no inhibition in *K. pneumoniae* (0%), 6.0% in *B. subtilis*, 22.3% in *S. aureus*, and 42.3% in *P. aeruginosa*. When tested at 100µg/ml, no inhibitory activity was detected in *K. pneumoniae*, *B. subtilis*, or *S. aureus*, while *P. aeruginosa* showed a 19% reduction. At the lowest concentration (10µg/ml), *K. pneumoniae* and *B. subtilis* exhibited no inhibition, whereas *S. aureus* and *P. aeruginosa* showed reductions of 2.4% and 20%, respectively.

Discussion:

Bacterial clumps known as biofilms are typically resistant to broad-spectrum antibiotics at normal therapeutic or even at higher dosages. Multi-drug resistance in biofilms, which cause the emergence of potentially fatal nosocomial infections, is on the rise and is becoming a major issue (5). From this study, the anti-adhesion effect of *T. africana* lectin extract indicates that it is limited at 4 hours but improved after 24 hours, without achieving complete inhibition. At 4 hours, the relatively higher reduction in biofilm observed in *S. aureus* compared to other isolates suggests that TAL lectin had a modest ability to interfere with early adhesion, whereas its negligible effect on *K. pneumoniae* and weak activity on *P. aeruginosa* reflect poor disruption of initial attachment. However, by 24 hours, the increased inhibition in *K. pneumoniae* and *B. subtilis* demonstrates that prolonged

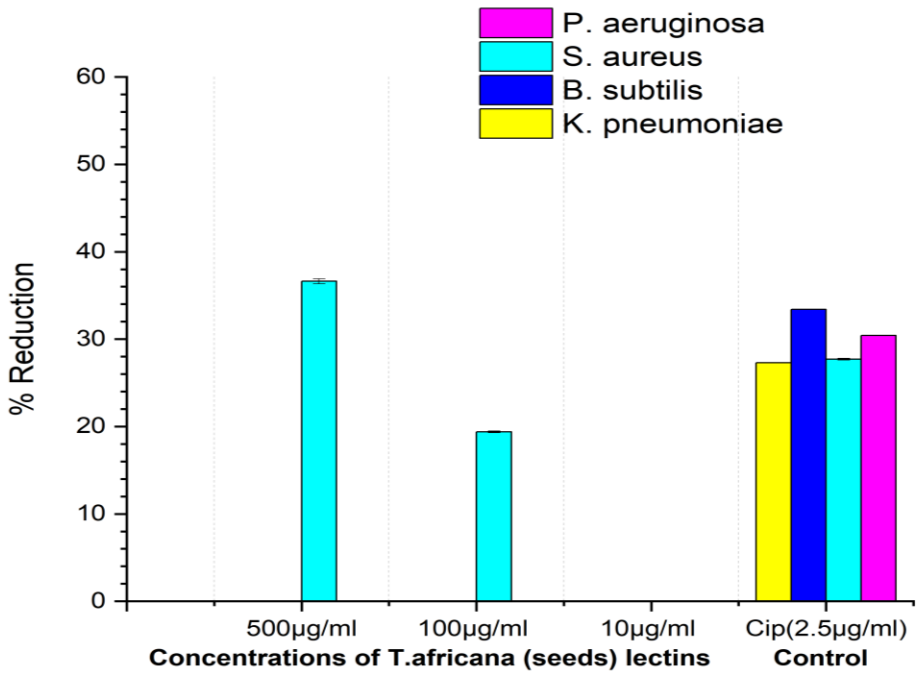

Fig 3a: Antiadhesion property of *Allium sativum* (bulbs) lectins at 4 hours exposure

