Revista Brasileira de Farmacognosia 26 (2016) 701–704

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Cucumol A: a cytotoxic triterpenoid from *Cucumis melo* seeds

Sabrin Ibrahim[a,b,*], Rwaida Al Haidari[a], Gamal Mohamed[c,d], Ehab Elkhayat[d], Mohamed Moustafa[e]

[a] *Department of Pharmacognosy and Pharmaceutical 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 and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*
[d] *Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut, Egypt*
[e] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*

ARTICLE INFO

Article history:
Received 31 January 2016
Accepted 2 March 2016
Available online 21 May 2016

Keywords:
Cucumis melo
Cucurbitaceae
Triterpenoid
Cucumol A
Cytotoxic activity

ABSTRACT

Phytochemical investigation of the MeOH extract of *Cucumis melo* L. var. *reticulates*, Cucurbitaceae, seeds led to the isolation of a new triterpenoid: cucumol A (27-hydroxy taraxerol-3β-ol), along with three known compounds: α-spinasterol and D:B-friedoolean-5-ene-3-β-ol. Their structures were established by extensive 1D (^{1}H, ^{13}C, and DEPT) and 2D (^{1}H–^{1}H COSY, HMQC, and HMBC) NMR, as well as IR and HRESIMS spectral analyses. Compound **3** displayed cytotoxic activity against L5178Y and Hela cancer cell lines with ED_{50} of 1.30 and 5.40 μg/ml, respectively compared to paclitaxel (0.07 and 0.92 μg/ml, respectively).

Introduction

Cucumis melo L. (cantaloupe) belonging to Cucurbitaceae family, is cultivated in temperate, subtropical, and tropical regions worldwide (Yasar et al., 2006; Ibrahim, 2010). Its fruits are consumed in the summer period because the pulp of the fruit is very refreshing, high nutritional, and sweet with a pleasant aroma, which may be used as an appetizer, a dessert or as salad (Melo et al., 2000; Baghaei et al., 2008). In Chinese folk medicine, the seeds of *C. melo* are used as digestive, febrifuge, antitussive, demulcent, and vermifuge (Duke and Ayensu, 1985; De Marino et al., 2009). Their extract can be used as an anti-diabetic and is useful in chronic eczema (Lal and Lata, 1980; Teotia and Ramakrishna, 1984). Seed kernel is commonly used in renal disorders such as kidney and bladder stones, ulcers in the urinary tract and stomach, painful and burning micturation, jaundice, vitiligo, ascites, suppression of urine, chronic fevers, inflammation of the liver and kidney, and in general debility in the Indian traditional medicine (Nayar and Singh, 1998; Baitar, 2003; Gill et al., 2011; Milind and Kulwant, 2011; Ibrahim, 2014; Ullah et al., 2015). The fruit's pulp possesses diuretic and anthelmintic properties (Ullah et al., 2015). Its lotion is employed for acute and chronic eczema. Roots are emetic agents. The fruits are used as a first aid treatment for burns and abrasions. Peduncle is used to manage anasarca and indigestion (Milind and Kulwant, 2011). *C. melo* revealed a wide range of biological activities such as antioxidant, analgesic, anti-inflammatory, and antimicrobial (Vouldoukis et al., 2004; Mariod and Matthaus, 2008; Gill et al., 2011; Ibrahim, 2014). Previous phytochemical studies on *C. melo* L. var. *reticulates* seeds resulted in the isolation of chromone derivatives, triterpene, and sterols (Ibrahim, 2010; Ibrahim, 2014, Ibrahim and Mohamed, 2015a,b). In the present work, investigation of the MeOH extract of *C. melo* L. var. *reticulates* seeds afforded a new triterpenoid: cucumol A (27-hydroxy taraxerol-3β-o1) (**3**), along with α-spinasterol (**1**) and D:B-friedoolean-5-ene-3-β-ol (**2**). The new compound was evaluated for its cytotoxic activity against L5178Y, PC12, and Hela cancer cell lines.

