ABSTRACT

Hyptis brevipes is one of the species of the Hyptis genus, known to contain a variety of bioactive compounds such as flavonoids, terpenoids, lactones, and steroids. The leaves of this plant are traditionally used to treat asthma, malaria, and show anticancer potential. The ethyl acetate fraction (F_{EA}) of Hyptis brevipes leaves has been reported to exhibit high antioxidant activity with an IC_{50} value of 8.03 µg/mL. This activity indicates the presence of potential phenolic compounds that have not yet been fully identified, including the possible presence of lithospermic acid A, which was previously isolated from Hyptis radicans, a closely related species in terms of chemotaxonomy. This study aims to isolate and identify phenolic compounds in the F_{EA} of Hyptis brevipes leaves. Phytochemical screening showed that F_{EA} contains flavonoids and phenolic compounds. The isolation process began with determining the best solvent system using thin-layer chromatography (TLC), followed by separation using gravity column chromatography (GCC) with a solvent mixture of ethyl acetate:methanol (5:1). One gram of F_{EA} yielded 90 small fractions, which were then grouped into five major fractions (A–E) based on TLC spot patterns. Fraction A was further separated by preparative TLC, yielding isolate A at 3 mg (0.3%). Isolate A showed a single spot on TLC with three different eluent systems, indicating the purity of the compound. UV-Vis spectroscopy showed maximum absorption at 291 and 327 nm, characteristic of aromatic compounds. FTIR analysis revealed the presence of O-H, C=O, aromatic C=C, and C-O groups, confirming that the compound belongs to the phenolic class. LC-MS/MS showed a dominant peak with a retention time of 2.4 minutes and a molecular ion $[M–H]^-$ of 537 m/z. Based on the combined spectroscopy data and comparison with references, isolate A was identified as lithospermatic acid A, a phenolic compound that may contribute to the antioxidant activity of F_{EA} from Hyptis brevipes leaves.

Keywords: *Lithospermic acid A; Hyptis brevipes; ethyl acetate fraction; isolation*