

ABSTRACT

Red spinach (Amaranthus tricolor L.) is a vegetable plant rich in secondary metabolites, particularly flavonoids, which exhibit potential antioxidant activity. This study aimed to identify the secondary metabolites present in red spinach, determine the total flavonoid and total phenolic contents, evaluate the antioxidant activity, and isolate flavonoid compounds from the ethyl acetate fraction of red spinach leaves. The study was initiated with plant identification, sample preparation, phytochemical screening, maceration extraction using ethanol, and fractionation to obtain the ethyl acetate fraction. The ethyl acetate fraction was analyzed for total flavonoid content using the aluminum chloride (AlCl₃) colorimetric method, total phenolic content using the Folin–Ciocalteu method, and antioxidant activity using the DPPH radical scavenging assay. Flavonoid isolation was carried out by thin-layer chromatography (TLC), gravity column chromatography, and preparative TLC. The purity of the obtained isolate was evaluated by analytical TLC using various solvent systems, followed by structural characterization using UV–Visible spectrophotometry and shift reagent analysis. The results showed that red spinach leaves contained flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids. The yield of the ethanol extract was 6.97%, while the ethyl acetate fraction yielded 15.7%. The ethyl acetate fraction exhibited a total flavonoid content of 37.287 ± 0.040 mg QE/g fraction, a total phenolic content of 67.2 ± 0.105 mg GAE/g fraction, and an IC₅₀ value of 175.95 mg/L, indicating weak antioxidant activity. Based on the UV–Visible spectral characteristics and the responses to shift reagents, isolate E2 was tentatively identified as a flavone containing hydroxyl groups at the C-5 and C-7 positions (5,7-dihydroxyflavone).

Keywords: *Amaranthus tricolor L., isolation, flavonoids, ethyl acetate fraction, antioxidant activity*