Leaf extract of *Murraya koenigii* disrupts the biofilm strengthening amyloids of *Staphylococcus aureus*

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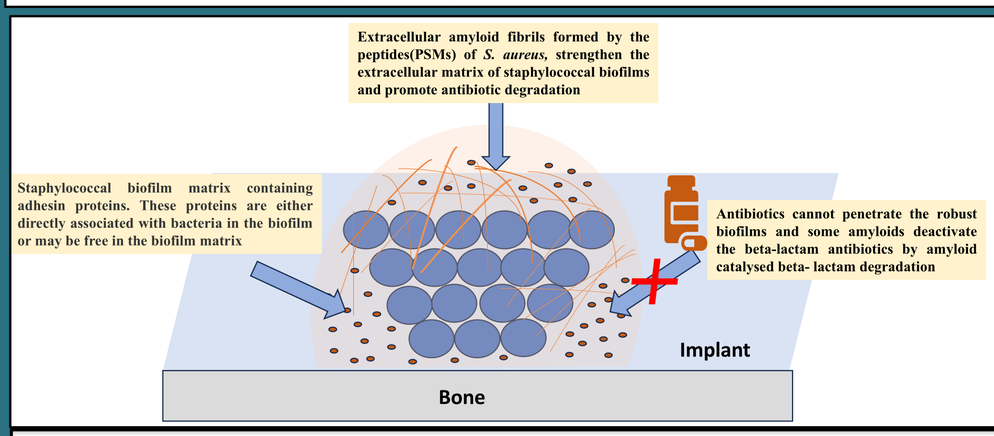
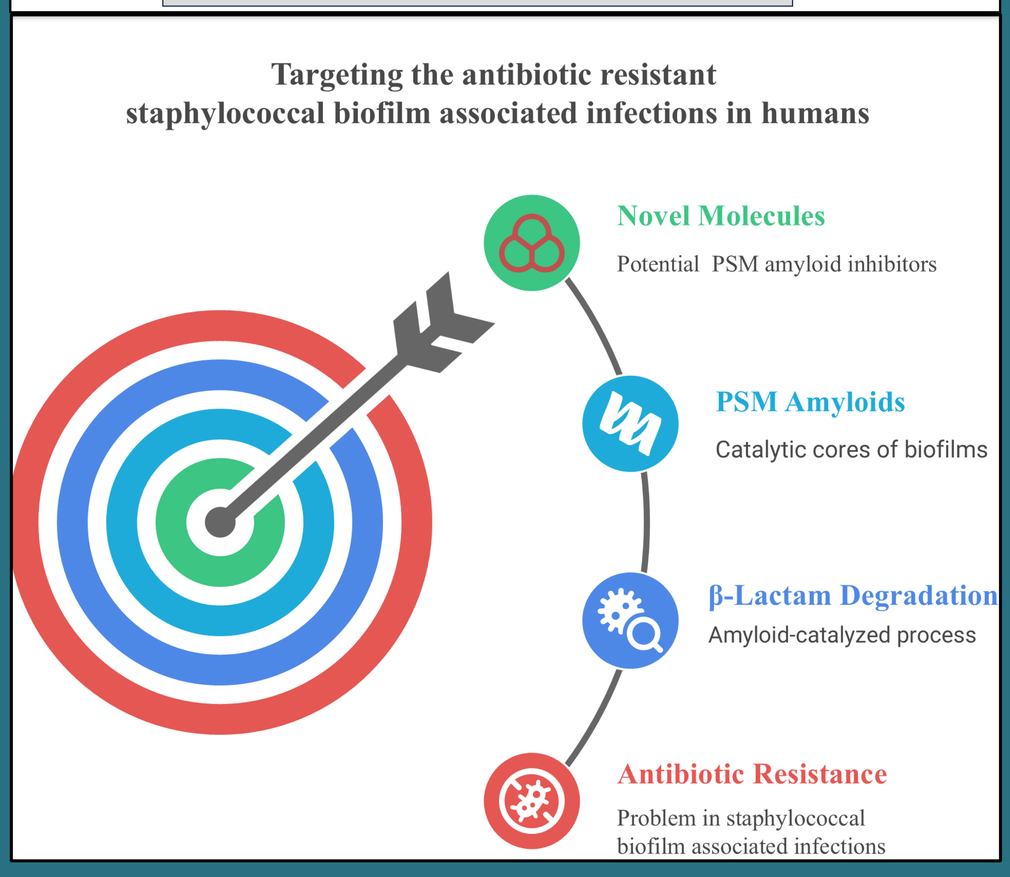
BACKGROUND