Materials and methods

General experimental procedures

Melting point was carried out using an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). Optical rotation was recorded on a Perkin-Elmer

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

http://dx.doi.org/10.1016/j.bjp.2016.03.012

Model 341 LC Polarimeter (Perkin-Elmer, Waltham, MA, USA). EIMS was recorded on JEOL JMS-SX/SX 102A mass spectrometer (Joel, Peabody, MA, USA). HRESIMS spectrum was recorded on a LTQ Orbitrap (Thermo Finnigan, Bremen, Germany). 1D and 2D NMR spectra were recorded on a Bruker DRX400 NMR spectrometer using standard Bruker software and C_5D_5N and $CDCl_3$ as solvents, with TMS as the internal reference (Bruker, Rheinstetten, Germany). Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm, Merck, Darmstadt, Germany). TLC was performed on precoated TLC plates with silica gel 60 F_{254} (layer thickness 0.2 mm, Merck, Darmstadt, Germany). The chromatograms were developed using the following solvent systems: hexane:EtOAc (95:5, S_1) and hexane:EtOAc (90:10, S_2). The compounds were detected by spraying with *p*-anisaldehyde/H_2SO_4 reagent and heating at 110 °C for 1–2 min.

Plant material

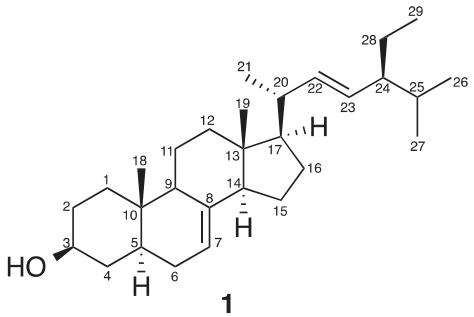
Seeds of *Cucumis melo* L. var. *reticulates*, Curcubitaceae, were obtained from the cultivated plants El-Galaa Village, Samalout, Minia, Egypt. The plant material was identified and authenticated (voucher specimen 2014-5) by Prof. Dr. Mohamed A. Farghali, Professor of Horticulture (Vegetable Crops), Faculty of Agriculture, Assiut University.

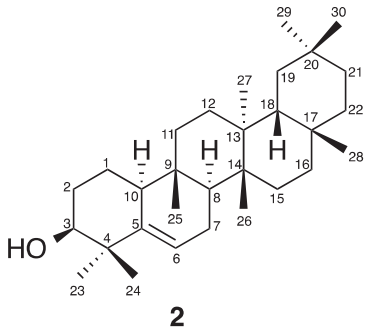
Table 1
NMR spectral data of compound **3** (C_5D_5N, 400 and 100 MHz).

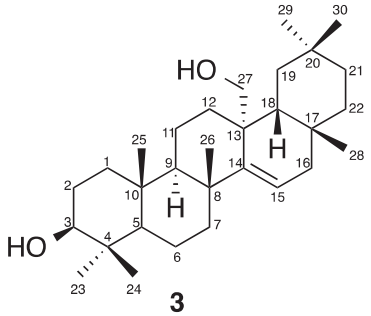
No.	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC
1	1.51 m 1.47 m	37.7 (CH_2)	–
2	2.05 m 1.83 m	28.0 (CH_2)	3
3	3.42 dd (9.2, 6.6)	78.1 (CH)	5, 24
4	–	39.2 (C)	–
5	0.81 dd (9.6, 1.3)	55.9 (CH)	1, 4, 6, 24
6	1.53 m 1.34 m	19.1 (CH_2)	–
7	1.92 m 1.89 m	41.6 (CH_2)	14
8	–	39.4 (C)	–
9	1.42 m	49.5 (CH)	–
10	–	38.7 (C)	–
11	1.64 m 1.41 m	17.8 (CH_2)	–
12	1.31 m 1.27 m	33.2 (CH_2)	–
13	–	41.1 (C)	–
14	–	158.6 (C)	–
15	5.62 dd (10.6, 4.3)	116.8 (CH)	8, 13, 17
16	2.68 dd (13.8, 10.6) 1.86 dd (13.8, 4.3)	31.7 (CH_2)	14, 15, 17, 18, 27, 15
17	–	41.1 (C)	–
18	0.70 dd (10.8, 4.8)	45.5 (CH)	14, 27
19	1.50 m 1.03 m	36.8 (CH_2)	–
20	–	38.7 (C)	
21	–	28.4 (CH_2)	–
22	1.62 m 1.46 m	33.8 (CH_2)	–
23	1.21 s	28.6 (CH_3)	3, 4, 5, 24
24	1.05 s	16.4 (CH_3)	3, 4, 5, 23, 25
25	0.92 s	15.6 (CH_3)	1, 5, 9, 24
26	1.08 s	26.2 (CH_3)	7, 9, 14
27	3.59 d (11.8) 3.44 d (11.8)	64.5 (CH_2)	12, 14, 16, 18, 16
28	1.02 s	33.7 (CH_3)	18, 19, 21
29	0.98 s	30.1 (CH_3)	19, 21, 30
30	1.09 s	21.9 (CH_3)	21, 22, 29
OH	5.96 brs	–	–
OH	5.75 brs	–	–

