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Determination of Volatile Constituents of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi Fruits and Evaluation of its Cytotoxic Activity

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ABSTRACT

Objectives: *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi plant is a member of family Cucurbitaceae. This study aimed to identify volatile components of the pulp of the fruit and also to evaluate its cytotoxic activity. **Methods:** Automated rapid headspace solid phase micro extraction (SPME) was applied for extraction of the volatile components combined with gas chromatography coupled to mass spectrometry GC/MS for identification of the compounds responsible for the specific aroma of the fruits, MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay) was used for the evaluation of the cytotoxic activity of both petroleum ether and defatted methanol extracts of the fruits' pulp against 3 different tumor cell lines; colon (Caco-2), hepatic (HepG2) and cervix (HeLa) carcinoma cells. **Results:** Twenty volatile components were identified in the pulp of the fruits which were dominated by esters as the major class of components, with hexyl acetate representing the major constituent (23.07%). Petroleum ether extract of the pulp displayed mild cytotoxic activity against HepG2 cell line with IC_{50} of (31.6) µg/ml, all results were standardized against doxorubicin positive control**. Conclusion:** This is the first report for the evaluation of the volatile constituents of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi fruits which can serve as a base for its differentiation from other similar varieties in the same family.

Keywords: *Cucumis melo; Cytotoxicity; Flavor; Volatile aroma*

INTRODUCTION

Family Cucurbitaceae comprises a wide variety of plants including 119 genera and 775 species distributed in tropical, warm regions and few in the temperate regions[1]. In the flora of Egypt, the family is represented by seventeen wild species in ten genera[2]. *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi (Ismailawy sweet melon) belonging to family Cucurbitaceae had originated in Upper Egypt. It was introduced to Ismailia in the early part of the 20th century and has been known as the most famous and popular cultivated fruits' crop in Egypt for many years[3]. Sweetness and aroma properties are two of the most important parameters that determine the quality and consumer preference of melon fruit[6-7]. Essential oils are the secondary metabolites that contain the volatile aroma compounds from natural sources. They may be also formed directly in the plant cell or they may result from the hydrolysis of certain glycosides[8]. Since ancient

times, essential oils are recognized for their medicinal value and they have been used as perfumes, flavors for foods and beverages, or to heal both body and mind for thousands of years[9].The main purpose of this study was to identify the aromatic profile of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi using quantitative method utilizing gas chromatography coupled to mass spectrometry (GC–MS), which can be used as the base for the differentiation of several *Cucumis* varieties. In addition to the evaluation of the cytotoxic activity of petroleum ether and defatted methanol extracts of the pulp of the fruits against 3 different tumor cell lines; colon (Caco-2), hepatic (HepG2) and cervix (HeLa) cell lines by the calculation of their half maximal inhibitory concentration (IC_{50}), where the results were standardized against doxorubicin.

MATERIAL AND METHODS

Plant Material

Cucumis melo L. var. *aegyptiacus* cv. Ismailawi fruits were collected in June 2016 from Saeed farm, 32 kilometers along Ismailia Port-said desert highway. Identification was done by Professor Dr. Mohamed Abd Al-Salam, Faculty of Agriculture - Suez Canal University & Dr. Ahmed Abd-Elmogali, a specialized taxonomist at Agriculture Research Center, Giza, Egypt. Dried specimens (117) were kept at the Herbarium of Floral & Phyto-taxonomy Researches – Horticultural Research Institute, Agriculture Research Center, Dokki, Cairo, Egypt.

Headspace solid phase micro extraction (SPME) of volatile constituents

SPME holder and fiber coated with 50 µm/30µm DVB-CAR-PDMS were supplied by Supelco (Oakville, ON, Canada). All other chemicals, volatile standards were provided from Sigma Aldrich (St. Louis, Mo., U.S.A.). Headspace volatiles analysis using SPME was applied according to the official method of analysis[10]. The pulp of the fruit was ground, and 3 g were placed inside 20ml clear glass vials. volatile organic compounds (VOCS) free from (Z)-3 hexenyl acetate, was used as an internal standard (IS), dissolved in water and added to each vial at a concentration of 1 µg/vial. Vials were then immediately capped and placed on a temperature controlled tray for 30 min at 50 ºC with the SPME fiber inserted into the headspace above the sample. Adsorption was timed for 30 min. A system blank containing no plant material was run as a control.

