Chemical and antioxidant characterization of the chemopreventive properties of fermented and unfermented rooibos tea against UVB induced skin damage

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Introduction: The use of rooibos (*Aspalathus linearis*), a South African herbal plant, as a natural remedy in the form of tea has significantly increased due to its unique polyphenol composition and health promoting properties such as anti-mutagenic, anti-cancer and, anti-inflammatory responses.

Aim: The current study aimed to compare the chemical composition of ethanol and aqueous extracts from fermented and unfermented rooibos by HPLC analyses. Additionally, extracts were assessed in UVB pre-exposure skin keratinocyte models as a preventative measure of UVB induced damage.

Methodology: Ethanol and aqueous rooibos extracts were prepared through steeping, filtering, and rotary evaporation/freeze-drying in a modified method. The ethanol extracts were further purified and fractionated through reverse-phase column chromatography with a XAD-4 matrix. TLC analysis was used to determine the presence of aspalathin and nothofagin in the fractions. Characterization of the chemical and antioxidant content of the aqueous and ethanol extracts was achieved through quantifying the monomeric flavonoids using HPLC and photometric analyses to determine the total phenolic content. The experimental conditions of the UVB pre-exposure model were optimized by determining the seeding efficiency of HaCaT cells, time (6hrs vs 24hrs), and treatment measures (0.5 % vs 1% DMSO for extract solubilisation). The aqueous rooibos extracts and ethanolic fractions were then screened for biological activity (IC50 concentration) against cell viability (ATP).

Results: Comparison of ethanol extracts indicated that the unfermented (EUF) rooibos contained the highest levels of polyphenols suggesting high levels of antioxidant activity. In contrast, the aqueous extracts of both unfermented (AUF) and fermented (AF) rooibos displayed significantly lower levels of polyphenols. Column fractionation of both EUF and fermented ethanol (EF) rooibos yielded 4 fractions (UF1 - UF4 and F1 - F4 respectively) of decreasing polarity with the most polar fractions UF1, UF2, F1, and F2 containing minimal traces of polyphenols. Fraction UF3 and F3 exhibited high levels of aspalathin followed by UF4 and F4 containing the highest nothofagin content. Optimal seeding efficiency for HaCaT cells was achieved at 24hrs, with 0.5% DMSO treatment. The biological activity of the aqueous extracts exhibited higher reductions in cell viability in a dose-dependent manner whereas the polyphenol enriched ethanol fractions F3 and UF 3, containing higher levels of aspalathin and nothofagin, exhibited lower activity.

Discussion and Conclusion: The ethanol extraction method proved more effective in extracting the major rooibos polyphenols aspalathin and nothofagin. Characterization of the polyphenol composition of both aqueous and ethanol fractionated rooibos extracts suggested that the recovery of specific bioactive polyphenols is dependent on the extraction methods and solvents. To further characterise the underlying anti-inflammatory mechanisms of rooibos in response to UVB irradiation, a detailed analysis of the proteome, lipidome, and selected transcriptomic profiles of both fermented and unfermented ethanol and aqueous rooibos teas in the UVB pre-exposure model will be investigated.