



Isolation of the Antioxidant Metabolite from the EAF

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ABSTRACT

EAF (4.13 g), which showed the most antioxidant activity, was subjected to column chromatography on a silica gel column chromatography (CC), employing a step gradient of CH₂Cl₂-CH₃OH (10:1, 10:2, 10:3, 10:5, 1:1, 0:1, v/v) and afforded eleven fractions (Fr. 1-Fr. 9) (Fig. 1). Fr. 2 was subjected to open silica gel CC using a successive elution of EtOAc-CH₃OH (10:1-0:1, v/v) to yield fractions Fr. 2.1-2.4. Fr. 2.2 and 2.4 were isolated using Sephadex LH-20 CC/ODS-HPLC to afford **6** (8.1mg, 0.20%) and **7** (20mg, 0.48%). Fr. 4 was subjected to polyamide CC using CH₂Cl₂-CH₃OH-HCOOH as eluent (10:2:1, v/v). Promising subfraction Fr. 4-3 was eluted using CH₃OH-H₂O (1:1-1:0, v/v) with RP C-18 CC. Final purification was achieved by polyamide CC using CH₂Cl₂-CH₃OH (10:4, v/v) to yield **1** (0.803 g, 19.44%). Fr. 5 was subjected to polyamide CC with CH₂Cl₂-EtOAc-CH₃OH(5:5:1,v/v) as the solvent. Fr. 5-3 and Fr. 5-5 were isolated using polyamide/RP C-18/Sephadex LH-20 CC to yield **5** (11mg, 0.27%), **11** (8mg, 0.19%) and **12** (5mg, 0.12%). Fr. 7.1 collected from Fr. 7 with EtOAc-CH₃OH(10:2, v/v) were passed through polyamide CC, and purified by ODS-HPLC using a gradient of CH₃OH-H₂O(3:7-9:1,v/v))as eluent to yield **3** (10mg, 0.24%) and **9** (5mg, 0.12%). Fr. 8-1, Fr. 8-3 and Fr. 8-4 obtained from Fr. 8 with CH₂Cl₂-CH₃OH(10:2,v/v) were isolated using ODS- HPLC/Sephadex LH-20 CC to yield **2** (150mg, 3.63%), **4** (5mg, 0.12%), **8** (3mg, 0.07%) and **10** (8mg, 0.19%). EAF (4.13 g), which showed the most antioxidant activity, was subjected to column chromatography on a silica gel column chromatography (CC), employing a step gradient of CH₂Cl₂-CH₃OH (10:1, 10:2, 10:3, 10:5, 1:1, 0:1, v/v) and afforded eleven fractions (Fr. 1-Fr. 9) (Fig. 1). Fr. 2 was subjected to open silica gel CC using a successive elution of EtOAc-CH₃OH (10:1-0:1, v/v) to yield fractions Fr. 2.1-2.4. Fr. 2.2 and 2.4 were isolated using Sephadex LH-20 CC/ODS-HPLC to afford **6** (8.1mg, 0.54‰) and **7** (20mg, 1.33‰). Fr. 4 was subjected to polyamide CC using CH₂Cl₂-CH₃OH-HCOOH as eluent (10:2:1, v/v). Promising subfraction Fr. 4-3 was eluted using CH₃OH-H₂O (1:1-1:0, v/v) with RP C-18 CC. Final purification was achieved by polyamide CC using CH₂Cl₂-CH₃OH (10:4, v/v) to yield **1** (0.803 g, 0.54%). Fr. 5 was subjected to polyamide CC with CH₂Cl₂-EtOAc-CH₃OH(5:5:1,v/v) as the solvent. Fr. 5-3 and Fr. 5-5 were isolated using polyamide/RP C-18/Sephadex LH-20 CC to yield **5** (11 mg, 0.73‰), **11** (8 mg, 0.53‰) and **12** (5 mg, 0.33‰). Fr. 7.1 collected from Fr. 7 with EtOAc-CH₃OH(10:2, v/v) were passed through polyamide CC, and purified by ODS-HPLC using a gradient of CH₃OH-H₂O(3:7-9:1,v/v))as eluent to yield **3** (10 mg, 0.66 ‰) and **9** (5 mg, 0.33‰). Fr. 8-1, Fr. 8-3 and Fr. 8-4 obtained from Fr. 8 with CH₂Cl₂-CH₃OH(10:2,v/v) were isolated using ODS- HPLC/Sephadex LH-20 CC to yield **2** (150 mg, 0.1%), **4** (5 mg, 0.33‰), **8** (3 mg, 0. 20‰) and **10** (8 mg, 0.53‰).

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