Extraction and isolation

Fruits were cut and seeds were removed from stringy piths. The seeds were rubbed by hand and washed quickly with tap water, then transferred to a colander and dried at room temperature. Dried seeds (200 g) were triturated in a ball mill and screened through a mesh of 0.5 mm diameter. The triturated seeds (175 g) were packed in a Soxhlet apparatus and defatted using hexane (3 × 1 l), then extracted with MeOH several times (4 × 1 l). The MeOH extract was evaporated and concentrated under reduced pressure to afford a dark brown residue (9.3 g). The latter was subjected to VLC (vacuum liquid chromatography) using hexane:EtOAc and EtOAc:MeOH gradients to afford five fractions: CA-I to CA-V; CA-I (2.6 g, hexane:EtOAc 75:25), CA-II (1.3 g, hexane:EtOAc 50:50), CA-III (2.1 g, hexane:EtOAc 25:75), CA-IV (0.7 g, EtOAc 100%), and CA-V (1.2 g, MeOH 100%). Fraction CA-I (2.6 g) was subjected to silica gel column chromatography (120 g × 50 × 2 cm) using hexane:EtOAc gradient to afford four subfractions CA-IA:CA-ID. Silica gel column chromatography (80 g × 50 × 2 cm) of subfraction CA-IB (0.55 g) using hexane:EtOAc as an eluent gave compound **1** (15 mg, white crystalline needles). Subfraction CA-IC (0.67 g) was chromatographed over silica gel column (90 g × 50 × 2 cm) using hexane:EtOAc gradient to yield compound **2** (10 mg, white crystals). Subfraction CA-ID was subjected to silica gel column using hexane:EtOAc (95:5–80:20) to afford compound **3** (7.5 mg, white needles).







Spectral data

Cucumol A (**3**): White needles (7.5 mg); mp 203–204 °C; $[\alpha]_D^{22}$ + 36.1 (*C* = 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 258 (4.25) nm; IR (KBr) γ_{max}: 3435, 2982, 1610, 1070 cm^{-1}; NMR data (C_5D_5N, 400 MHz and 100 MHz), see Table 1: HRESIMS *m*/*z* 443.38840 (calcd for $C_{30}H_{51}O_2$ [M+H(proton)]$^+$, 443.38836).