The prevalence of biofilm-associated multi-drug resistance among hospital acquired infections (HAIs) ranges from 17.9% to 100.0% worldwide. WHO has predicted that 1 in every 10 affected patients die from their HAI.

With the increasing reports of staphylococcal biofilm-associated HAIs, there is an impending urge of abrogating the assembly of functional amyloids which stabilize the biofilm architecture. Functional amyloids formed by the aggregation prone proteins and peptides of *Staphylococcus aureus*, fortify the biofilms and promote antibiotic degradation. These aggregates reinforce staphylococcal biofilms primarily by developing an extracellular amyloid fibrillar structure.

Antimicrobial resistance triggered by the biofilms of *S. aureus* is a global concern and emphasizes on a need to develop novel therapeutic agents. Herein, we have deciphered the role of phytocompound(s) present in the methanolic leaf extract of the Indian curry leaf plant, *Murraya koenigii* and we discovered a novel amyloid remodeling activity of this extract against the amyloids of *S. aureus* biofilm-associated peptides, phenol soluble modulins (PSM).

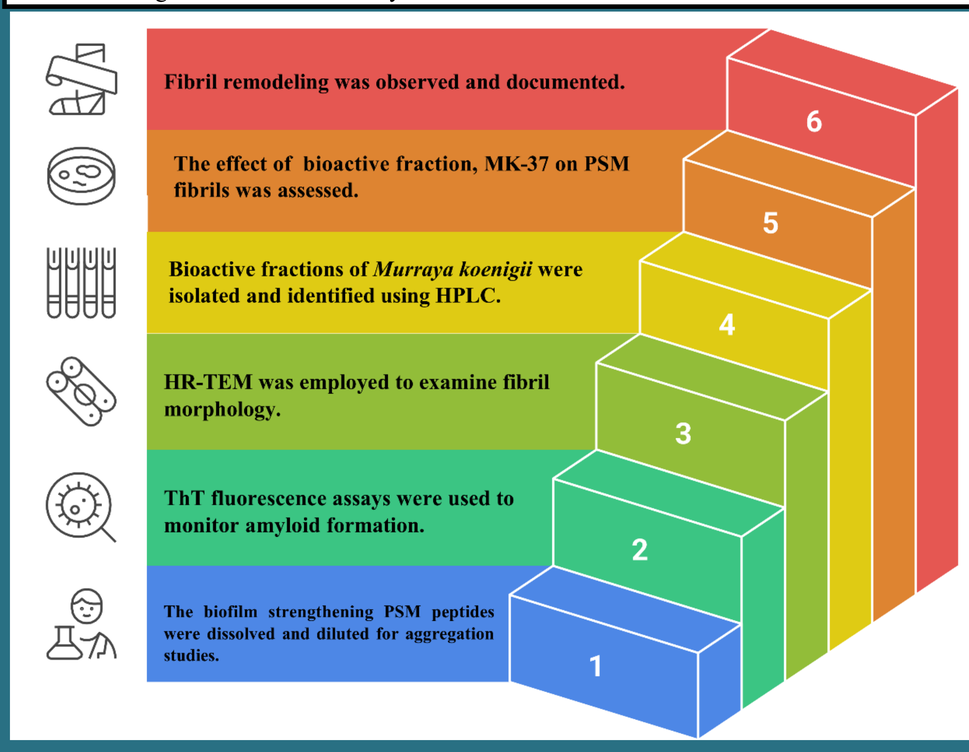




METHODS

We studied the novel amyloid modulatory and putative anti-biofilm potential of the methanolic leaf extract of *Murraya koenigii* against the PSM α1 amyloid fibrils using biophysical assays and high-end microscopy. The effective subfraction of *Murraya koenigii* leaf extract obtained after chromatographic separations, showing potent fibril remodeling activity has been named as MK-37.

