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Short communication

A cytotoxic *C*-glycosylated derivative of apigenin from the leaves of *Ocimum basilicum* var. *thyrsiflorum*

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ABSTRACT

The standardized 80% ethanolic extract of the leaves of *Ocimum basilicum* var. *thyrsiflorum* (L.) Benth., Lamiaceae, growing in KSA, exhibited a significant antioxidant activity compared to the ethyl acetate and butanol extracts, which was correlated to its higher phenolic and flavonoid contents. Chromatographic separation of the 80% ethanol extract resulted in the isolation of ten known compounds; cinnamic acid, gallic acid, methylgallate, ellagic acid, methyl ellagic acid, apigenin, luteolin, vitexin, isovitexin, and 3″-*O*-acetylvitexin. Compound 3″-*O*-acetylvitexin, a *C*-glycosylated derivative of apigenin, was isolated for the first time from genus *Ocimum*. The 80% ethanolic extract and 3″-*O*-acetylvitexin showed significant cytotoxic activities against the HCT_{116} human colon cancer cell line [IC_{50} values 22.3 ± 1.1 and 16.8 ± 2.0 μg/ml (35.4 μM), respectively].

Introduction

Herbs provided us with some of the very important life saving drugs used in the armamentarium of modern medicine (Goyal et al., 2007). Some world's population depends on traditional medicine because of scarcity, high cost of orthodox medicine and unpleasant side effects (Agrawal et al., 2011). Among the plants known for their medicinal value are the plants of genus *Ocimum*, family Lamiaceae, which are rich in phenolic constituents and are very useful for their therapeutic potentials (Nahak et al., 2011). Several studies have shown various activities of Ocimum species including bactericidal, antiulcer, antidiarrheal, antiinflammatory, antioxidative, anticancer, for cough and kidney malfunction, hypoglycemic, nervous system stimulation and protection from radiation (Elansary and Mahmoud, 2015; Kadan et al., 2016). The pharmaceutical potentiality of *Ocimum* species may be attributed to their profound biological effects due to the presence of active polyphenols, as hydroxycinnamic acids (caffeic acid and rosmarinic acid) and flavonoids, mainly in the form of derivatives such as esters and glycosides (Wang et al., 2004). An interesting plant which belongs to genus *Ocimum* is *O. basilicum* L. which is native to India and is cultivated in other regions of Asia, Africa, the Mediterranean region and KSA.

Chemical and biological investigation of *O. basilicum* var. *thyrsiflorum* growing in KSA was carried out. The cytotoxic activity of a *C*-glycosylated derivative of apigenin was studied against human colon cancer cell lines, along with the parent standardized ethanolic extract.

Materials and methods

General

NMR (^1H and ^{13}C NMR) spectra were recorded at 300 MHz for ^1H and 125 MHz for ^{13}C on a Varian Mercury 300. ESI-MS spectra were measured using mass spectrometer connected to an ESI-II ion source (Finnigan, Lc-MSLCQdeca. Advantage MAX, Finnigan Surveyor LC pump). The UV analyses for pure compounds were recorded on a Shimadzu UV 240 spectrophotometer. UV-VS spectrophotometer (Milton Roy 601) was used for determination of total phenolic content. Stationary phases used were polyamide 6S (Seelze Hannover, Germany), sephadex LH-20 (Fluka, Switzerland) and cellulose (Pharmacia, Uppsala, Sweden). Purity of the isolated compounds was tested by HPLC/DAD (Hewlett Packard, Agilent

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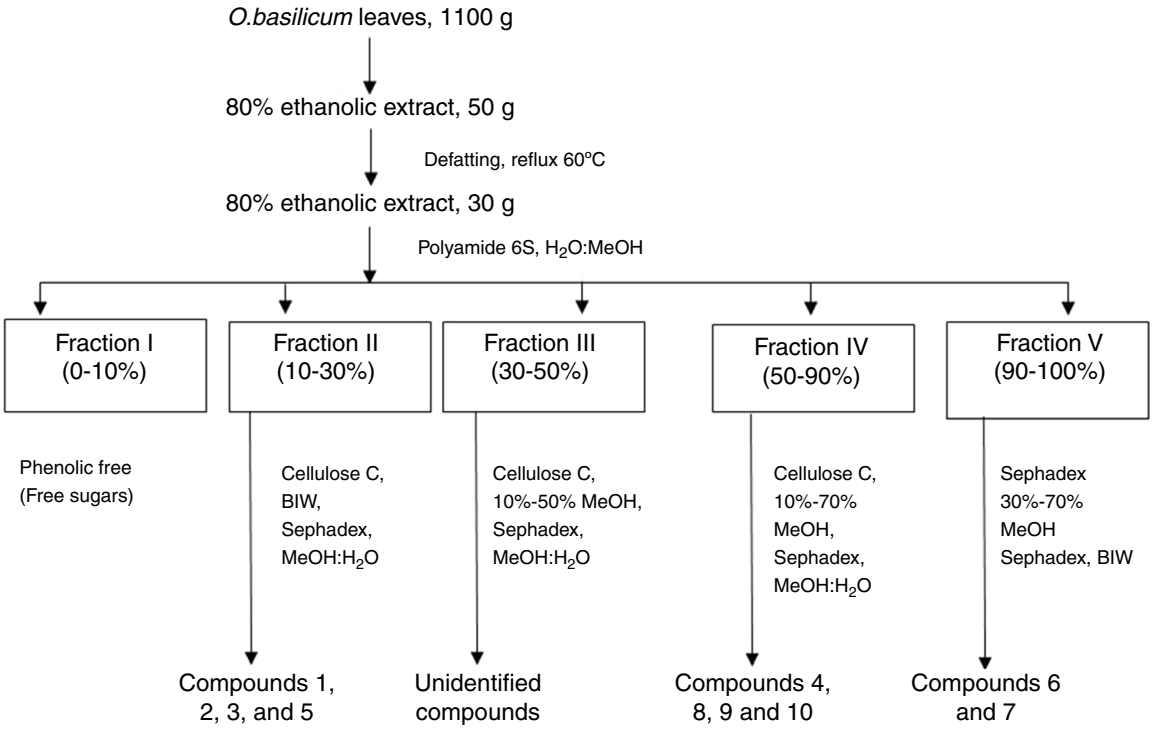


