Flavonoids Exhibit Anti-Biofilm Activity Against Multispecies Biofilm Commonly Present in the Cystic Fibrosis Lung

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Antibiotic resistant biofilms represent a major public health threat. Particularly, *Pseudomonas*, *Staphylococcus*, and *Burkholderia* species form mixed biofilms in cystic fibrosis (CF) lungs that drive inflammatory response. Flavonoids have exhibited anti-biofilm activity against some *Staphylococcus* and gram-negative species, although less explored against multispecies biofilm. The aim was to evaluate the anti-biofilm activities of myricetin, quercetin, rutin, and epigallocatechin gallate (EGG) flavonoids against common, mixed species biofilms in the CF lung. Biofilm formation of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia pyrocinnia*, both individually and co-cultured (including a combination of all three pathogens), was assayed in 96-well polystyrene plates, incubated in the presence or absence of flavonoids. Quantification was achieved via crystal violet staining (absorbances recorded at 570 nm). Biofilm viability was assessed using the resazurin cell viability (AlamarBlue) assay, and a cytotoxicity assay on A549 human alveolar epithelial cells was conducted to confirm treatment safety. Mixed species biofilms were resistant to various broad-spectrum antibiotics including, amoxicillin/clavulanic acid, Ceftazidime, Fosfomycin, Linezolid, and Gentamicin. Conversely, all flavonoids demonstrated >60% inhibition efficacy at <20 µg/mL against individual species. Of flavonoids inhibiting >50% of biofilm at <20 µg/mL against mixed species, myricetin (10 µg/mL) demonstrated greatest (81% ± 1%) efficacy against *P. aeruginosa-S. aureus* formation with 82% ± 1% reduced viability. Rutin (10 µg/mL) exhibited greatest (86% ± 2%) efficacy against *P. aeruginosa-B. Pyrocinnia* and 83% ± 2% against *S. aureus-B. pyrocinnia* with reduced viabilities of 89% ± 1% and 79% ± 1%, respectively. Combination of all flavonoids (10 µg/mL) exhibited 92% ± 1% efficacy against *P. aeruginosa-S. aureus-B. pyrocinnia* biofilm with 90% ± 1% reduced viability. Treatment safety was supported by mammalian cytotoxicity assay. These findings support the efficacy of a combinatorial flavonoid-based therapy in conjunction with antibiotics to alleviate multispecies biofilm proliferation. Effective treatment strategies are crucial for combating multidrug-resistant infections within the diverse and biofilm-centric CF lung microbiome.