The amyloid transformation of PSM α1 was monitored using Thioflavin T (ThT) kinetics and the formation of mature fibrils was confirmed using transmission electron microscopy (TEM). The fibril disaggregating potency of the methanolic leaf extract of *Murraya koenigii* was monitored and confirmed using ThT fluorescence assay and TEM.



RESULTS

1. Amyloid transformation of PSM α1, a biofilm scaffolding peptide

A PSM α1 peptide sequence: MGIIAGIIKVIKSLIEQFTGK

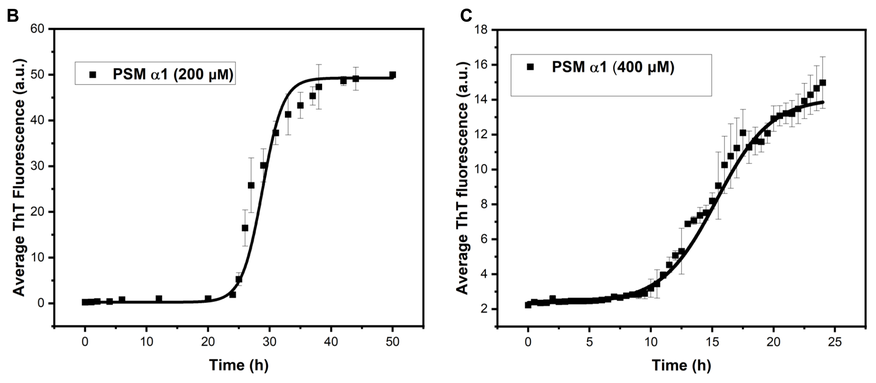
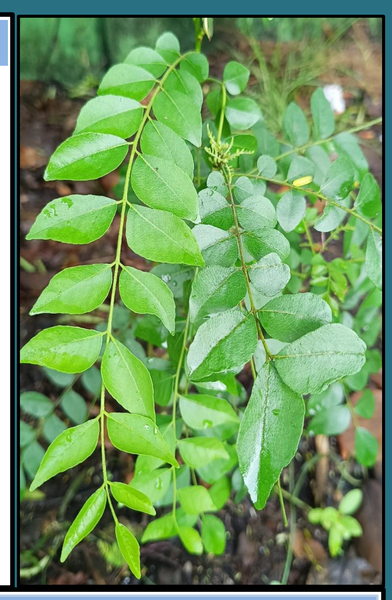


Figure: A Sequence of PSM α1. B Amyloid transformation of PSM α1 aggregating alone (black traces)showing a typical sigmoidal kinetics with high ThT maxima (200 μM concentration), C ThT kinetics of PSM α1 at 400 μM concentration.