Cytotoxicity assay

The cytotoxic activity of compound **3** was examined towards mouse lymphoma (L5178Y), rat brain (PC12), and human cervix (Hela) cancer cell lines using MTT assay as described earlier (Mohamed, 2014; Mohamed et al., 2013). Exponentially growing cells were harvested, counted, and diluted appropriately. Of the cell suspension, 50 μl containing 3750 cells were pipetted into 96-well microtiter plates. Subsequently, 50 μl of a solution of the tested sample was added to each well. The test plates were incubated at 37 °C with 5% CO_2 for 72 h. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at 5 mg/ml in phosphate buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4) and from this solution, 20 μl was pipetted into each well. The yellow MTT penetrates the healthy living cells and in the presence of mitochondrial dehydrogenases, MTT is transformed to its blue formazan complex. After an incubation period of 4 h at 37 °C in a humidified incubator with 5% CO_2, the medium was centrifuged (15 min, 20 °C, 210 × *g*) with 200 μl DMSO, the cells were lysed to liberate the formed formazan product. Cell viability was evaluated by measurement of the absorbance 520 nm using a scanning microtiter-well spectrophotometer. Compound concentrations that produce 50% cell growth inhibition (ED_{50}) were calculated from curves constructed by plotting cell survival (%) versus drug concentration (μg/ml). All experiments were carried out in triplicates and repeated three times. As negative controls, media with 0.1% (v/v) EtOH were included in all experiments. The paclitaxel was used as a positive control (Ibrahim et al., 2015c).

Results and discussion

Compound **3** was obtained as white needles. It gave a positive Liebermann-Burchard's test, indicating its triterpenoidal nature (Ibrahim et al., 2012; Sayed et al., 2007; Reinhold, 1935). Its HRESIMS spectrum showed a quasi-molecular ion peak at *m*/*z* 443.3884 [M+H]$^+$, suggesting a molecular formula $C_{30}H_{50}O_2$, which implied six degrees of unsaturation. The IR spectrum showed absorption bands at 3435 and 1070 cm^{-1} characteristic for the presence of a hydroxyl group (Silverstein and Webster, 1998; Al Musayeib et al., 2014). This was confirmed by the appearance of two exchangeable protons signals at δ_H 5.96 (1H, brs) and 5.75 (1H, brs) in ^{1}H NMR spectrum. ^{13}C NMR and DEPT spectra of **3** revealed the presence of resonances for thirty carbons: seven methyls, eleven methylenes one of them is oxygen-bonded at δ_C 64.5 (C-27), five methines, and seven quaternary carbons. The ^{1}H and ^{13}C NMR spectra exhibited seven methyl groups signals at δ_H 1.21 (H-23)/δ_C 28.6 (C-23), 1.05 (H-24)/16.4 (C-24), 0.92 (H-25)/15.6 (C-25), 1.08 (H-26)/26.2 (C-26), 1.02 (H-28)/33.7 (C-28), 0.98 (H_3-29)/30.1 (C-29), and 1.09 (H-30)/21.9 (C-30), suggesting a pentacyclic triterpenoidal nature of **3** (Mahato and Kundu, 1994; Laphookhieo et al., 2004; Al Muqarrabun et al., 2014). Moreover, signals for *tri*-substituted double bond at δ_H 5.62 (dd, *J* = 10.6, 4.3 Hz, H-15)/δ_C 116.8 (C-15) and 158.6 (C-14) were observed. Its position at C_{14}–C_{15} was established by the ^3J HMBC cross peaks of H-7, H-16, and H-18 to C-14 and H-15 to C-8, C-13, and C-17. The *Z* geometry of the double bond was assigned based on the coupling constant value $J_{15,16ax}$ = 10.6 and

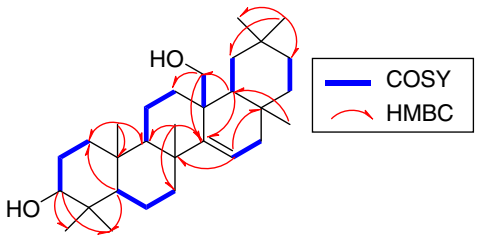


Fig. 1. Some key ^{1}H–^{1}H COSY and HMBC correlations of compound **3**.

