INTRODUCTION:

The link between Streptococcus mutans and dental caries is well documented. The use of natural plant products in the treatment of oral diseases is gaining popularity. One plant that has gained recognition as a source of traditional medicine is Dodonaea viscosa var. angustifolia. The aim of this study was to analyse the phytochemical constituents of D. viscosa var. angustifolia (DVA) and establish their beneficial effects against S. mutans.

MATERIALS AND METHODS

Cultures of S. mutans ATCC 10923 and SM1 were obtained from the Oral Microbiology laboratory and the DVA was collected from the Pypeklipberg, Mkhunyane Eco Reserve, South Africa. Dry DVA leaves were extracted with methanol. The crude extract was fractionated into six fractions (F1-F6) using silica gel column chromatography and thin layer chromatography. The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of the crude extract and six fractions were determined using microtitre plate dilution technique. The effect of the crude extract and fractions on biofilm formation and acid production were investigated using standard techniques. The bioautography technique was also used to identify fractions with bioactive compounds. The most active fraction (F5) was further fractionated and purified into two subfractions, 5.1 and 5.2. Both subfractions were further screened to identify the most beneficial subfraction (5.1). Subfraction 5.1 was identified and elucidated using GC-MS and NMR. The effect of the purified compound on biofilm formation and acid production on S. mutans was repeated to establish reproducibility of the results. Cytotoxic effect of the crude extract and identified subfraction (5.1) was studied using human
embryonic kidney cells (HEK). The results were analyzed using Wilcoxon rank-sum test (Mann-Whitney).

Results

The MIC and MBC of the six fractions and crude extract ranged from 0.39 to 12.5 mg/ml. On preliminary screening of 6 fractions, F5 showed lowest MBC of 0.39 mg/ml and highest total activity value of 2000. In addition, at 0.2 mg/ml, F5 reduced biofilm formation by 93.3% and reduced acid production in S. mutans. Purification of F5 produced subfraction 5.1 and 5.2. Subfraction 5.1 showed higher antimicrobial activity (MIC-0.05 mg/ml) compared to the crude extract (MIC-0.78 mg/ml) and subfraction 5.2 (MIC-0.78 mg/ml). At a concentration of 0.05 mg/ml, subfraction 5.1 exhibited an inhibitory effect on biofilm formation at both 6 hours (94% reduction) and 24 hours (99% reduction) which was higher compared to the crude extract (87% reduction at 0.78 mg/ml after 6 hours). Subfraction 5.1 also exhibited a higher inhibitory effect on acid production compared to the crude extract. Subfraction 5.1 was identified as, 5,6,8-Trihydroxy-7,4\textsuperscript{1}-dimethoxyflavone. Cytotoxicity analysis of the crude extract and subfraction 5.1 (5,6,8-Trihydroxy-7,4\textsuperscript{1}-dimethoxyflavone) on HEK 293 cells showed IC\textsubscript{50} values of 0.09 mg/ml and 0.03 mg/ml respectively.

Conclusion

Phytochemical analysis of D. viscosa var. angustifolia produced an anticariogenic constituent, 5,6,8-Trihydroxy-7,4\textsuperscript{1}-dimethoxyflavone. The compound showed improved antimicrobial and anticariogenic activity at lower concentrations than the crude extract. At subinhibitory concentrations, the compound significantly inhibited biofilm formation and acid production by S. mutans. Cytotoxicity analysis established the safe use of this newly isolated compound therefore it has potential to be used in the oral cavity to prevent dental caries.