Revista Brasileira de Farmacognosia 25 (2015) 462–464

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

# Cucumin S, a new phenylethyl chromone from *Cucumis melo* var. *reticulatus* seeds

Sabrin R.M. Ibrahim[a,b,*], Gamal A. Mohamed[c,d]

[a] Department of Pharmacognosy and Medicinal Chemistry, College of Pharmacy, Taibah University, Al Madinah Al Munawwarah, Saudi Arabia
[b] Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[c] Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia
[d] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut, Egypt

ARTICLE INFO

*Article history:*
Received 29 May 2015
Accepted 30 June 2015
Available online 21 July 2015

*Keywords:*
*Cucumis melo*
Cucurbitaceae
Chromone
Flavonoid
Antioxidant

ABSTRACT

A new phenylethyl chromenone, cucumin S [(R)-5,7-dihydroxy-2-[1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl]chromone] (**1**), along with five known compounds: 5,7-dihydroxy-2-[2-(4-hydroxyphenyl)ethyl]chromone (**2**), 5,7-dihydroxy-2-[2-(3,4-dihydroxyphenyl)ethyl]chromone (**3**), luteolin (**4**), quercetin (**5**), and 7-glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl)ethyl]chromone (**6**) were isolated from the EtOAc fraction of *Cucumis melo* var. *reticulatus* Ser., Cucurbitaceae, seeds. Their structures were determined by spectroscopic means (1D and 2D NMR), as well as HRESIMS, optical rotation measurement, and comparison with literature data. The isolated compounds **1**–**6** were assessed for their antioxidant activity using DPPH assay. Compounds **3**, **4**, and **5** showed potent activities compared to propyl gallate at concentration 100 μM.

© 2015 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

## Introduction

Family Cucurbitaceae includes pumpkins, squash, musk melons, watermelons, cucumbers, and grounds, which are known as vine crops. Cucurbit fruits are significant source of dietary fibers, beta-carotene (pro-vitamin A), minerals (potassium), and vitamin C (Fleshman et al., 2011; Adams and Richardson, 1981). Naturally, they are low in sodium, fat, and cholesterol (Lester, 1997). *Cucumis melo* var. *reticulatus* Ser., Cucurbitaceae (netted muskmelon or cantaloupe) is one of the most cultivated cucurbits. It is an orange-fleshed, sweet, and aromatic melon that is highly popular for its nutritive and medicinal properties. It is a worm-season crop grows in all tropical and subtropical regions of the world. *C. melo* has potential in the treatment of pain, inflammation, cough, dysuria, diabetes, liver diseases, and cardiovascular disorders (Ibrahim, 2010, 2014). It exhibited antioxidant, antimicrobial, and anti-inflammatory activities (Ibrahim, 2010, 2014; Vouldoukis et al., 2004). Previous studies on *C. melo* L. led to isolation of hydrocarbons (Velcheva and Donchev, 1997), peptides (Ribeiro et al., 2007), fatty acids, volatile sesquiterpenes (Lewinsohn et al., 2008; Portnoy et al., 2008), phenylethyl chromone derivatives, triterpenes, sterols, and one triacylglyceride (Ibrahim, 2010, 2014). In the present work, we reported the isolation and structural characterization of a new phenylethyl chromone derivative: (*R*)-5,7-dihydroxy-2-[1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl]chromone (**1**), along with five known compounds from the EtOAc fraction of *C. melo* seeds. The antioxidant activity of compounds **1**–**6** was evaluated using DPPH assay.

