

Antioxidant Potential and Chemical Profiling of *Citrus hystrix*

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Structured Abstract

Background: *Citrus hystrix* (kaffir lime) is traditionally used in Southeast Asia, yet its antioxidant properties are not well studied. This research evaluated the antioxidant activity and chemical composition of *C. hystrix* essential oil (EO) for its potential use in food, cosmetics, and pharmaceuticals. Four in vitro antioxidant assays were used: DPPH, FRAP, β -carotene bleaching (BCB), and total phenolic content (TPC). While the DPPH assay showed a negative scavenging value (−74.23%), the FRAP assay indicated strong reducing activity (851.33 μ M Trolox equivalent), and the BCB assay demonstrated 74.32% inhibition, close to L-ascorbic acid (75.33%). TPC was 1.741 mg gallic acid equivalent/g EO. GC-MS identified major constituents like d-limonene (48.64%), β -pinene (22.22%), and sabinene (14.56%). Statistical analysis revealed significant differences between assays ($p < 0.05$), with a positive correlation between TPC, FRAP, and BCB values.

Methods: The essential oil was extracted via hydrodistillation at FRIM. Antioxidant activity was tested using four assays. EO was dissolved in DMSO (1 mg/mL). In DPPH assay, samples were incubated with 0.1 mM DPPH solution and absorbance measured at 517 nm. FRAP assay used TPTZ, FeCl_3 , and acetate buffer, with readings at 593 nm. BCB assay measured absorbance at 470 nm. TPC assay used Folin–Ciocalteu reagent with absorbance at 765 nm. GC-MS analysis identified compounds using the NIST2011 library. All tests were run in triplicate and analyzed via SPSS (one-way ANOVA, $p < 0.05$).

Results: EO showed high antioxidant activity via FRAP and BCB. TPC confirmed the presence of phenolics. DPPH assay yielded a negative value due to compound interference. GC-MS identified 19 compounds, with limonene and β -pinene as major constituents.

Conclusion: *C. hystrix* EO exhibits antioxidant potential, making it a promising natural antioxidant source for further application and research.

Keywords: *Citrus hystrix*, Essential Oil, Antioxidant Activity, GC-MS, Natural Antioxidants

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