Gas chromatography-mass spectrometry (GC-MS) analysis

SPME fibers were desorbed at 210 ºc for 1 min in the injection port of a Shimadzu Model GC-17A gas chromatograph interfaced with a Shimadzu model QP-5000 mass spectrometer (Japan). Volatiles were separated on a DB5-MS column (30 m length, 0.25mminner diameter, and 0.25 µm film). Injections were made in the split-less mode for 30 s. The gas chromatograph was operated at a rate of 38 ºC/min with column oven at 220 ºC for 3 min, then programmed at a rate of 12 ºC/min to 180 ºC, kept at 180 ºC for 5 min, and finally ramped at a rate of 40 ºC/ min to 220 ºC and kept for 2 min, He carrier gas at 1 ml/min. The transfer line and ion-source temperatures were adjusted at 230˚C and 180 ºC, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at m/z 40-500.

Identification of volatile constituents

Volatile components peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its kovat retention index (KI) relative to n-alkanes (C6-C20), mass spectrum matching to NIST, WILEY library database and with authentic standards when available.

Extraction and sample preparation

C. melo L. var. *aegyptiacus* cv. Ismailawi fruits were cut into small pieces after peeling and seeds were removed from stringy pith. The pulp of fruits was defatted by maceration in petroleum ether then filtration to prepare petroleum ether extract followed by extraction with 70% methanol to prepare defatted methanol extract for the pulp of the fruits. All samples were prepared by dissolving in dimethylsulfoxide (DMSO) at 100 mM and stored at -20°C. Different concentrations of each of the tested extracts (6.25, 12.5, 25, 50, 100 µg/ml) were used[12].

Maintenance of the human cancer cell lines in the laboratory

A cryotube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37°C. The tube was opened under strict aseptic conditions and its contents were supplied by 5 ml supplemented medium drop by drop in a 50 ml sterile falcon tube. Then incubation was done for 2 hours followed by centrifugation at 1200 rpm for 10 minutes. The supernatant was discarded and the cell pellet was suspended and seeded in 5 ml supplemented medium in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated and followed up daily. The supplemented medium was replaced every 2-3 days. Incubation was continued until a confluent growth was achieved and the cells were freshly sub-cultured before each experiment to be in the exponential phase of growth.

Table 1. Identified volatile components in the pulp of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi and their relative percentage using SPME-GCMS

Rt (min)	Identified compounds	KI	Molecular Formula	Molecular weight	Relative area (%)
4.115	Acetic acid, isobutyl ester	1056	$C_6H_{12}O_2$	116	1.78
4.500	Ethyl butyrate	1090	$C_6H_{12}O_2$	116	5.09
4.600	Ethyl isobutyrate	1100	$C_6H_{12}O_2$	116	9.56
4.656	Ethyl isocyanide	1104	C_3H_5N	55	0.03
4.808	Acetic acid butyl ester	1118	$C_6H_{12}O_2$	116	4.34
4.883	Isobutyl acetate	1125	$C_6H_{12}O_2$	116	13.78
5.450	Unidentified compound	-	-	-	1.98
5.533	Butanoic acid 2-methyl- ethyl ester	1183	$C_7H_{14}O_2$	130	4.44
6.125	2-Methyl-1-butyl acetate	1235	$C_7H_{14}O_2$	130	14.73
6.518	Isobutyric acid, propyl ester	1270	$C_7H_{14}O_2$	130	0.34
6.833	Unidentified compound	-	-	-	0.45
7.277	Unidentified compound	-	-	-	0.16
7.550	Unidentified compound	-	-	-	0.13
7.839	Unidentified compound	-	-	-	0.11
8.275	Hexanoic acid ethyl ester	1427	$C_8H_{16}O_2$	144	7.03
8.408	4-Hexen-1-ol acetate	1438	$C_8H_{14}O_2$	142	5.67
8.500	Hexyl acetate	1447	$C_8H_{16}O_2$	144	23.07
8.527	Butanoic acid 3 methyl butyl ester	1449	$C_9H_{18}O_2$	185	0.09
8.776	Limonene	1471	$C_{10}H_{16}$	136	0.27
9.250	Propanoic acid, 2-methyl, 3- methyl butyl ester	1513	$C_9H_{18}O_2$	136	0.26
10.042	Unidentified compound	-	-	-	0.35
10.909	2,6-Nonadienal (E,Z)-	1662	$C_9H_{14}O$	138	0.33
11.283	3-Hexenyl iso-butyrate	1680	$C_{10}H_{18}O_2$	170	0.84
11.442	Butanoic acid hexyl ester	1685	$C_{10}H_{20}O_2$	172	2.12
11.975	Hexanoic acid, 3-methyl butyl ester	1759	$C_{11}H_{22}O_2$	186	0.23
12.350	Unidentified compound	-	-	-	0.19
12.745	Unidentified compound	-	-	-	0.004
13.642	Hexyl caproate	1919	$C_{12}H_{24}O_2$	200	0.96
14.284	Unidentified compound	-	-	-	0.45
15.317	Unidentified compound	-	-	-	0.27
16.142	Unidentified compound	-	-	-	0.30
16.220	Unidentified compound	-	-	-	0.47
17.727	Unidentified compound	-	-	-	0.17
19.133	Unidentified compound	-	-	-	0.006
	Total esters	-	-	-	**93.37%**
	Total Monoterpene	-	-	-	**1.23%**
	Total acetate esters	-	-	-	**57.25%**
	Total cyano-compounds	-	-	-	**0.03%**
	Total aldehyde	-	-	-	**0.33%**
	Total identified compounds	-	-	-	**94.96%**
	Total unidentified compounds	-	-	-	**5.04%**

