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Chemical Composition and Anticancer activity of the Essential Oil Extracted from Fresh Orange Peels of *Citrus sinensis*

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Abstract. Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil (EO) separated from fresh orange peels (*Citrus sinensis* L.) revealed the presence of eight components and representing 99.33% of the total oil composition. Their identification was based on their retention times and mass spectral fragmentation patterns. D-limonene (40.45%), α-Terpineol (25.26%), γ-terpinene (16.28%), Geranyl acetate (5.80%) and Linalool (5.60%) represent major components. MTT assay was conducted to test the anticancer activity of the EO against four human tumor cell lines namely, Hepatocellular carcinoma (HEPG-2), Mammary gland breast cancer (MCF-7), Epitheliod Carcinoma (Hela) and Human prostate cancer (PC-3). IC_{50} value of essential oil for HEPG-2 cell line was 41.44 µg/ml, for MCF-7 cell line 53.23µg/ml, for PC-3 cell line was 63.32 µg/ml while for Hela cell line was 36.52 µg/ml. In conclusion, EO of *Citrus sinensis* may be a good natural anticancer agent and its major components may be responsible for the anticancer activity.

Keywords: Orange peels; *Citrus sinensis*; Essential oil; GC-MS; Anticancer activity.

1. INTRODUCTION

Cancer is the most leading cause of morbidity and mortality worldwide; it can be defined as a rapid uncontrolled growth of body cells and must be fight via surgery or chemotherapy [1-3]. Essential oils (EOs) are the volatile constituents that occur in plants and responsible for their characteristic odor. The chemical profile of the EOs is very complicated, it contained large number of mixed components i.e., monoterpenes, sesquiterpenes, diterpenes and their derivatives. Moreover, EOs possess wide spectrum of biological and pharmalogical potentials including antioxidant, anticancer and antimicrobial [4,5]. The genus *Citrus*, belonging to the family (Rutaceae), comprises of about 140 genera and 1,300 species. Most *Citrus* are growing in tropical and subtropical regions around the world especially Mediterranean region [6,7]. *Citrus* as an edible plant have numerous medicinal and nutritional benefits [8]. Orange peels commonly known as sweet oranges and its volatile constituents are mainly; esters, aldehydes, alcohols, terpenes, terpenols and ketones [9,10]. Essential oils from orange peels have a broad spectrum of uses like; aroma flavour in alcoholic and nonalcoholic beverages, marmalades, gelatins, sweets, soft drinks, ice creams, dairy products, candies, and cakes [11]. Therefore, the current study aims to the isolation and characterization of the essential oil from fresh peels of the *Citrus sinensis* L. growing in Egypt as well as to investigate its anticancer activity.

2. EXPERIMENTAL/MATERIALS AND METHODS

2.1. Plant material

Fresh sweet orange fruits (*Citrus sinensis* L.; Rutaceae) were purchased from market in Giza, Egypt country during November 2017. The plant was identified by Mrs. Threase Labib consultant of plant taxonomy at the Ministry of Agriculture; formerly, the head of taxonomist specialists at the garden. The fruits were stored at 4 °C, then washed and peeled within 2 days. The orange peels were fragmented into small pieces and stored at -20 °C before extraction.

2.2. Essential oil isolation

The fresh sweet orange peels (0.5 Kg) were subjected to hydrodistillation using Clavenger apparatus [5], to extract the essential oil which was determined as mean of triplicate. The chemical composition of the collected oil was determined qualitatively via GC-MS by comparing their retention times and mass spectral fragmentation patterns with the previously reported data [12].

2.3. GC/MS Analysis

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS and TGSMS Fused Silica Capillary Column (30m, 0.251 mm, 0.1 mm Film thickness), National Research Center, Giza, Egypt. For GC/MS detection, an electron ionization system with ionization energy for 70 ev was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C was a final temperature at an increasing rate of 5°C /min (hold 5 min). The identified components were investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY Library data of the GC/MS system [13].