$J_{15,16eq}$ 4.3 Hz (Ibrahim, 2014). The ^{1}H NMR spectrum revealed signals for an oxymethine at δ_H 3.42 (1H, dd, *J* = 9.2, 6.6 Hz, H-3) and oxymethylene at δ_H 3.59 and 3.44 (each d, *J* = 11.8 Hz, H-27), which correlated with the carbon signals resonating at δ_C 78.1 (C-3) and 64.5 (C-27), respectively in HMQC spectrum. The HMBC cross peaks of H-2, H-23, and H-24 to C-3, H-16 and H-18 to C-27, and H-27 to C-12, C-14, and C-18 established the positions of the oxymethine and oxyemthylene moieties at C-3 and C-27, respectively. Three methine proton signals at δ_H 0.81 (dd, *J* = 9.6, 1.3 Hz, H-5), 1.42 (m, H-9), and 0.70 (dd, *J* = 10.8, 4.8 Hz, H-18), which correlated with the carbon signals resonating at δ_C 55.9 (C-5), 49.5 (C-9), and 45.5 (C-18), respectively in HMQC spectrum were observed. Their assignment was established based on the observed correlation in the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 1). On the basis of the above spectra evidence and by comparison with literature (Mahato and Kundu, 1994; Laphookhieo et al., 2004), the structure of compound **3** was established as 27-hydroxy taraxerol-3β-o1 and named cucumol A.

The known compounds were identified by analysis of the spectroscopic data (^{1}H, ^{13}C NMR, COSY, and HMQC) and comparison of their data with those in the literature to be: α-spinasterol (**2**) (Ibrahim, 2014) and D:B-friedoolean-5-ene-3-β-ol (**3**) (Ibrahim, 2014).

The cytotoxic effect of compound **3** was tested towards L5178Y, PC12, and Hela cancer cell lines. Compound **3** was found to display cytotoxic activity towards L5178Y and Hela cancer cell lines with ED_{50} values of 1.30 and 5.40 μg/ml, respectively compared to paclitaxel (0.07 and 0.92 μg/ml, respectively). While, it was inactive against PC12 cancer cell line.

Conclusion

A new triterpenoid: cucumol A (**3**) and three known compounds were isolated from *C. melo* seeds afforded. Their structures were established by different spectroscopic analyses. Compound **3** showed cytotoxic activity towards L5178Y and Hela cells.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

GAM collected the plant sample. SRMI, RAA, MAAM, and GAM contributed in running the laboratory work, analysis of the spectro-

scopic data, and writing the manuscript. SRMI and ESE have revised and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to express our deep thanks to Dr. Volker Brecht for acquiring NMR and MS spectroscopic data. We are grateful to Prof. Dr. W. E. G. Müller (Institute für Physiologische Chemie, Dues bergweg 6, D-55099 Mainz, Germany) for carrying out cytotoxicity assay.

References

Al Muqarrabun, L.M.R., Ahmat, N., Aris, S.R.S., Norizan, N., Shamsulrijal, N., Yusof, F.Z.M., Suratman, M.N., Yusof, M.I.M., Salim, F., 2014. A new triterpenoid from *Sapium baccatum* (Euphorbiaceae). Nat. Prod. Res. 28, 1003–1009.

Al Musayeib, N.M., Mothana, R.A., Ibrahim, S.R.M., El Gamal, A.A., Al-Massarani, S.M., 2014. Klodorone A and klodorol A: new triterpenes from *Kleinia odora*. Nat. Prod. Res. 28, 1142–1146.

Baghaei, H., Shahidi, F., Varidi, M.J., Mahallati, M.N., 2008. Orange-cantaloupe seed beverage: nutritive value, effect of storage time and condition on chemical, sensory and microbial properties. World Appl. Sci. J. 3, 753–758.

Baitar, I.E., 2003. Aljamaiul Mufradat-ul Advia Wal Aghzia. CCRUM, New Delhi, pp. 248.

De Marino, S., Festa, C., Zollo, F., Iorizzi, M., 2009. Phenolic glycosides from *Cucumis melo* var. *inodorus* seeds. Phytochem. Lett. 2, 130–133.

Duke, J.A., Ayensu, E.S., 1985. Medicinal Plants of China, ISBN 0-9177256-20-4.

Gill, N.S., Bajwa, J., Dhiman, K., Sharma, P., Sood, S., Sharma, P.D., Singh, B., Bali, M., 2011. Therapeutic potential of traditionally consumed *Cucumis melo* seeds. Asian J. Plant Sci. 10, 86–91.