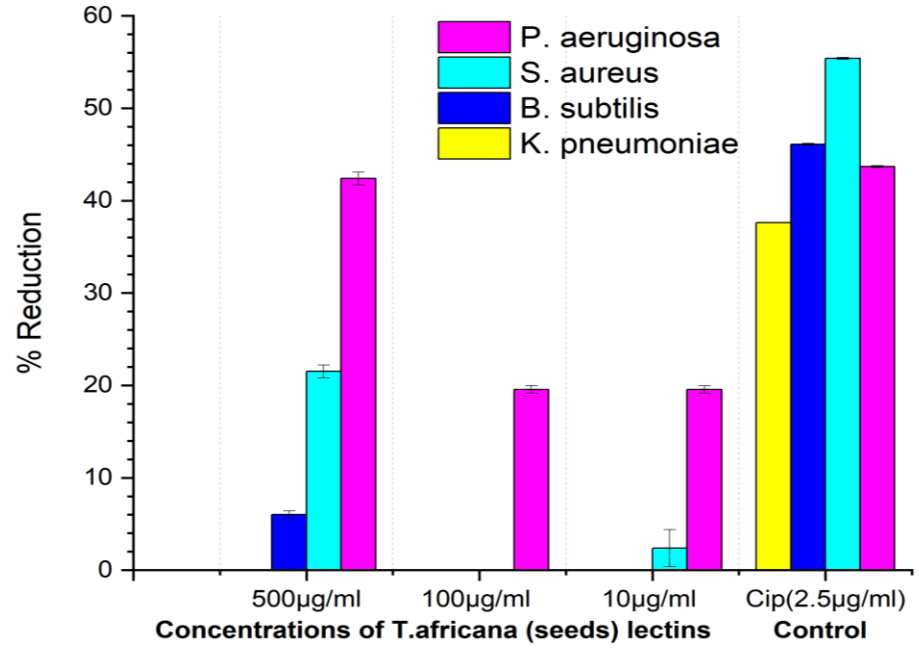

Fig 3b: Result showing antiadhesion property of *Allium sativum* (bulb) lectins at 24 hours exposure

exposure enhanced lectin interaction with bacterial surfaces, possibly affecting extracellular polymeric substance formation and cell signaling mechanisms essential for biofilm development (4,7,9).

Although this time-dependent improvement is partial, it supports the understanding that biofilm-associated bacteria possess adaptive resistance that limits complete inhibition (5). Furthermore, the differential response among the tested bacteria suggests that the activity of TAL is strongly influenced by bacterial cell

wall architecture. The relatively higher biofilm reduction in *S. aureus* and *B. subtilis* compared to *K. pneumoniae* and *P. aeruginosa* implies that structural accessibility of binding sites plays a role in lectin efficacy. In this context, the outer membrane of Gram-negative bacteria may restrict lectin penetration, whereas the exposed surface components in Gram-positive bacteria may facilitate stronger interactions, thereby explaining the observed variation in inhibition patterns.

Similarly, the results for *M. oleifera* lectin show a moderate but inconsistent anti-adhesion effect that varied with both time and bacterial species. At 4 hours, the strong inhibition observed in *P. aeruginosa* indicates that MOL lectin effectively interfered with early adhesion in this organism; however, the marked decline in activity at 24 hours suggests that this effect was not sustained as the biofilm matured. In contrast, the increased inhibition recorded for *K. pneumoniae* and *B. subtilis* at 24 hours compared to 4 hours indicates that prolonged exposure enhanced lectin–cell interactions in these species. Meanwhile, the relatively stable but moderate inhibition of *S. aureus* across both time points reflect partial susceptibility and adaptive resilience. Moreover, the inability of MOL lectin to completely prevent adhesion and the occasional reduction in effectiveness over time, reinforce the complexity of lectin–bacteria interactions and the adaptive capacity of biofilms. This observation aligns with earlier reports that disrupting initial adhesion is often more effective than targeting mature biofilms (22). Additionally, the variability in response among the isolates further supports the role of bacterial surface composition and biofilm-forming ability in determining lectin activity, consistent with the growing recognition of plant lectins as modulators of microbial attachment (23).

Meanwhile, the results for *A. sativum* lectin demonstrate comparatively weak and highly selective anti-adhesion activity. At 4 hours, the inhibition observed primarily in *S. aureus* indicates limited effectiveness in disrupting early adhesion in most organisms, while the absence of activity against *K. pneumoniae*, *B. subtilis*, and *P. aeruginosa* suggests minimal interaction with their cell surfaces. However, at 24 hours, moderate inhibition in *P. aeruginosa* indicates a delayed and organism-specific response, although the overall activity remained low. This restricted effectiveness contrasts with previous findings (24), highlighting variability in lectin activity across plant sources and bacterial targets. Nevertheless, the persistence of biofilm formation despite ASL lectin treatment emphasizes the resilience of biofilm-forming bacteria and the limited capacity of lectins to achieve complete inhibition. The weak performance of ciprofloxacin further supports this observation and underscores the challenge of biofilm-associated resistance (5). In addition, the possibility that lectins may enhance adhesion under certain conditions, as reported by Ofek et al., (25), provides a plausible explanation for the inconsistent inhibitory patterns, while the findings remain comparable to earlier reports on lectin activity against biofilms (26,27).

Conclusion:

The results of this study are significant because plant lectin may be used as a natural anti-adhesion agent to reduce early bacterial colonization, which is a crucial stage in developpment of infections.

Contributions of authors:

OUU and ESC were involved in all aspects of the study, including conceptualization, experimental design, data collection, analysis, and manuscript preparation; EK contributed to plant extraction as well as the preparation of stock solutions and standardization of test bacteria; NUV was responsible for plant extraction; AT and AAB handled statistical analysis; BRA was responsible for sourcing of plant materials; OB carried out the preparation of stock solutions and standardization of test bacteria; while EJO, OCS, EEC and EGO contributed to data interpretation and manuscript editing. All authors approved the manuscript submitted for publication.

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