## Materials and methods

### General experimental procedures

Melting points were carried out using an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd., Essex, England). Optical rotation was recorded on a Perkin-Elmer Model 341 LC Polarimeter. Shimadzu 1601 UV/VIS and Shimadzu Infrared-400 (Shimadzu, Kyoto, Japan) spectrophotometers were used to measure the Ultraviolet (UV) and Infrared (IR) spectra, respectively. HRESIMS spectra were measured with a Micromass Qtof 2 mass spectrometer (ThermoFinnigan, Bremen, Germany). Bruker Avance DRX 500 and 700 (Bruker BioSpin, Billerica, MA, USA) were used to record NMR spectra. Chromatographic separation was achieved using $RP_{18}$ (0.04–0.063 mm), silica gel 60 (0.04–0.063 mm), and Sephadex LH-20 (0.25–0.1 mm) (Merck, Darmstadt, Germany). TLC analysis was performed on pre-coated silica gel 60 $F_{254}$TLC plates (0.2 mm, Merck, Darmstadt, Germany).

* Corresponding author.
*E-mail:* sribrahim@taibahu.edu.sa (S.R.M. Ibrahim).

http://dx.doi.org/10.1016/j.bjp.2015.06.006
0102-695X/© 2015 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

The compounds were detected by UV absorption at $\lambda_{max}$ 255 and 366 nm followed by spraying with *p*-anisaldehyde/$H_2SO_4$ reagent and heating at 110 °C for 1–2 min. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and propyl gallate were purchased from Sigma Chemical Co. (Taufkirchen, Germany).

*Plant material*

Seeds of *Cucumis melo* var. *reticulatus* Ser., Cucurbitaceae, were obtained from cultivated plants at Mankabad, Assiut, Egypt. Plant material was identified and authenticated (voucher specimen 2013-5) by Prof. Dr. Mohamed A. Farghali, Professor of Horticulture (Vegetable Crops), Faculty of Agriculture, Assiut University.

*Extraction and isolation*

The dried seeds (263 g) were defatted in a Soxhlet apparatus using *n*-hexane (3 × 1 l), then extracted with MeOH (4 × 2 l). The MeOH extract was concentrated under reduced pressure to afford a dark brown residue (18.9 g). The latter was subjected to silica gel vacuum liquid chromatography (VLC) using *n*-hexane and EtOAc to afford 12.6 and 2.9 g, respectively. The EtOAc fraction was subjected to silica gel VLC using *n*-hexane:EtOAc gradient to give five subfractions: C-1 (0.93 g, *n*-hexane:EtOAc 8:2), C-2 (0.41 g, *n*-hexane:EtOAc 6:4), C-3 (0.48 g, *n*-hexane:EtOAc 4:6), C-4 (0.24 g, *n*-hexane:EtOAc 2:8), and C-5 (0.51 g, EtOAc). Subfraction C-2 (0.41 g) was chromatographed over silica gel column (80 g × 50 × 3 cm) using *n*-hexane:EtOAc gradient to obtain **1** (4.9 mg, yellow crystals). Silica gel column chromatography (70 g × 50 × 3 cm) of subfraction C-3 (0.48 g) using *n*-hexane:EtOAc gradient afforded **2** (15 mg, yellow crystals) and **3** (9 mg, yellow crystals). Subfraction C-4 (0.24 g) was chromatographed over silica gel column (50 g × 30 × 2 cm) using *n*-hexane:EtOAc gradient to yield **4** (6.2 mg, yellow powder) and **5** (11 mg, yellow powder). Compound **6** (8.1 mg, yellow powder) was isolated from subfraction C-5 (0.51 g) through silica gel column (50 g × 50 × 2 cm) using $CHCl_3$:MeOH gradient elution.

*Antioxidant activity*

The antioxidant activity of compounds **1**–**6** was evaluated using 2,2′-diphenylpicrylhydrazyl (DPPH) assay as previously outlined (Mohamed et al., 2013,2014). The decrease in the absorption of each compound (100 μM) in DPPH solution was monitored at 517 nm using a spectrophotometer. The absorbance of DPPH in MeOH (with or without compounds) was measured after 2 min. The antioxidant activity of each compound was measured in relation to propyl gallate (a standard antioxidant). Determinations were performed in triplicate. The % activity was calculated by the following equation:

$$\text{Antioxidant activity} = 100 \times 1 - \frac{\text{Compound absorbance}}{\text{Blank absorbance}}$$

*Spectral data*

Cucumin S (**1**). Yellow crystals, mp 115–116 °C; $[\alpha]^{25}_D$ + 51.6 (*c* 0.5, $CHCl_3$), + 49.7 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 255 (3.89), 352 (3.15) nm; IR (KBr) $\nu_{max}$ 3445, 2978, 1655, 1620, 1584, 1065 $cm^{-1}$; NMR data ($CD_3OD$, 500 and 125 MHz), see Table 1; HRESIMS *m/z* 345.0976 $[M+H]^+$ (calc. for $C_{18}H_{17}O_7$, 345.0974).