Fig. 1. Scheme demonstrating the fractionation of the 80% ethanolic extract of *Ocimum basilicum*. BIW, butanol isopropanol water.

1100, quaternary pump G 1311A, vacuum degasser G 1322A, column oven G 1316A, photodiode array detector G 1315A, column C18 silica 10 μm particle size, Lichrocart, Water Ireland).

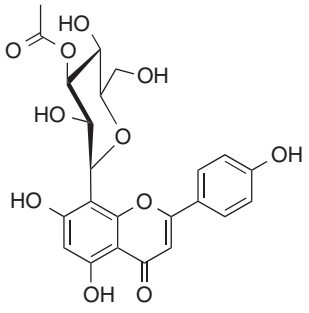
DPPH (1.1-diphenyl-2-picrylhydrazyl) was purchased from Sigma-Aldrich Co. (St Louis, MO). Sodium phosphate, ammonium molybdate, Folin-Ciocalteu's reagent, ascorbic acid, gallic acid were purchased from Merck Chemical Co (Darmstadt, Germany).

Leaves of *Ocimum basilicum* var. *thyrsiflorum* (L.) Benth., Lamiaceae, were freshly collected from Taif, KSA. The samples were collected in May 2012. A sample of the plant was identified by Prof. Dr. Mohamed M. Milad, Department of Biology, Faculty of Applied Sciences, Umm Alqura University, KSA. A voucher specimen (no. 402) was deposited at the herbarium of Faculty of Pharmacy, Helwan University.

Three separate portions (50 g each) of the air dried ground leaves were extracted with 80% aqueous ethanol (80% EtOH), ethyl acetate (EtOAc) and *n*-butanol saturated with water (*n*-BuOH) (500 ml × 3) under reflux (70 °C), yielding three extracts of 2, 1.2 and 1.5 g, respectively. For the isolation of pure compounds, the dried leaves (1100 g) were extracted with 80% EtOH (2.5 l × 5) under reflux (70 °C). Fractionation and isolation of compounds was carried out as shown in Fig. 1.

*8-C-β-D-(3″-O-acetyl) glucopyranosylapigenin (**1**)*

Pale yellow amorphous powder (21 mg); R_f values, 0.29 (S_1), 0.56 (S_2), dark purple spot under UV light which turned into green with $FeCl_3$ and greenish yellow with Naturstoff spray reagents; UV (MeOH): λ_{max} nm: 269, 301, 335, (+NaOMe): 287, 336, 401, (+NaOAC): 282, 305, 386, (+$AlCl_3$): 275, 304 (sh), 350, 390, (+$AlCl_3$/HCl): 277, 302 (sh), 346, 391; Negative ESI-MS: *m/z* 473.3743 $[M-H]^-$ 431.1153 $[M\text{-}COCH_3]^-$ (calculated for $C_{23}H_{22}O_{11}$, 474); ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 13.25 (1H, s, H-bonded OH-5), 8.03 (2H, d, H-2′/6′), 6.90 (2H, d, H-3′/5′), 6.63 (1H, s, H-3), 6.27 (1H, s, H-6), 5.17 (1H, m, H-3″), 4.97 (1H, brs, H-1″), 4.37 (1H, brt, H-2″), 3.94 (1H, m, H-4″), 3.83 (1H, m, H-5″), 3.53 (2H, d, H-6″), 1.96 (3H, s, methyl gp of the acetyl); ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 182.54 (C-4), 164.86 (C-2), 163.04 (C-7), 161.59 (C-5), 160.83 (C-4′), 156.44 (C-9), 129.41 (C-2′/6′), 122.05 (C-1′), 116.26 (C-3′/5′), 104.47 (C-8), 105.06 (C-10), 102.88 (C-3), 98.59 (C-6), 82.29 (C-5″), 79.11(C-3″), 73.84 (C-1″), 71.29 (C-2″), 71.26 (C-4″), 61.74 (C-6″), 24.14 (CH_3 of acetyl), 169.47 (CO of acetyl).



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The total phenolic contents of 80% EtOH, EtOAc and *n*-BuOH extracts of *O. basilicum* were determined using Folin-Ciocalteu reagent and gallic acid as a reference standard according to the method described by Kumar et al. (2008). The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g extract.

The total flavonoid contents of the three extracts were determined using the procedure described by Kumaran and Karunakaran (2006) using quercetin as a standard. The total flavonoid content in each extract was determined as mg quercetin equivalent (QE)/g extract.

The ability of 80% EtOH, EtOAc and BuOH extracts of *O. basilicum* to scavenge DPPH radicals was evaluated according to the procedure described by Mensor et al. (2001). Ascorbic acid was used as a reference standard.

The cytotoxic activity of 80% EtOH extract of *O. basilicum* as well as the isolated compound (**1**) was assessed using the sulforhodamine-B colorimetric assay (Skehan et al., 1990), against

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