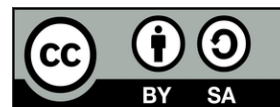




Research article



ANTIOXIDANT POTENTIAL AND PHENOLIC COMPOSITION OF COLUMN-CHROMATOGRAPHIC FRACTIONS FROM *GYMNEMA INODORUM* LEAF EXTRACT

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ABSTRACT

Gymnema inodorum (Lour.) Decne, a plant traditionally used in Northern Thailand for its antidiabetic properties has shown potential as a source of bioactive compounds with antioxidant activity. Despite its traditional use, the phenolic profile and specific antioxidant contributions of *Gymnema inodorum* fractions remain inadequately characterized. Given the growing interest in identifying plant-based molecules to combat oxidative stress, further exploration is necessary. This study aims to explore the antioxidant properties and phenolic profile of column chromatographic fractionated *G. inodorum* leaf extract. The ethanolic reflux extracts of *G. inodorum* leaves were subjected to column chromatography for the isolation of bioactive molecules. Total phenolic and flavonoid contents were assessed using Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively. Total antioxidant activity was evaluated by employing different antioxidant assays, including DPPH, ABTS scavenging assay and FRAP assay. High-Performance Liquid Chromatography (HPLC) with a C18 column was employed to determine the level of gymnemic acid, while Liquid Chromatography Quadrupole Time-of-Flight/Mass Spectrometry (LC-QTOF/MS) was used to identify potential phenolic compounds. The three separated fractions obtained after column chromatographic separation were designated as Fraction A (fraction 1-4, eluted with low polarity mobile phase), Fraction B (fractions 5-10, intermediate polarity), and Fraction C (fractions 11-12, higher polarity) based on UV absorbance profiles. These fractions exhibited remarkable antioxidant activity, with total phenolic content of 0.294 ± 0.056 , 0.085 ± 0.039 , and 0.227 ± 0.041 mg QE/mL, and total flavonoid content of 0.274 ± 0.050 , 0.309 ± 0.110 , and 0.384 ± 0.007 mg QE/mL, respectively. Fraction A exhibited the highest DPPH and ABTS scavenging activity, while fraction C demonstrated significantly higher ferric-reducing power. HPLC analysis revealed a highest concentration of gymnemic acid in fraction C (0.393 ± 0.003 mg/g) compared to fraction B (0.159 ± 0.003 mg/g) and fraction A (0.142 ± 0.000 mg/g). The LC-QTOF/MS phenolic screening showed seven potential phenolic compounds in fraction A, seven compounds in fraction B,

and four compounds in Fraction C. Pearson correlation analysis using the individual replicate measurements underlying Table 1 and Table 2 ($n = 9$) showed a significant positive correlation between total phenolic content and DPPH scavenging ($r = 0.68$, $p = 0.044$), and significant correlations between total flavonoid content and DPPH ($r = -0.79$, $p = 0.012$), ABTS ($r = -0.95$, $p < 0.001$), and FRAP ($r = 0.81$, $p = 0.009$), suggesting that phenolic and flavonoid compounds are important contributors to the antioxidant activity of *G. inodorum*.
