

**DEVELOPMENT OF A HIGH-PERFORMANCE THIN
LAYER CHROMATOGRAPHY (HPTLC)-
DENSITOMETRIC METHOD FOR THE ESTIMATION OF
QUERCETIN AND KAEMPFEROL IN *Euphorbia hirta*
(TAWA-TAWA) EXTRACT**

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ABSTRACT

A sensitive, fast, efficient and repeatable high-performance thin-layer chromatographic (HPTLC) method has been developed for simultaneous analysis of quercetin and kaempferol from the extracts of the aerial parts of *Euphorbia hirta*. In the development of the HPTLC method, different extraction parameters were evaluated and quantification of quercetin and kaempferol in the extract of *E.hirta* was performed using a densitometric software, Quantiscan™. Among the different combinations of mobile phases used, best separation was achieved in toluene: ethyl acetate: formic acid: methanol (5: 6: 2: 1) solvent system. *E.hirta* was extracted using the developed extraction method which involves the use of 80% ethanol, 8 minutes of sonication and serial extraction until 3rd extraction. This determined method provides a maximum yield of the compounds at minimum time and cost. The method was also validated for linearity and precision specifically repeatability. The results show that the produced calibration is linear, as supported by the obtained correlation coefficient (r) of > 0.999 and coefficient of determination (R^2) of > 0.997. Moreover, the obtained slopes of the calibration curves show that the method is most sensitive towards quercetin and least sensitive to gallic acid. The method was also found out to be repeatable and precise as the %RSD for both the inter-day and intra-day trials obtained a value less than $\leq 2\%$. The present method is being reported for the first time and can be used for routine quality control and quantification of quercetin and kaempferol in various plant samples, extracts, and market formulations.