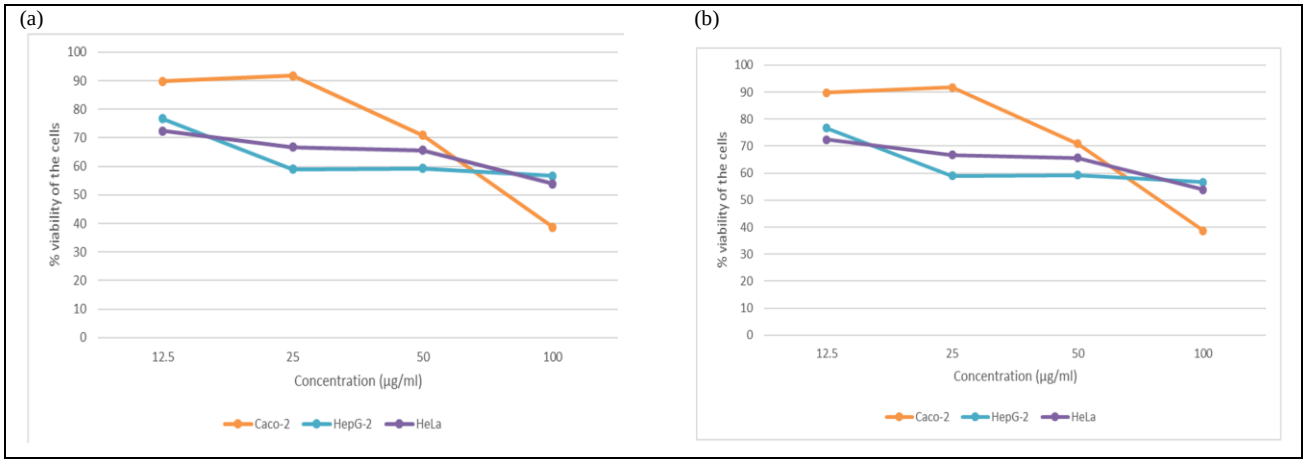

Figure 1. (a) Effect of the Petroleum ether extract of the pulp of the fruits of *C. melo* L. var. *aegyptiacus* cv. Ismailawi against Caco-2, HepG2 and HeLa cell lines. (b) Effect of the defatted methanol extract of pulp of the fruits of *C. melo* L. var. *aegyptiacus* cv. Ismailawi against Caco-2, HepG2 and HeLa cell lines

Collection of cells by trypsinization

The medium was discarded. The monolayer cells were washed twice with 5 ml phosphate buffered saline. All the adherent cells were dispersed from their monolayer by the addition of 1 ml trypsin solution (0.25% trypsin w/v) for 2 minutes.

Procedure of the MTT assay

In flat bottom 96 well-microplates, cells (0.5×105) were cultured in 180 µl/well RPMI media supplemented with 10% fetal bovine serum, 2µmol/ml L-glutamine, 250 ng/ml fungi zone, 100 units/ml penicillin streptomycin solutions at 37°C in a CO_2 incubator. The plates were incubated for 24 h at 37°C in a humidified 5% CO_2 atmosphere to allow cells to settle down. After incubation, the media were removed, and 180 µl/well fresh serum free medium were added to each well. Cells are then treated with 20 µl of different concentrations of the tested extracts dissolved in DMSO (100 – 0.8 µg/ml) or doxorubicin as standard drug. The plates were then incubated for 24 h at 37°C in a humidified 5% CO_2 atmosphere. After incubation, the media were removed and MTT solution 40 µl/well was added and incubated for additional 4 h. MTT crystals were solubilized by adding acidified isopropanol (160 µl/well) and the plate was shaken at room temperature. This was followed by colorimetric determination of the absorbance at 570 nm using the microplate ELISA reader (FLUOstar Omega, BMG, Labtech, Germany). The absorbance of the resulting color is directly proportional to the number of the viable cells in each sample[12]. The percentage of relative viability was calculated using the following equation:

[Absorbance of treated cells/ Absorbance of control cells)] X 100

RESULTS AND DISCUSSION

Volatile constituents' analysis

Thirty four volatile components were detected, of which twenty components were tentatively identified in the extracted volatiles from the pulp of *C. melo* L. var. *aegyptiacus* cv. Ismailawi fruit representing 94.96% identification. The volatiles composition, relative area percentage, chemical formula and molecular weight of each identified component are represented in **Table 1** and arranged corresponding to their retention time (Rt). In the present study, it was found that the volatile components were dominated by esters representing up to 93.37% of the volatiles composition with nearly up to more than half this percentage is formed of acetate esters indicating the completion of maturity of the tested fruits as immature fruits are usually predominated by aldehydes[13]. Volatile esters are major components of the aroma of different fruits such as strawberries, bananas, apples, sometimes being the compounds mainly responsible for the well-flavor appreciated by consumers[14, 15]. In *C. melo* L. var. *aegyptiacus* cv. Ismailawi fruit, esters of aliphatic acids are the main constituents, which can be used as a base for its differentiation from other *Cucumis* varieties having volatiles lacking the presence of esters in their composition such as *C. melo* L. var. Cantaloupensis[16] and *C. melo* L. cv. Miyabi[17]. It is worth mentioning that thioesters, which appear to be derived biogenetically from methionine and which play an important role in the aroma profile of *Cucumis*, although being reported in many varieties of melons[5,7,17], are completely absent in the tested volatiles under investigation. Moreover, five components of the identified constituents; ethyl isocyanide, 4-hexen-1-ol acetate, 3-hexenyl iso-

butyrate, butanoic acid hexyl ester and hexyl caproate were detected for the first time in *C. melo* L.

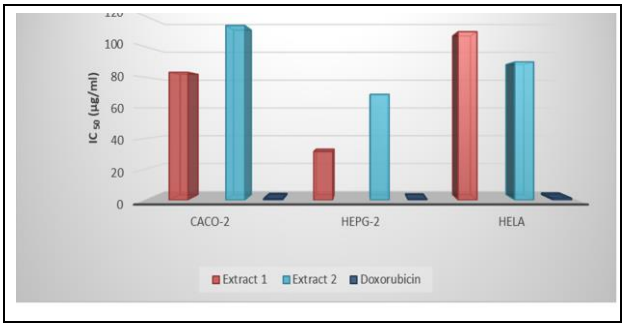

Figure 2. IC_{50} of the petroleum ether extract and defatted methanol extract of the pulp of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi fruits and doxorubicin against HeLa, HepG2 and Caco-2 cell lines.

Cytotoxic activity

The IC_{50} of the petroleum ether and the defatted methanol extracts of the pulp of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi fruits against HeLa, HepG2 and Caco-2 cell lines are recorded in **Table 2** using doxorubicin as a positive standard. The results are graphically represented in **Figures** (**1a**, **1b** and **2**). The best cytotoxic activity for both tested extracts was seen against HepG2 cell line with IC_{50} of 31.6 µg/ml for the petroleum ether extract and 68.9 µg/ml for the defatted methanol extract. Although previous reports indicated potential medical anti-carcinogenic properties of some separated compound from Japanese pickling melon, *C. melo* var. *conomon*[18], as well as for *C. melo*. Linn against prostate cancer cell lines (PC-3)[19], the present study showed a mild activity for the tested fractions in comparison to doxorubicin positive standard. According to the National Cancer Institute (USA), vegetables crude extracts are considered cytotoxic when their IC_{50} values are less than 30µg/ml and pure compounds with IC_{50} values below 4 µg/ml in tumor cell line assays are promising[20].

Table 2. IC_{50} of Petroleum ether extract & defatted methanol extract of the pulp of *C. melo* L. var. *aegyptiacus* cv. Ismailawi against Caco-2, HepG2 and HeLa cell lines

Cell lines	IC_{50} of the extracts (µg/ml)		
	Extract (1) *	Extract (2) **	Dox***
Caco-2	83.2	113.7	0.97
HepG-2	31.6	68.9	0.7
HeLa	109.6	89.9	1.3

*****Extract (1): Petroleum ether extract of pulp*
***Extract (2): Defatted methanol extract of pulp*
****Dox: Doxorubicin (Standard)*

CONCLUSION

This is the first report for the determination of volatile composition of *Cucumis melo* L. var. *aegyptiacus* cv. Ismailawi showing the major volatile components to be dominated by esters (93.37%). Five compounds were isolated for the first time from *C. melo* L. fruits with the complete absence of thioesters. This volatile components profiling can be used as a fingerprint for *C. melo* L. var. *aegyptiacus* cv. Ismailawi. The investigated cytotoxic activity for both the petroleum ether and defatted methanol extracts against Caco-2, HepG2 and HeLa cell lines showed that the best activity was seen against HepG2 cell line for the petroleum ether extract with an IC_{50} (31.6 µg/ml).

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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