Isoeugenol Modulates Expression Pattern of Transcriptional Regulators *MedA*, *SomA* and Conidial Hydrophobin Gene *RodA* in *Aspergillus fumigatus*

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Introduction

Aspergillus fumigatus is one of the major pathogenic fungal species, causing life-threatening infections. Due to a limited spectrum of available antifungals, exploration of new drug targets as well as potential antifungal molecules has become pertinent. Rodlet layer plays an important role in adherence of fungal conidia to hydrophobic cell surfaces in host, which also leads to *A. fumigatus* biofilm formation, contributing factor to fungal pathogenicity. The rodlet layer is composed of multiple hydrophobin (Rod) proteins encoded by their respective genes (*RodA-G*). RodA is essential hydrophobin responsible for outer layer permeability, stability, hydrophobicity and immune-inertia of the conidial cell wall surface. Also, regulatory proteins governing conidiation, adherence, cell wall homeostasis and biofilm formation in *A. fumigatus* includes Medusa (MedA) and transcription factor SomA.

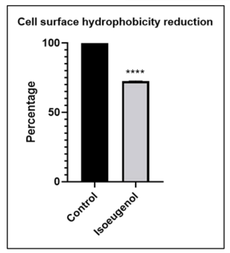
From decades, natural sources have been known for the development of new active molecules. Isoeugenol is a phenylpropanoid and isomer of eugenol which is generally found in plants like *Syzygium aromaticum*, *Myristica fragrans etc.* It has been reported to have antimicrobial, antioxidant, anti-tumor activities. Antifungal activity of isoeugenol has been reported against various *Aspergillus spp.* However, the mechanism of inhibitory action of isoeugenol against *A fumigatus* is yet to be explored. The present study investigates the effect of isoeugenol on genes responsible for hydrophobins (*RodA*), adhesion as well as biofilm formation (*MedA* and *SomA*) of *A. fumigatus*.

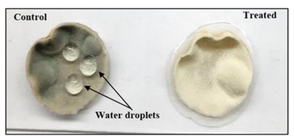
Experimental Section and Results

1. **The *in-vitro* antifungal drug susceptibility of the isoeugenol was calculated against *A. fumigatus* ATCC 45546 and its biofilm via CLSI M38-A2 microbroth dilution method for filamentous fungi:** Minimum inhibitory concentration (MIC) and subinhibitory concentration (IC50) of isoeugenol were calculated as 1.90 mM and 0.95 mM, respectively against *A. fumigatus*. Phenotypic estimation showed characteristic greenish-grey *A. fumigatus* conidia in positive control whereas isoeugenol treated well at IC_{50} depicted white pigment-less conidia.

2. **All biochemical, molecular assays and microscopic analysis of *A. fumigatus* were conducted to determine the hydrophobicity at calculated IC50 of isoeugenol.**

Biochemical Assays





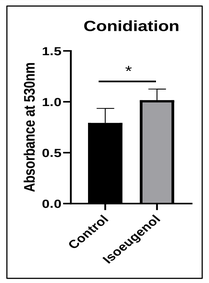


Fig. 1 A) Reduction in cell surface hydrophobicity (CSH) percentage of *A. fumigatus* conidia in the presence of isoeugenol. B) 10 µL of sterile water was dropped onto the surface of isoeugenol treated and untreated *A. fumigatus* culture. Loss of hydrophobicity is indicated by absorption of the water droplet into the culture.

Fig. 2 Evaluation of *A. fumigatus* conidia formation in presence of isoeugenol and graph depicted statistical increase in number of *A. fumigatus* conidia after treating with isoeugenol.

Molecular Assay (Relative gene expression via qRT-PCR**)**

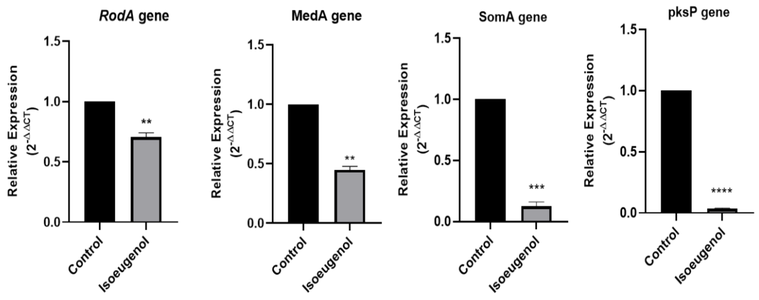


Fig. 3 Relative quantification of *RodA, MedA SomA* and *pksP* gene expression (normalised to house-keeping gene β-tubulin) in *A. fumigatus* treated with IC_{50} of isoeugenol. Data reported as mean of fold changes with standard deviation from three independent experiments amplified in triplicates. $p \leq 0.05$ was considered statistically significant.