2.4. Cell lines

Four human tumor cell line namely, Hepatocellular carcinoma (HEPG-2), Mammary gland breast cancer (MCF-7), Epitheliod Carcinoma (Hela) and Human prostate cancer (PC-3). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was used as a standard anticancer drug for comparison.

2.5. Chemical reagents

The reagents RPMI-1640 medium, MTT and DMSO (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

2.6. MTT assay

The cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeds in a 96-well plate at a density of $1.0x10^4$ cells/well at 37°C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100 [14,15].

3. RESULTS AND DISCUSSION

3.1. Identification of the EO constituents via GC-MS analysis

EOs are a diverse group of natural products that are composed of different classes of volatile constituents. The first class is constituted of terpenes and terpenoids, the second include phenolic (phenylpropanoids) constituents, the third one includes aliphatic components like alkanes, alkenes as well as their oxygenated derivatives (alcohols, aldehydes, and ketones) and other constituents such fatty acids, oxides, and sulfur derivatives [16,17].

Herein, qualitative investigation of the EO of *C. sinensis* resulted in the identification of eight components (**Table 1, Figure 1**) representing 99.33% of the total oil composition based on their retention times and mass spectral fragmentation patterns. D-limonene (40.45%), α-Terpineol (25.26%), γ-terpinene (16.28%), Geranyl acetate (5.80%) and Linalool (5.60%) were the major identified components.

Several *Citrus* species have been investigated for their essential oil content and evaluated for biological activity. Lin et al (2010) identified nine volatile constituents from the fresh sweet orange fruits (Citrus *sinensis*) namely, α-terpineol, β-pinene, myrcene, linalool, limonene, neryl acetate, α-pinene, γ-terpinene and geranyl acetate [18]. The GC-MS analysis of the volatile oil obtained from *Citrus sinensis* revealed the presence of chemical ingredients including α-terpineol (35.39%), D-limonene (17.74%), linalool (9.73%), citronellol (4.88%), γ-muurolene (4.44%) and isopiperitenone (3.58%) [19]. Also, the GC-MS investigations of citrus oil extracted from orange peels led to the identification of volatile constituents including α -Pinene (1.24%), Octanol (0.84%), β-Mycrene (3.79%) and D-limonene (94.13%) [20]. Moreover, fourteen components were identified in the volatile oil of *citrus reticulata* by GC-MS analysis including monoterpene constituents (68.1%), monoterpenic hydrocarbons i.e., *α*-thujene (3.1%), *α*-pinene (4.2%), *α*-camphene (1.8%), *β*-pinene (10.8%) and Sabinene (0.9%), monoterpenic alcohol i.e., *α*-terpineol (0.8%), monoterpenic ketones [21]. Colecio-Juárez et. al., [22] reported the identification of 46 components in the volatile oil of *Citrus limetta* including linalool, sabinene, and bergamol [22].

Table 1. Chemical compositions of the essential oil of fresh orange peels of *C. sinensis*

Peak No.	RT (min)	Area (%)	M.wt.	M.F.	Identified compounds
1	6.62	1.25	136	$C_{10}H_{16}$	α -Pinene
2	11.53	3.75	136	$C_{10}H_{16}$	β-Mycrene
3	12.01	0.94	222	$C_{15}H_{26}O$	Elemol
4	13.0	5.60	154	$C_{10}H_{18}O$	Linalool
5	19.46	40.45	136	$C_{10}H_{16}$	D-limonene
6	22.32	25.26	154	$C_{10}H_{18}O$	α-Terpineol
7	26.27	16.28	136	$C_{10}H_{16}$	γ-terpinene
8	28.45	5.80	196	$C_{12}H_{20}O_2$	Geranyl acetate

