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Short Communication

Rapid isolation of rosmarinic acid from *Ocimum basilicum* using flash chromatography

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ABSTRACT

In this study; it was aimed at the isolation of rosmarinic acid (RosA) from *Ocimum basilicum* (OB) with a selective extraction and rapid isolation technique using flash chromatography (FC). For this purpose, dried leaves of OB were extracted with n-hexane, dichloromethane, and methanol, respectively. The methanol extract (ME) was extracted with dichloromethane and ethyl acetate using separation funnel by suspending with water. The ethyl acetate layer was dried with anhydrous Na_2SO_4 and the solvent was evaporated to give a rosmarinic acid-rich extract. Then the ethyl acetate extract (EAE) was separated over silica gel column using FC, and thus RosA was isolated with high purity.

Keywords: Rosmarinic acid, flash chromatography, *Ocimum basilicum.*

Flash kromatografi kullanarak Reyhan bitkisinden rozmarinik asitin hızlı izolasyonu

ÖZ

Bu çalışmada, Reyhan bitkisinden (*Ocimum basilicum*) seçici bir ekstraksiyon ve hızlı izolasyon tekniği ile flash kromatografi kullanılarak rozmarinik asitin izolasyonu amaçlanmıştır. Bu amaçla; kurutulmuş reyhan yaprakları sırası ile hekzan, diklorometan ve methanol ile ekstrakte edildi. Methanol ekstraktı su ile çözülerek ayırma hunisinde diklormetan etil asetat ile ekstrakte edildi. Etil asetat fazı susuz Na_2SO_4 ile kurutuldu ve rosmarik asitçe zengin ekstrakt vermek üzere çözücü evapore edildi. Daha sonra, etil asetat ekstraktı flash kromatografi kullanarak silica jel ile ayrıma tabi tutuldu ve böylece rozmarinik asit yüksek saflıkta elde edildi.

Anahtar Kelimeler: Rozmarinik asit, flash kromatografi, Reyhan bitkisi.

1. INTRODUCTION

Natural products play an important role for drug discovery and development process due to their bioactive contents.[1-8] *Ocimum basilicum* belongs to the *Lamiaceae* family known to be rich sources of polyphenolic compounds, particularly phenolics acids.[9] The plant extensively includes substances that high antioxidant activity due to the content of phenolic acids especially rosmarinic acid as a major constituent.[10] RosA is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid and is a widely occurring natural antioxidant in many plant kingdom with interesting biological activities e.g. antitumor,[11] antidiabetic,[12] antioxidant,[13] antiproliferative,[14,15] antiviral and anti-inflammatory.[16] Antioxidants were called as bioactive substances that prevent the formation of free radicals or neutralize free radicals in living organisms.[17,18] To prevent oxidative degradation of food, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutylhydroquinone (TBHQ) are widely used in the food industry, but BHA and BHT are suspected of being responsible for liver damage and carcinogenesis.[19] Therefore, the development and utilization of more effective antioxidants from the natural origin are desired.

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2. EXPERIMENTAL

2.1. Plant material

Ocimum basilicum was cultivated in Gaziosmanpasa University Field Crops in Faculty Application sites, and harvested in July 2011.

2.2. Extraction of RosA

The air-dried aerial parts of plant material (400 g) were extracted sequentially with hexane, dichloromethane and methanol (every 24 hours, 4 Lx3). The methanol extract (13 g) suspended in water and partitioned between dichloromethane and water. The organic layer was removed. Then the water phase was partitioned between water and ethyl acetate. The ethyl acetate phase was separated and concentrated using evaporator after drying with anhydrous sodium sulfate to give slurry extract (6.30 g) (Figure 1).

2.3. Flash Chromatography conditions

Flash chromatography is a useful technique for separation in both natural and synthetic products. The constant flow rate of mobile phases and enabling detection with UV detector increases the efficiency of separation and reproducibility when compared with common column chromatography techniques. A Combiflash companion Flash chromatography instrument was used for purification of RosA. The UV detector was set up at 330 nm. As a mobile phase, dichloromethane-ethyl acetate system was used with a linear gradient system at 10 mL/min flow rate. A manually packed with silica gel GF_{254} glass column (5 cm X 50 cm, diameter X length) was used. The detected fractions at 330 nm were collected in 10 mL volumes.