## Results and discussion

Compound **1** was obtained as yellow crystals. Its molecular formula $C_{18}H_{16}O_7$ was determined from the HRESIMS (positive ion-mode): *m/z* 345.0976 $[M+H]^+$ (calc. for $C_{18}H_{17}O_7$, 345.0974). The UV absorption maxima at 255 and 352 nm and the IR absorption bands at 1655, 1620, and 1584 $cm^{-1}$ suggested the presence of a chromone moiety in **1** (Ibrahim, 2010, 2014). The IR spectrum showed a band at 3445 $cm^{-1}$ characteristic for hydroxyl groups. The $^1$H NMR spectrum (Table 1) showed signals for an olefinic proton at $\delta_H$ 6.47 (1H, s, H-3) and two *meta*-coupled protons for 1,2,3,5-tetrasubstituted phenyl moiety at $\delta_H$ 6.24 (1H, d, *J* = 2.5 Hz, H-8) and 6.06 (1H, d, *J* = 2.5 Hz, H-6). They correlated to the carbon signals at $\delta_C$ 110.6, 95.8, and 98.2, respectively in the HSQC spectrum. Furthermore, the $^1$H and $^{13}$C NMR spectra displayed diastereotopic methylene protons at $\delta_H$ 3.26 (dd, *J* = 14.5, 6.0 Hz, H-7′a) and 2.73 (dd, *J* = 14.5, 3.5 Hz, H-7′b)/$\delta_C$ 40.6 (C-7′) and an oxymethine at $\delta_H$ 5.91 (dd, *J* = 6.0, 3.5 Hz, H-8′)/$\delta_C$ 85.8 (C-8′), indicating the presence of an AMX spin system. It was confirmed by the observed $^1$H-$^1$H COSY and HMBC correlations (Fig. 1). The oxymethine was located at C-8′ based on the HMBC cross peaks of H-8′ to C-2, C-3, and C-1′ and absence of cross peaks with C-2′ and C-6′. Also, the presence of a trisubstituted phenylethyl moiety was evident by the signals at $\delta_H$ 7.23 (dd, *J* = 7.5, 2.5 Hz, H-6′)/$\delta_C$ 119.8 (C-6′), 7.28 (d, *J* = 2.5 Hz, H-2′)/115.6 (C-2′), and 6.78 (d, *J* = 7.5 Hz, H-5′)/117.8 (C-5′). This moiety was confirmed by the $^1$H-$^1$H COSY and HMBC cross peaks. Moreover, the singlet signal at $\delta_H$ 3.87, correlating with the carbon signal at $\delta_C$ 57.3 in the HSQC experiment was assigned to a methoxyl group. The $^{13}$C NMR and HSQC spectra of **1** revealed the presence of 18 carbons: one methoxyl, one methylene, seven methines, and nine quaternary carbons including carbonyl carbon at $\delta_C$ 181.7 (C-4). The connectivity of the phenylethyl moiety at C-2 of the chromone ring was secured through the HMBC correlations