2. Morphology of PSM α1 amyloid aggregates formed at the end-stage of ThT kinetics

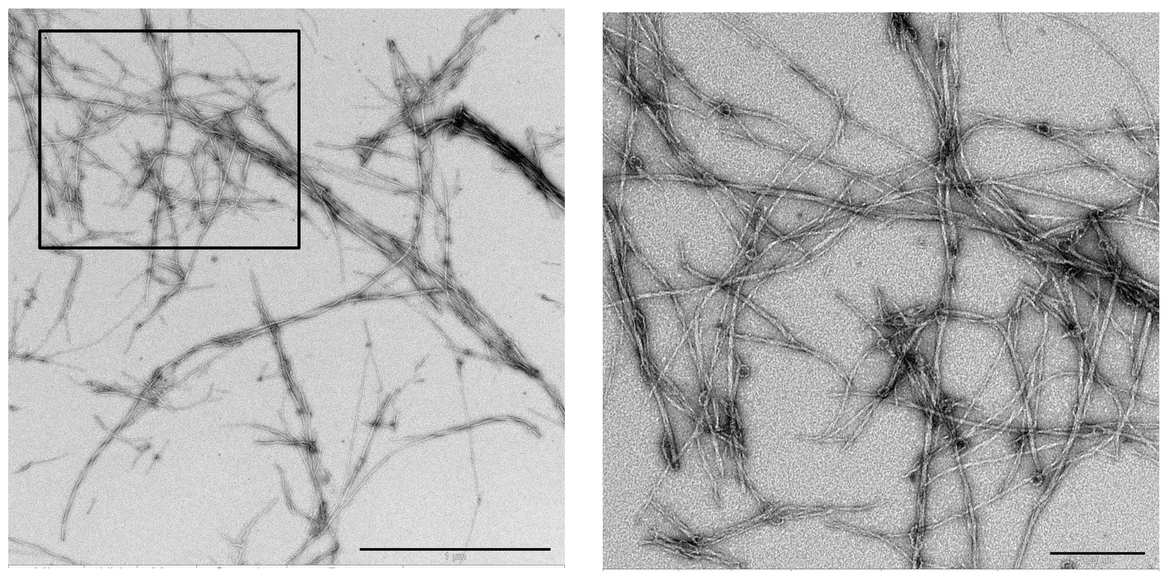


Figure: TEM Micrographs of end stage PSM α1 aggregates obtained at the saturation-stage of ThT kinetics. PSM α1 aggregating alone shows dense, laterally stacked fibrillar network. These mature amyloid fibrils formed by PSM α1 , strengthen the biofilms of *S. aureus.*