2.4. LC-TOF-MS analysis

LC-TOF-HRMS analysis was performed using an Agilent 6210 LC-TOF instrument according to the method described previously.[20] Briefly; elution was performed using Poroshell column 120 EC-C18 (3.0 × 50 mm, 2.7 µm I.D.). The mobile phases were water with 0.1% formic acid and 5 mM ammonium format (Solvent A) and acetonitrile (solvent B) with 0.7 mL/min flow rate. The gradient program was used as follows: 0–1 min, 10% B; 1–8 min, 10% B; 8–11.1 min, 95% B; 11.1–13 min, 10% B; 13–14 min, 10% B. TOF analyses were carried out in negative ion mode; gas temperature, 325 °C and column temperature, 35 °C; drying gas flow, 0.7 ml/min; fragmentor voltage, 175 V.

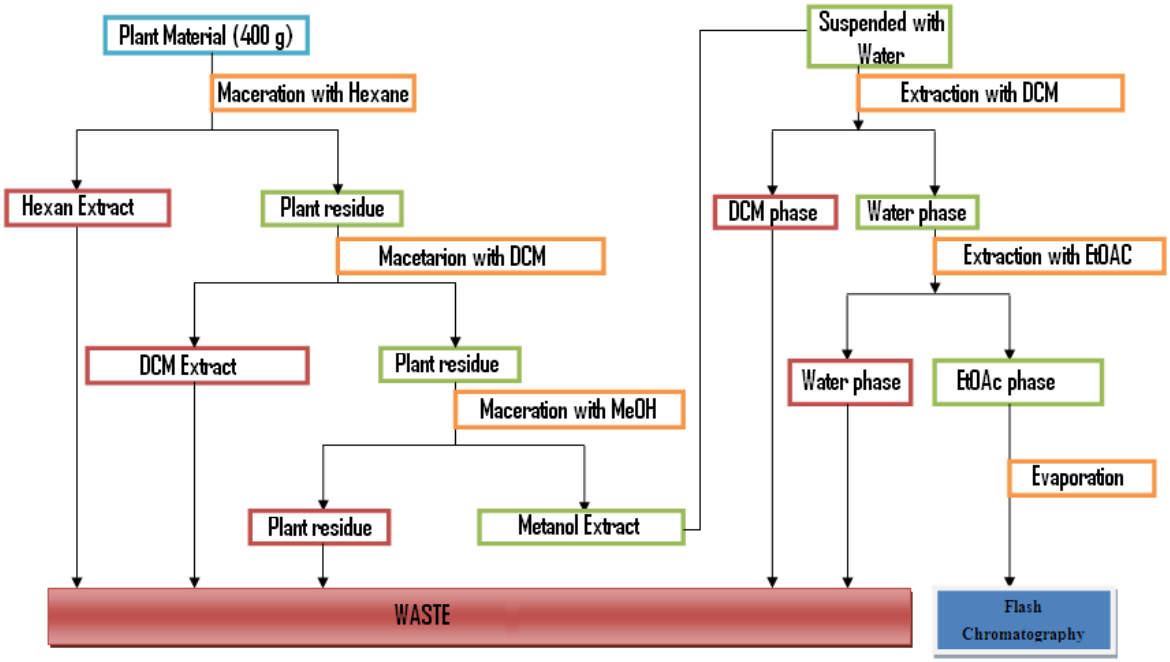

Figure 1. Extraction flowchart of RosA from *O. basilicum*.

