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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].

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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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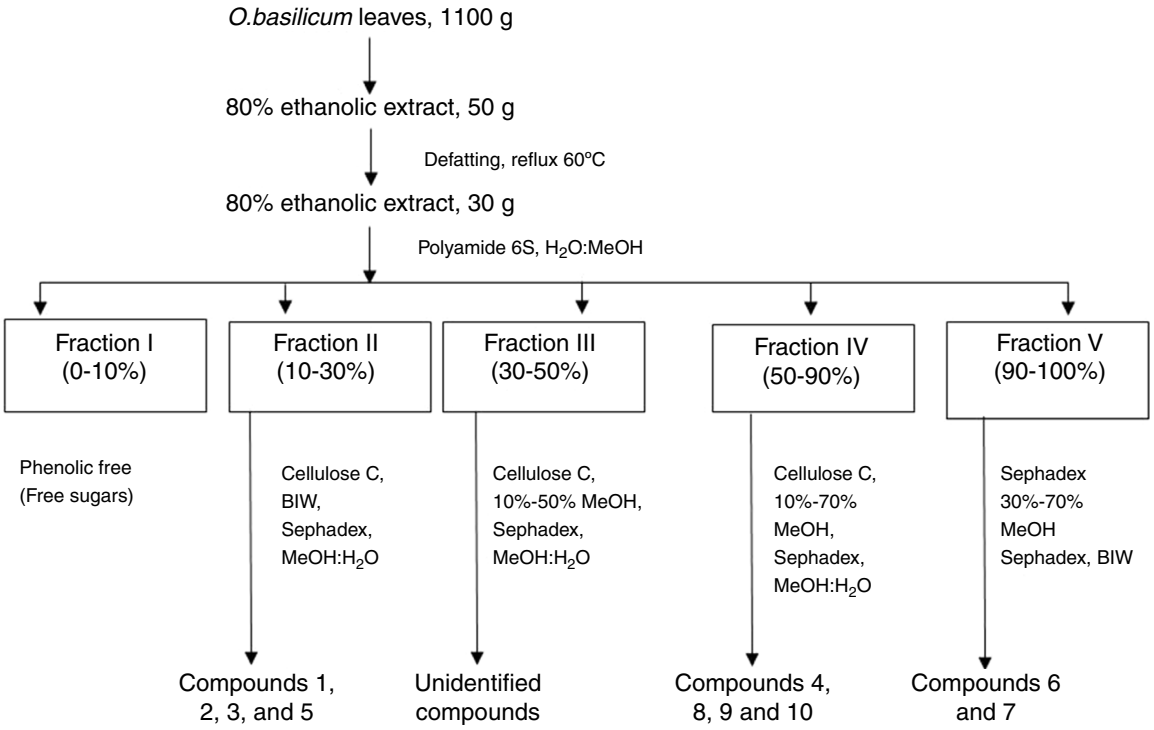


Fig. 1. Scheme demonstrating the fractionation of the 80% ethanolic extract of *Ocimum basilicum*. BIW, butanol isopropanol water.

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).

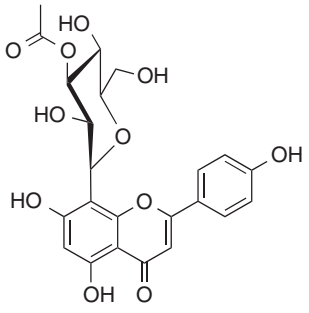
DPPH (1.1-diphenyl-2-picrylhydrazyl) was purchased from Sigma-Aldrich Co. (St Louis, MO). Sodium phosphate, ammonium molybdate, Folin-Ciocalteu's reagent, ascorbic acid, gallic acid were purchased from Merck Chemical Co (Darmstadt, Germany).

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

Table 1a
DPPH radical scavenging activity of *Ocimum basilicum* extracts.