**Table 1**
NMR spectral data of compound **1** ($CD_3OD$).

| No. | $\delta_H$ [mult., *J* (Hz)][a] | $\delta_C$ (mult.)[a] | HMBC[b] |
|---|---|---|---|
| 2 | – | 168.6 (C) | – |
| 3 | 6.47 s | 110.6 (CH) | 2, 4, 10, 8′ |
| 4 | – | 181.7 (C) | – |
| 5 | – | 161.5 (C) | – |
| 6 | 6.06 d (2.5) | 98.2 (CH) | 5, 7, 8, 10 |
| 7 | – | 164.2 (C) | – |
| 8 | 6.24 d (2.5) | 95.8 (CH) | 7, 9, 10 |
| 9 | – | 158.6 (C) | – |
| 10 | – | 102.3 (C) | – |
| 1′ | – | 129.6 (C) | – |
| 2′ | 7.28 d (2.5) | 115.6 (CH) | 1′, 4′, 6′, 7′ |
| 3′ | – | 149.0 (C) | – |
| 4′ | – | 145.9 (C) | – |
| 5′ | 6.78 d (7.5) | 117.8 (CH) | 1′, 3′, 4′, 6′ |
| 6′ | 7.23 dd (7.5, 2.5) | 119.8 (CH) | 1′, 4′, 7′ |
| 7′ | 3.26 dd (14.5, 6.0)<br>2.73 dd (14.5, 3.5) | 40.6 ($CH_2$) | 2, 1′, 2′, 6′, 8′ |
| 8′ | 5.91 dd (6.0, 3.5) | 85.8 (CH) | 2, 3, 1′ |
| 3′-$OCH_3$ | 3.87 s | 57.3 ($CH_3$) | 3′ |

[a] Measured at 500 and 125 MHz.
[b] Measured at 700 MHz.

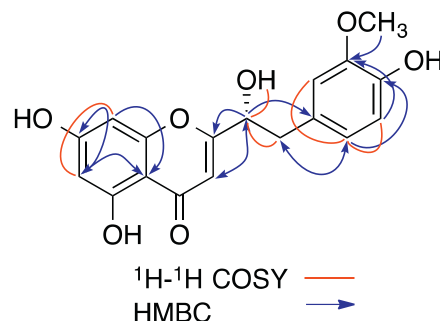


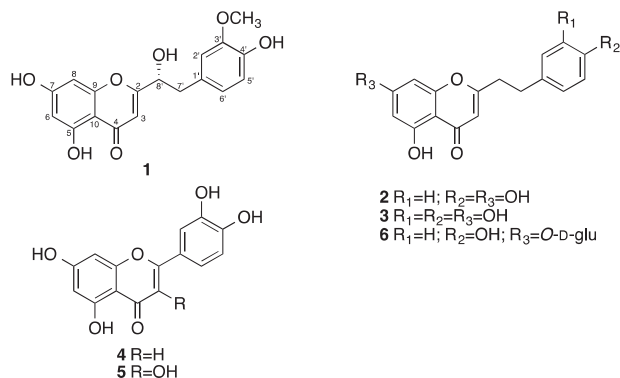

**Fig. 1.** Some important HMBC correlations of compound **1**.

**Table 2**
The DPPH radical scavenging activity results.

| Sample* | Inhibition% |
|---|---|
| PG | 97.39 ± 1.8 |
| 1 | 63.12 ± 1.6 |
| 2 | 67.09 ± 1.8 |
| 3 | 86.20 ± 0.9 |
| 4 | 87.16 ± 0.7 |
| 5 | 91.24 ± 0.8 |
| 6 | 56.07 ± 1.2 |

* Conc. (100 μM); Each value represents the mean ± S.D., $n$ = 3.

of H-7′ to C-2 and H-8′ to C-2 and C-3 (Fig. 1). The HMBC cross peaks of H-8 to C-7 ($\delta_C$ 164.2), C-9 ($\delta_C$ 158.6), and C-10 ($\delta_C$ 102.3), H-6 to C-5 ($\delta_C$ 161.5), C-7 ($\delta_C$ 164.2), C-8 ($\delta_C$ 95.8), and C-10 ($\delta_C$ 102.3), H-5′ to C-3′ ($\delta_C$ 149.0), and H-2′ and H-6′ to C-4′ ($\delta_C$ 145.9) supported the presence of hydroxyl groups at C-5, C-7, and C-4′. The HMBC correlation of the methoxy group at $\delta_H$ 3.87 to the carbon at $\delta_C$ 149.0 established its attachment at C-3′. Due to the scarcity of the compound, the relative configuration at C-8′ was assigned to be *R*-form based on the comparison $^1$H chemical shift and coupling constant of H-8′ as well as optical rotation of **1** with those of series of analogous compounds (Bernhardt et al., 2000; Kim et al., 2010; Park et al., 2010; Ibrahim et al., 2014). On the basis of these findings, **1** was identified as (*R*)-5,7-dihydroxy-2-[1-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl]chromone and named cucumin S.