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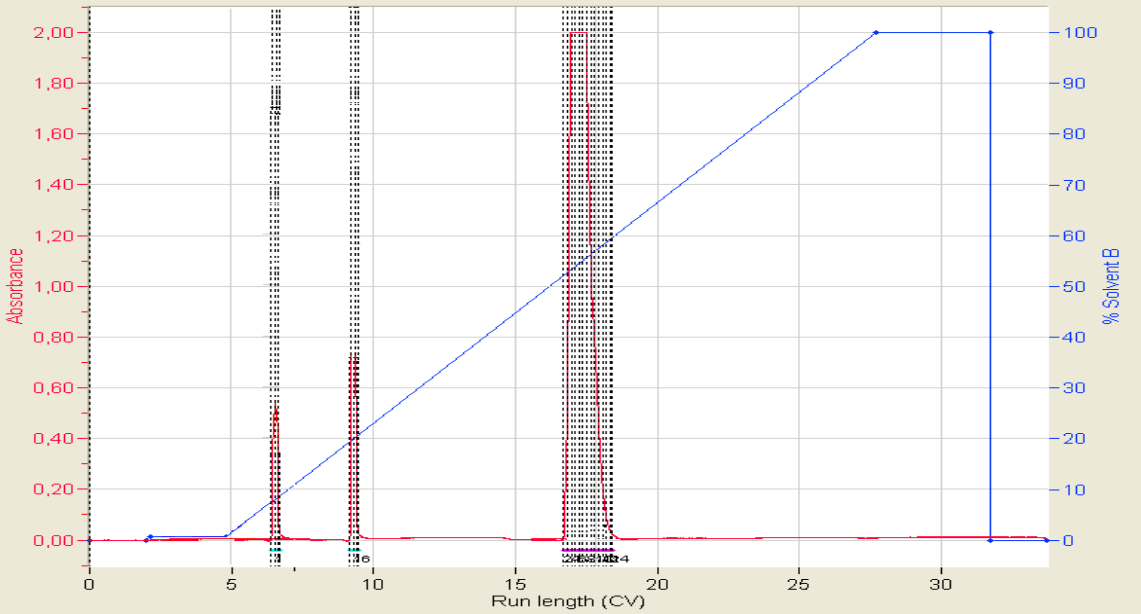

Figure 2. Flash chromatography chromatogram of the ethyl acetate phase.

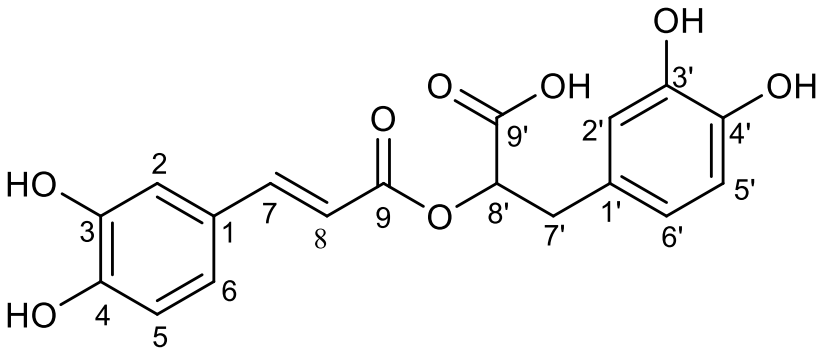

Figure 3. Chemical structure of RosA.

3. RESULTS AND DISCUSSION

The collected fractions during 16-18 CV (column volume) were combined and evaporated to give yellowish amorphous solid (The main peak observed on LC-chromatogram: See Figure 2).

20 mg of collected fractions were solved in DMSO-d6 and recorded ^{1}H and ^{13}C-NMR spectra. The data obtained as follows:

