ACTIVITY-GUIDED PARTIAL PURIFICATION OF FREE RADICAL SCAVENGING COMPONENTS OF *Citrus hystrix*

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ABSTRACT

Citrus fruits have high antioxidant activity due to active compounds such as vitamins, carotenoids, flavonoids and phytochemicals. Besides the flesh, citrus peels are also rich source of bioactive compounds such as flavonoids and phytochemicals. The main objective of this study was to partially purify and separate the free radical scavenging compounds and phenolic compounds in the peels of *Citrus hystrix*. The peels of *Citrus hystrix* were extracted with 77% ethanol. The crude extract was then partially purified into hexane, chloroform, *n*-butanol, and formic acid fractions using liquid-liquid partitioning chromatography. Among all the fractions as well as crude extract, the chloroform fraction showed the highest antioxidant activity. The chloroform fraction was then subjected to further purification using column chromatography, giving sub-fractions I, II, III, and IV. The eluents from the column was monitored using thin layer chromatography. All the sub-fractions showed the presence of free radical scavenging compounds and phenolic compounds when bioautographic reagents were sprayed. SF III and SF IV showed higher DPPH radical scavenging activities than SF I and SF II, whereas SF IV showed highest phenolic content among all the sub-fractions. After that, HPLC identification and quantification were performed on all the sub-fractions. It was found that ascorbic acid, catechin, hesperetin, apigenin and tannic acid were present, which were believed to contribute to the antioxidant activity.