The known compounds were identified by analysis of the spectroscopic data ($^1$H and $^{13}$C NMR) and comparison of their data with those in the literature, in addition to *co*- chromatography with authentic samples to be: 5,7-dihydroxy-2-[2-(4-hydroxyphenyl)ethyl]chromone (**2**) (Ibrahim, 2010; Ibrahim and Mohamed, 2014), 5,7-dihydroxy-2-[2-(3,4-dihydroxyphenyl)ethyl]chromone (**3**) (Ibrahim, 2010; Ibrahim and Mohamed, 2014), luteolin (**4**) (Harborne, 1988; Mohamed, 2008), quercetin (**5**) (Harborne, 1988; Mohamed, 2008), and 7-glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl)ethyl]chromone (**6**) (Ibrahim, 2010).

DPPH radical is widely used as a model system to investigate the scavenging activity of several natural compounds. DPPH is scavenged by the antioxidants through the donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance.

Compounds **1**–**6** showed a concentration dependent scavenging activity by quenching DPPH radical. They exhibited maximum inhibition of DPPH free radical that ranged from 91.24 to 56.07% (Table 2). Their antioxidant effects were related to the number of free hydroxyl groups especially that located at C-3′ and C-4′ in their structures. Compounds **3**, **4**, and **5** showed potent activities compared to propyl gallate at the same concentration. However, absence or blocking of the hydroxyl group by a methyl or glucose moiety leads to a decrease in the activity as in **1**, **2**, and **6** (Dugas et al., 2000; Al-Musayeib et al., 2014).

## Authors' contributions

SRMI and GAM contributed in collecting the plant sample, running the laboratory work, analysis of the spectroscopic data, and writing the manuscript. GAM carried out the antioxidant activity measurement. SRMI has revised and approved the submission.

## Conflicts of interest

The authors declare no conflicts of interest.

## References


Adams, C.F., Richardson, M., 1981. Nutritive value of foods. USDA Home and Garden Bul. 72. Government Printing Office, Washington DC, USA, pp. A2–A35.

Al-Musayeib, N.M., Mohamed, G.A., Ibrahim, S.R.M., Ross, S.A., 2014. New thiophene and flavonoid from *Tagetes minuta* leaves growing in Saudi Arabia. Molecules 19, 2819–2828.

Bernhardt, M., Shaker, K.H., Elgamal, M.H.A., Seifert, K., 2000. The new bishomoflavone ononin and its glucoside from *Ononis vaginalis*. Z. Naturforsch. C 55, 516–519.

Dugas, A.J.J., Castañeda-Acosta, J., Bonin, G.C., Price, K.L., Fischer, N.H., Winston, G.W., 2000. Evaluation of the total peroxyl radical-scavenging capacity of flavonoids: structure–activity relationships. J. Nat. Prod. 63, 327–331.

Fleshman, M.K., Lester, G.E., Riedl, K.M., Kopec, R.E., Narayanasamy, S., Curley Jr., R.W., Schwartz, S.J., Harrison, E.H., 2011. Carotene and novel apocarotenoid concentrations in orange-fleshed *Cucumis melo* melons: determinations of β-carotene bioaccessibility and bioavailability. J. Agric. Food Chem. 59, 4448–4454.

Harborne, J.B., 1988. The Flavonoids. Advances in Research Since 1980. Chapman & Hall, New York.

