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The effect of flavonoids from Malaysian propolis in regulating *Enterococcus faecalis* biofilm virulent gene expressions

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Background: *E. faecalis* is a gram positive facultative anaerobe associated with nosocomial infections, implanted devices and dental infections. The pathogenesis of *E. faecalis* is enhanced by its ability to form biofilms which is a virulent factor. Propolis is composed of resins, essential oils, bioflavonoids, phenols and aromatic compounds and is known to have bactericidal properties derived from bee hives. This study addresses the effect of Malaysian propolis on biofilm production and anti-bacterial activity.

Materials Extracts: Malaysian propolis was obtained from local bee farms and extracted using ethanol or ethyl acetate (20% w/v).

HPLC: The components in the extracts were evaluated by analytical HPLC methods using High Resolution column (RP-18e 100x4.6 mm).

Biofilms: *E. faecalis* was grown in tryptic soy broth/media with glucose in 96-well/ 24-well plates for 24 hours to form biofilms with/without different concentrations of propolis extracts. *E. faecalis* biofilm formation was evaluated by crystal violet staining and enumeration of colony forming units in the biofilm. OD₆₀₀ was used for planktonic forms.

QPCR: The expression of the genes involved in biofilm formation was analyzed by quantitative real time PCR using 16s rRNA gene expression as internal control.

Results: Ethanol and ethyl acetate extracts (20 µg-100 µg/ml) of Malaysian propolis were able to inhibit biofilm formation by *E. faecalis* at lower concentrations and were bactericidal at higher concentrations *in vitro*. Ethanolic extract was found to be more effective than ethyl acetate extract. Analytical studies using HPLC identified the presence of flavonoids, however their quantities were different between the ethanol and ethyl acetate extracts. QPCR analysis was done to the effect of propolis in modulating the expression of genes associated with virulence and biofilm formation in *E. faecalis* ((BopD, RecA, Efa, EBpA, AsA, CylB, CylL1, Gls24, etc).

Conclusion: Genes associated with biofilm formation from *E. faecalis* are potent virulent factor. Propolis proves to be a potent natural antibiotic that can inhibit biofilm formation, thereby rendering the bacteria sensitive to antibiotic treatment. The Malaysian propolis extracts can be developed as prospective agents for therapeutic interventions.

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