^{1}H-NMR (400 MHz, DMSO-d6) δ_H: d 7.46 (*d*, 1H, H-7, *J*= 15.8 Hz), 7.06 (*d*, 1H, H-2, *J*= 1.4 Hz), 7.02 (*d*, 1H, H-6, *J*= 8.12 Hz), 6.79 (*d*, 1H, H-5, *J*= 8.12 Hz), 6.69 (*d*, 1H, H-2', *J*= 1.5 Hz), 6.54 (*d*, 1H, H-5', *J*= 7.92 Hz), 6.65 (*d*, 1H, H-6', *J*= 7.92 Hz), 6.24 (*d*, 1H, H-8, *J*= 15.8 Hz), 5.08 (*dd*, 1H, H-8', *J*= 10 Hz, *J*= 2.8 Hz), 2.98 (*dd*, 1H, H- 7a', *J*= 10.1 Hz, *J*= 1.10 Hz), 2.98 (*d*, 1H, H-7b', *J*= 10 Hz); **^{13}C-NMR (100 MHz, DMSO-d6) δ_C:** d 171.33 (C-9'), 166.43 (C-9), 146.07 (C-4), 149.10 (C-3), 146.39 (C-7), 144.50 (C-3'), 145.41 (C-4'), 127.7 (C-1'), 125.9 (C-1), 122.02 (C-6), 115.85 (C-6'), 117.17 (C-2'), 116.23 (C-5), 120.53 (C-5'), 115.28 (C- 2), 113.66 (C-8), 73.18 (C-8'), 36.61 (C-7'). According to ^{1}H and ^{13}C-NMR data, the compound identified as RosA with good agreement with data given in the literature.[21, 22]

The LC-TOF-HRMS chromatogram of crude ethyl acetate extract, isolated RosA and standard RosA is given in Figure 4. LC-TOF analysis showed that the molecular weight of RosA was 360.0848 g/mol (calc. for $C_{18}H_{16}O_8$:360.0845 g/mol) with high purity.

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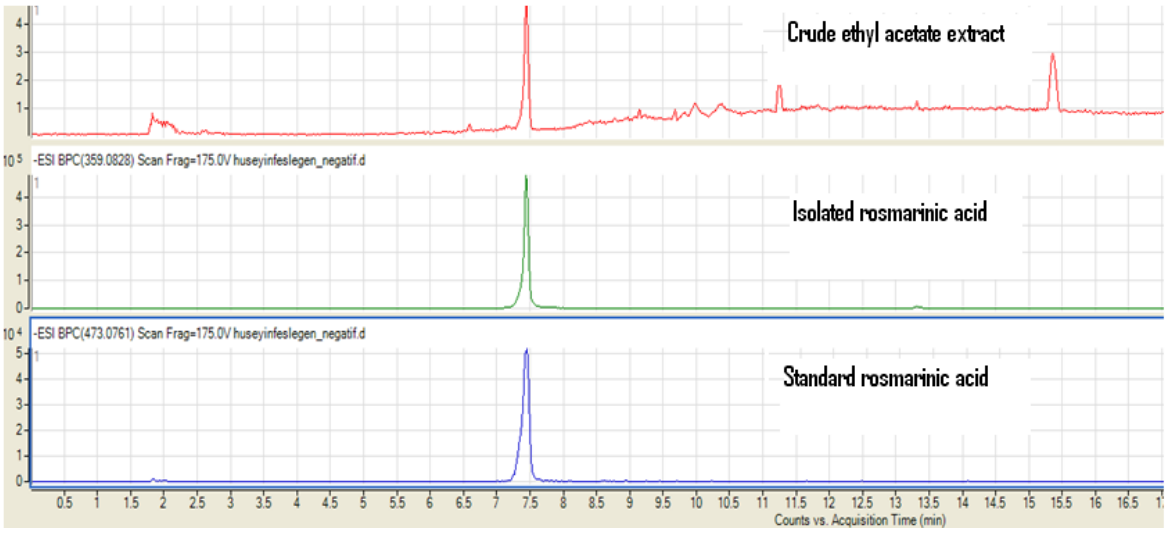

Figure 4. Chromatogram of TOF-LC/MS instrument of crude ethyl acetate extract, isolated RosA and standard RosA.

4. CONCLUSIONS

In this study, rosmarinic acid was extracted from *Ocimum basilicum* using a selective extraction technique. Purification of RosA was achieved by using flash chromatography. The extraction and purification process can be suggested to the isolation of RosA rapidly and efficiently from natural sources.

Conflict of interests

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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