Microscopic analysis

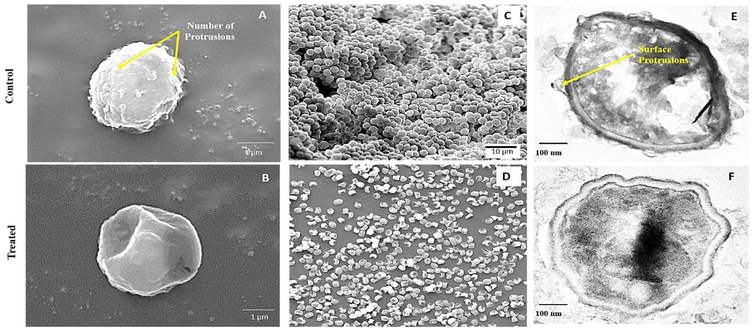


Fig. 4 Visualisation of scanning electron micrographs of *A. fumigatus* conidial surface with (A) presence of protrusions on wild-type conidia, whereas (B) surface morphology with absence of protrusions in isoeugenol treated conidia at magnification of 40K × (C) closely packed hydrophobic wild-type conidia (D) isoeugenol treated *A. fumigatus* conidia at 5K × magnification (E) and (F) loss of protrusions and electron dense melanin layer on *A. fumigatus* conidial cell wall in comparison to control (untreated) under transmission electron microscope.

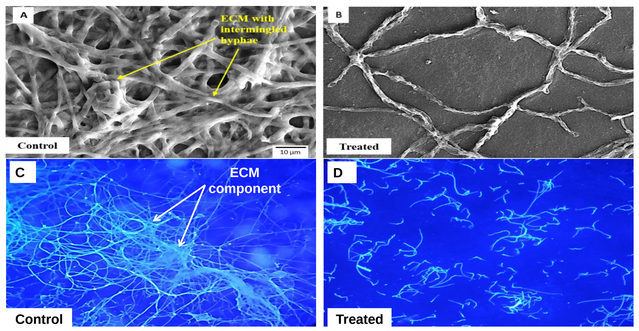


Fig. 5 Electron micrographs of *A. fumigatus* biofilm morphology (A) wild type control (without treatment); (B) isoeugenol treatment with lack of ECM and reduced hyphae at 2K × magnification; Fluorescence microscope image of *A. fumigatus* biofilm depicted (C) ECM components stained with calcofluor white dye in control; (D) disintegrated hyphae without ECM in isoeugenol treated sample at 40 × magnification.

***In-silico* studies**

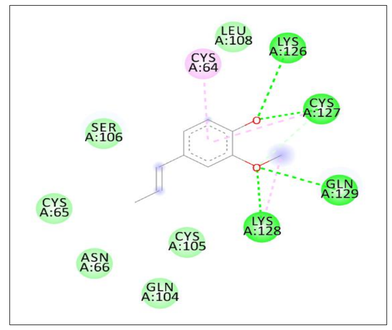


Fig. 6 Using AutoDock4 tool, binding interactions of isoeugenol with the active site of RodA hydrophobin protein target site (PDB ID: 6GCJ). Docking score of isoeugenol was -4.54 Kcal/mol. Green dotted lines depicted hydrogen bonds; pink dotted line showed pi-pi bond.

4. *In-silico* ADME/Tox study of phenolic compound Isoeugenol:

Phenolic compound isoeugenol has properties to become a drug, its drug likeliness score is -0.76. Isoeugenol showed drug-likeliness properties with no side effects on cardiovascular, lungs, liver, gastrointestinal systems. Similar results were reported by National Toxicology Program report. It has been approved for food use by the Food and Drug Administration when used in the minimum quantity required to produce its intended effect.

Conclusion

The present study concluded that isoeugenol is capable of inhibiting hydrophobin formation on *A. fumigatus* conidia, which is one of the crucial factors for adherence as well as initiation of infection in the host cell. The compound also prevents its biofilm formation. It downregulated the expression of *RodA* gene responsible for rodlet formation and transcriptional regulators *MedA* and *SomA* that regulates downstream genes responsible for adherence, virulence and biofilm formation in *A. fumigatus*. The compound also inhibits gene expression of *pksP* which is first gene responsible for DHN-melanin pigmentation in *A. fumigatus*.

References

1. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution testing of filamentous fungi, Approved standard- 2nd Ed. CLSI document M38-A2, Wayne, Pennsylvannia, USA, 2008.
2. Ferreira SB, Dantas TB, Silva DF, de Melo TR, Lima EO. *In vitro* antifungal effect of isoeugenol against *Penicillium citrinum* strains. MOL2NET, 2017; 3: 1-7.
3. Gravelat FN, Ejzykowicz DE, Chiang LY, et al (2010) Aspergillus fumigatus MedA governs adherence, host cell interactions and virulence. Cell Microbiol 12:473–488. https://doi.org/10.1111/j.1462-5822.2009.01408.x
4. Kumar CG, Mongolla P, Pombala S, et al (2011) Physicochemical characterization and antioxidant activity of melanin from a novel strain of Aspergillus bridgeri ICTF-201. Lett Appl Microbiol 53:350–358. https://doi.org/10.1111/j.1472-765X.2011.03116.x
5. Manavathu EK, Vager DL, Vazquez JA (2014) Development and antimicrobial susceptibility studies of in vitro monomicrobial and polymicrobial biofilm models with Aspergillus fumigatus and Pseudomonas aeruginosa. BMC Microbiology 14:53. https://doi.org/10.1186/1471-2180-14-53

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