Concentration (μg/ml)	Ascorbic acid	80% EtOH	EtOAc	*n*-BuOH
12.5	28.59 ± 0.25	15.23 ± 1.10	13.33 ± 0.54	9.43 ± 1.46
25	37.03 ± 0.40	27.62 ± 1.76	19.56 ± 1.30	14.64 ± 1.88
50	54.26 ± 0.21	59.35 ± 2.67	39.47 ± 1.22	23.43 ± 2.72
100	87.43 ± 0.35	76.48 ± 2.36	54.41 ± 1.87	40.21 ± 3.12
IC_{50} (μg/ml)	44.16 ± 0.9	53.85 ± 0.5	83.59 ± 2.1	127.37 ± 0.8

the human colon cancer cell lines HCT_{116}, using Doxorubicin® as a positive control. Data were analyzed by one-way analysis of variance (ANOVA). Differences were considered significant when p values were <0.05.

Results and discussion

The 80% EtOH, EtOAc and *n*-BuOH extracts from the leaves of *O. basilicum* were standardized to their total phenolic and flavonoid contents. The 80% EtOH extract was standardized to contain the highest content of phenolics and flavonoids (77.3 ± 3.0 GAE/g, 43.6 ± 1.5 QE/g, respectively), followed by EtOAc (48.3 ± 2.1 GAE/g, 32.6 ± 2.2 QE/g) and *n*-BuOH extracts (29.2 ± 1.4 GAE/g, 15.4 ± 2.6 QE/g).

The standardized extracts were assessed for their capacity to scavenge DPPH free radical along with ascorbic as a positive control (Table 1a). The 80% EtOH extract of the leaves of *O. basilicum* exhibited pronounced antioxidant activity (IC_{50} = 53.85 ± 0.5 μg/ml), followed by EtOAc and *n*-BuOH extracts (IC_{50} = 83.59 ± 2.1 and 127.37 ± 0.8 μg/ml, respectively) compared to ascorbic acid (IC_{50} = 44.16 ± 0.9 μg/ml), owing to its higher phenolic and flavonoidal contents. It is well known that there is a strong relationship between total phenol content and antioxidant activity, as phenols possess strong scavenging ability for free radicals due to their hydroxyl groups. Thus, the phenolic content of plants may directly contribute to their antioxidant action (Abdel Motaal and Shaker, 2011).

Ten compounds were isolated from the active 80% EtOH extract where nine of them were chemically identified using UV, ^{1}H NMR, ^{13}C NMR, or negative ESI-MS, and by comparison with previously published data (Meyer et al., 2006; López-Lázaro, 2009; Li et al., 2011; Choo et al., 2012; Kubacey et al., 2012). The compounds were cinnamic acid, gallic acid, methylgallate, ellagic acid, methyl ellagic acid, apigenin, luteolin, vitexin, isovitexin, where vitexin and isovitexin are the 8- and 6-*C*-glucosides of apigenin, respectively. Compound **1** was isolated as a pale yellow amorphous powder. According to the chromatographic properties and UV spectral data, compound **1** was expected to be *C*-glycosylapigenin. The UV spectrum in MeOH exhibited the two characteristic absorbtion bands at λ_{max} 269 nm (band II) and 335 nm (band I) of apigenin nucleus. Upon addition of NaOAc, a bathochromic shift of band II (≈+8) was diagnostic for a free 7-OH group. The remaining diagnostic shift reagents were in complete accordance with 5,7,4′-trihydroxy-*C*-glycosyl flavone structure (Mabry et al., 1970). Negative ESI-MS spectrum exhibited the molecular ion peak at *m/z* 473 $[M-H]^-$ (calculated for $C_{23}H_{22}O_{11}$, 474) and fragment ion peak at *m/z* 431 after loss of an acetyl moiety indicating an apigenin acetylhexoside structure. ^{1}H NMR spectrum showed an AX coupling system of two ortho doublets, each integrated for two protons at δ_H 8.03 and 6.90 assigned to H-2′/6′ and H-3′/5′, respectively, of 1′, 4′-disubstituted ring-B. In addition the two singlet signal resonances at δ_H 6.63 and 6.27 assignable to H-3 and H-6, respectively, showed characteristics of an apigenin moiety missing an H-8 resonance signal. The anomeric proton appeared as broad singlet at δ_H 4.97 giving the suggestion of the presence of a *C*-glucoside moiety. The absence of H-8 gave the expectation of *C*-glucosidation on C-8. This evidence