Ibrahim, S.R.M., Fouad, M.A., Abdel-Lateff, A., Okino, T., Mohamed, G.A., 2014. Alnuheptanoid A: a new diarylheptanoid derivative from *Alnus japonica*. Nat. Prod. Res. 28, 1765–1771.

Ibrahim, S.R.M., 2010. New 2-(2-phenylethyl)chromone derivatives from the seeds of *Cucumis melo* L var. *reticulatus*. Nat. Prod. Commun. 5, 403–406.

Ibrahim, S.R.M., 2014. New chromone and triglyceride from *Cucumis melo* seeds. Nat. Prod. Commun. 9, 205–208.

Ibrahim, S.R.M., Mohamed, G.A., 2014. Natural occurring 2-(2-phenylethyl) chromones, structure elucidation and biological activities. Nat. Prod. Res., http://dx.doi.org/10.1080/14786419.2014.991323.

Kim, J.M., Ko, R.K., Jung, D.S., Kim, S.S., Lee, N.H., 2010. Tyrosinase inhibitory constituents from the stems of *Maackia fauriei*. Phytother. Res. 24, 70–75.

Lester, G., 1997. Melon (*Cucumis melo* L.) fruit nutritional quality and health functionality. HortTechnology 7, 222–227.

Lewinsohn, E., Portnoy, V., Benyamini, Y., Bar, E., Harel-Beza, R., Gepstein, S., Giovannoni, J.J., Schaffer, A.A., Burger, Y., Tadmor, Y., Katzir, N., 2008. Sesquiterpene biosynthesis in melon (*Cucumis melo* L.) rinds. In: Pitrat, M. (Ed.), Cucurbitaceae Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. INRA, Avignon, France, pp. 249–255.

Mohamed, A.G., 2008. Alliuocide G, a new flavonoid with potent α-amylase inhibitory activity from *Allium cepa* L. ARKIVOC xi, 202–209.

Mohamed, G.A., Ibrahim, S.R.M., Ross, S.A., 2013. New ceramides and isoflavone from the Egyptian *Iris germanica* L. rhizomes. Phytochem. Lett. 6, 340–344.

Mohamed, G.A., Ibrahim, S.R.M., Al-Musayeib, N.M., Ross, S.A., 2014. New anti-inflammatory flavonoids from *Cadaba glandulosa* Forssk. Arch. Pharm. Res. 37, 459–466.

Park, D., Kim, H.J., Jung, S.Y., Yook, C., Jin, C., Lee, Y.S., 2010. A new diarylheptanoid glycoside from the stem bark of *Alnus hirsuta* and protective effects of diarylheptanoid derivatives in human HepG2 cells. Chem. Pharm. Bull. 58, 238–241.

Portnoy, V., Benyamini, Y., Bar, E., Harel-Beza, R., Gepstein, S., Giovannoni, J.J., Schaffer, A.A., Burger, Y., Tadmor, Y., Lewinsohn, E., Katzir, N., 2008. The molecular and biochemical basis for varietal variation in sesquiterpene content in melon (*Cucumis melo* L.) rinds. Plant Mol. Biol. 66, 647–661.

Ribeiro, S.F.F., Agizzio, A.P., Machado, O.L.T., Neves-Ferreira, A.G.C., Oliveira, M.A., Fernandes, K.V.S., Carvalho, A.O., Perales, J., Gomes, V.M.A., 2007. A new peptide of melon seeds which shows sequence homology with vicilin: partial characterization and antifungal activity. Sci. Hortic. 111, 399–405.

Velcheva, M.P., Donchev, C., 1997. Isoprenoid hydrocarbons from the fruit of extant plants. Phytochemistry 45, 637–639.

Vouldoukis, I., Lacan, D., Kamate, C., Coste, P., Calenda, A., Mazier, D., Conti, M., Dugas, B., 2004. Antioxidant and anti-inflammatory properties of *Cucumis melo* LC. Extract rich in superoxide dismutase activity. J. Ethnopharmacol. 94, 67–75.