Ibrahim, S.R.M., 2010. New 2-(2-phenylethyl)chromone derivatives from the seeds of *Cucumis melo* L var. *reticulates*. Nat. Prod. Commun. 5, 403–407.

Ibrahim, S.R.M., Mohamed, G.A., 2015a. Cucumin S, a new phenylethyl chromone from *Cucumis melo* var. *reticulatus* seeds. Rev. Bras. Farmacogn. 25, 462–464.

Ibrahim, S.R.M., Mohamed, G.A., 2015b. Natural occurring 2-(2-phenylethyl)chromones, structure elucidation and biological activities. Nat. Prod. Res. 29, 1489–1520.

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., Zayed, M.F., Sayed, H.M., 2015c. Ingenines A and B, two new alkaloids from the Indonesian sponge *Acanthostrongylophora ingens*. Drug Res. 65, 361–365.

Ibrahim, S.R.M., Mohamed, G.A., Shaala, L.A., Banuls, L.M.Y., Van Goietsenoven, G., Kiss, R., Youssef, D.T.A., 2012. New ursane-type triterpenes from the root bark of *Calotropis procera*. Phytochem. Lett. 5, 490–495.

Lal, S.D., Lata, K., 1980. Plants used by Bhat community for regulating fertility. Econ. Bot. 34, 273–275.

Laphookhieo, S., Karalai, C., Ponglimanont, C., 2004. New sesquiterpenoid and triterpenoids from the fruits of *Rhizophora mucronata*. Chem. Pharm. Bull. 52, 883–885.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids A compilation and some salient features. Phytochemistry 37, 1517–1575.

Mariod, A., Matthaus, B., 2008. Investigations on fatty acids, tocopherols, sterols, phenolic profiles and oxidative stability of *Cucumis melo* var. *agrestis* oil. J. Food Lipids 15, 56–67.

Melo, M.L.S., Narain, N., Bora, P.S., 2000. Characterisation of some nutritional constituents of melon (*Cucumis melo* hybrid AF-522) seeds. Food Chem. 68, 411–414.

Milind, P., Kulwant, S., 2011. Musk melon is eat-must melon. Int. Res. J. Pharm. 2, 52–57.

Mohamed, G.A., 2014. New cytotoxic cycloartane triterpene from *Cassia italica* aerial parts. Nat. Prod. Res. 28, 976–983.

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.

Nayar, N.M., Singh, R., 1998. In: Nayar, N.M., More, T.A. (Eds.), Taxonomy, Distribution and Ethnobotanical Uses in Cucurbits. Science Publishers, Inc., USA, pp. 1–18.

Reinhold, J.G., 1935. Liebermann-Burchard reaction velocities of sterols: I. Differences between free and ester cholesterol applied to the determination of cholesterol esters, II. A test for the presence of coprostenol in plasma. Am. J. Med. Sci. 189, 302.

Sayed, H.M., Mohamed, M.H., Farag, S.F., Mohamed, G.A., Proksch, P., 2007. A new steroid glycoside and furochromones from *Cyperus rotundus* L. Nat. Prod. Res. 21, 343–350.

Silverstein, R.M., Webster, F.X., 1998. Spectrometric Identification of Organic Compounds, 6th ed. New York, John Wiley.

Teotia, M.S., Ramakrishna, P., 1984. Chemistry and technology of melon seeds. J. Food Sci. Technol. 21, 332–340.

Ullah, N., Khan, S., Khan, A., Ahmad, W., Shah, Y., Ahmad, L., Ullah, I., 2015. A prospective pharmacological review of medicinal herbs, *Cucumis melo* and *Berberis vulgaris*, commonly used in the treatment of renal diseases in Pakistan. Acta Poloniae Pharm. Drug Res. 72, 651–654.

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.

Yasar, F., Kusvuran, S., Ellialtioglu, S., 2006. Determination of antioxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress. J. Hortic. Sci.