3. Effect of *Murraya koengii* leaf extract on the mature amyloid fibrils of PSM α1

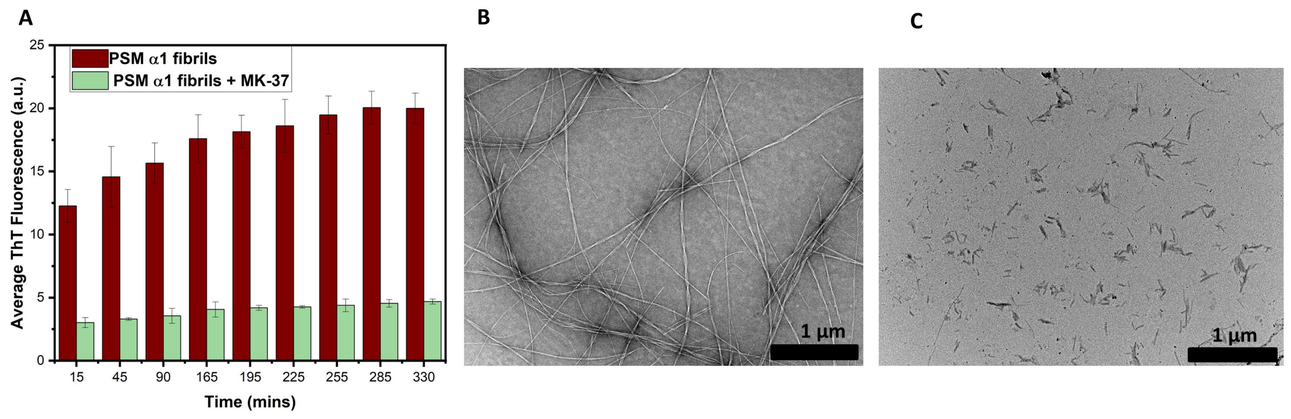
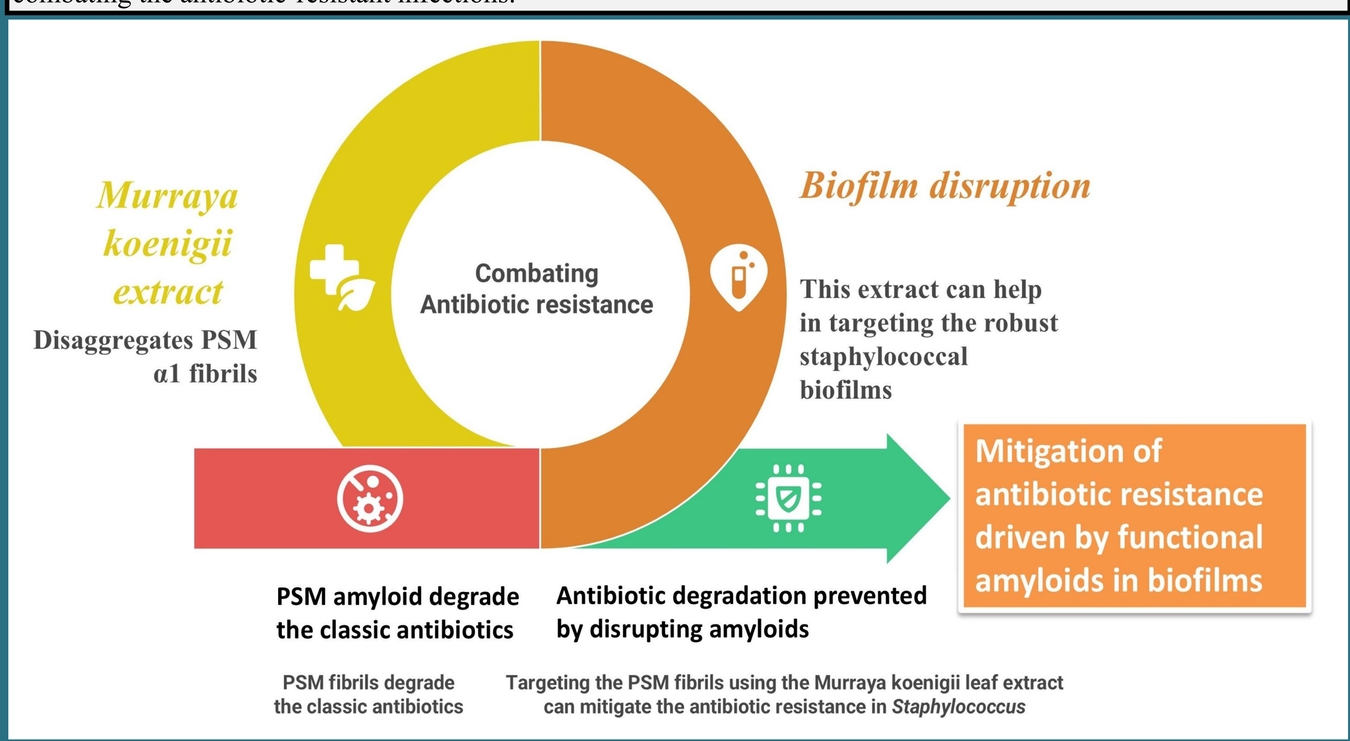


Figure: Effect of methanolic leaf extract of *Murraya koenigii* (MK-37) on the pre-formed amyloid fibrils of PSM α1. A. ThT fluorescence in the absence of MK-37 shows a high fluorescence indicating mature amyloid fibrils (red traces), but the matured amyloid fibrils treated with MK-37 show a remarkable decrease in the ThT binding depicted by a subdued fluorescence (light green traces). B. Mature amyloid fibrils of PSM α1. C. Disaggregated fibrils of PSM α1 after treatment with MK-37.

CONCLUSION

A novel activity has been identified in the methanolic leaf extract of the plant *Murraya koenigii.* The purified phytocompound(s) from this extract effectively interact with the mature amyloid fibrils of PSM α1 and promotes their disaggregation. The *in vitro* efficacy of purified phytocompound(s) in preventing the antibiotic degradation by PSM α1 fibrils will be determined in the further studies. These phytocompounds can be used as a scaffold for the rational discovery of potent biofilm inhibitors targeting the biofilm strengthening amyloids which can further help in combating the antibiotic-resistant infections.



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