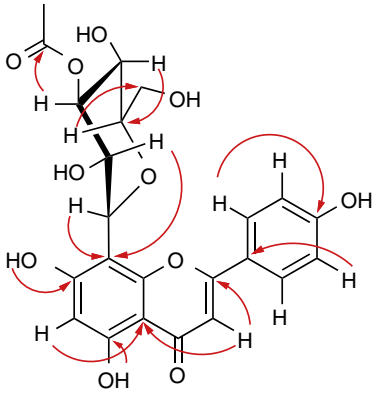

Fig. 2. Structure and key HMBC (H → C) of compound **10**.

was confirmed from the downfield shift of ^{13}C-resonance of C-8 to δ_C 104.47 and the upfield of C-7 and C-9 to δ_C 163.04 and 156.44, respectively. Moreover, the *C*-glucoside moiety was confirmed as β-glucopyranoside depending on the characteristic upfield location of C-1″ at δ_C 73.84 and downfield locations of C-5″ and C-3″ at δ_C 82.29 and 79.11, respectively. The downfield shift of C-3″ at δ_C 79.11 indicated the position of acetyl moiety with respect to those of *C*-glucoside. HMBC approved this structure and the linkage between the acetyl moiety and C-3″ (Fig. 2). All ^{1}H and ^{13}C resonances were assigned by comparison with the corresponding values of structurally related compounds of previously published data (Kim et al., 2005; Zhou et al., 2013).

Thus compound **1** was identified as 8-*C*-β-D-(3″-*O*-acetyl) glucopyranosylapigenin or 3″-*O*-acetylvitexin and was isolated for the first time from genus *Ocimum*.

Recently apigenin became interesting as a beneficial health promoting agent because of its low intrinsic toxicity. Its *C*-glycosylated derivatives, vitexin and isovitexin, were reported to possess antidiabetic, anti-Alzheimer's disease, and anti-inflammatory activities (Choi et al., 2014). Vitexin-2-*O*-xyloside previously showed a higher activity against colon cancer cell lines CaCo-2 (IC_{50} 120 ± 9) than against breast cancer cells MCF-7 (IC_{50} 350 ± 48) (Papi et al., 2013).

The 80% EtOH extract and compound **1** were tested against HCT_{116} colon carcinoma and showed a significant cytotoxic activity (IC_{50} value 22.3 ± 1.1 and 16.8 ± 2.0 μg/ml corresponding to

Table 1b
Cytotoxic activities of the 80% ethanol extract and compound **10** of *Ocimum basilicum* against HCT_{116} colon cell lines.

Concentration (μg/ml)	Survival fraction (mean ± SD)	
	80% EtOH extract	Compound **10**
5	0.824 ± 0.021	0.761 ± 0.034
10	0.657 ± 0.013	0.582 ± 0.007
25	0.467 ± 0.032	0.401 ± 0.003
50	0.346 ± 0.016	0.317 ± 0.031
100	0.303 ± 0.042	0.234 ± 0.026
IC_{50} (μg/ml)	22.3 ± 1.1	16.8 ± 2.0

Results shown are mean ± SD of triplicate experiments.

35.4 μM, respectively, Table 1b) compared to Doxorubicin® (IC_{50} value 3.7 ± 0.21 μg/ml corresponding to 6.4 μM).

Conclusion

The ethanolic extract of the leaves of *O. basilicum* var. *thyrsiflorum* showed potent antioxidant activity owing to its relatively high phenolic and flavonoid contents, compared to the ethyl acetate and *n*-butanol extracts. Fourteen compounds were isolated from the active ethanolic extract, where compound **1**, a *C*-glycosylated derivative of apigenin, was isolated for the first time from *Ocimum* genus and identified as 8-*C*-β-D-(3″-*O*-acetyl) glucopyranosylapigenin (or) 3″-*O*-acetyl-vitexin. Both compound **1** and its parent extract showed significant cytotoxic activities against the HCT_{116} human colon cancer cell line.

Author contributions

MI and AA participated in study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and drafted the manuscript. MI carried out the extraction, fractionation and isolation of pure compounds.

Conflicts of interest

The authors declare no conflicts of interest.

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