RT: Retention time
M.wt.: Molecular weight
M.F.: Molecular formula

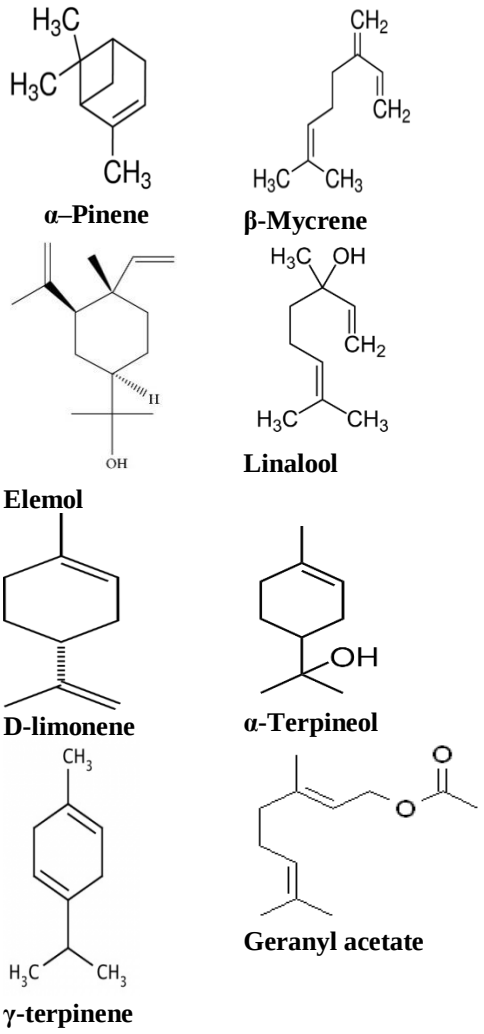

Figure 1. Chemical structures of the identified compounds.

3.2. Anticancer activity of EO

Essential oils have acquired increasing attention as potential sources for bioactive natural molecules. Several studies revealed their possible use as alternative medications for the treatment of many health disorders like inflammations, infectious diseases, and cancer [23]. Our findings revealed that the essential oil isolated from fresh peels of the *C. sinensis* exhibited high anticancer activity against, Hela (IC_{50}= 36.52 µg/ml), followed by HepG-2 (IC_{50}= 41.44 µg/ml), MCF-7 (IC_{50}= 53.23 µg/ml) and PC-3 (IC_{50}= 63.32 µg/ml) **(Table 2, 3; Figure 2)**.

Reviewing literature survey revealed that the anticancer activity of essential oil of Iranian *C. sinsensis* peels was evaluated using two human tumor cell lines (MCF-7 and Hela). IC_{50} value of essential oil for MCF-7 cell line was 0.5 µg/ml, while for Hela cell line 3 µg/ml [24].

The anticancer therapeutic potential of EOs includes two pathways, chemoprevention, and cancer suppression. In the same context, different modes of actions are possible like; activation of detoxification enzymes, modulation of DNA repair signaling, antimetastasis, and antiangiogenesis [23,25].

Table 2. Anticancer activity of the EO of *C. sinensis* against four human tumor cells

Sample	*In vitro* Cytotoxicity IC_{50}[1]			
	HePG2	MCF-7	PC3	Hela
DOX[2]	4.50 ± 0.2	4.17 ± 0.2	8.87 ± 0.6	5.57 ± 0.4
EO	41.44 ± 2.6	53.23 ± 3.0	63.32 ± 3.4	36.52 ± 3.1

[1] ***IC_{50} (µg/ml)****: 1-10 (very strong), 11-20 (strong), 21-50 (moderate), 51-100 (weak) and above 100 (non-cytotoxic)*
[2] ***DOX:*** *Doxorubicin*

Table 3. Average of relative viability of cells (%) using different concentrations from Doxorubicin and EO

Conc. (µg/ml)	HePG-2	MCF-7	PC-3	Hela
DOX				
100	6.3	6.2	8.8	7.3
50	11.2	10.9	16.3	12.1
25	14.1	14.3	21.7	18.9
12.5	28.3	26.9	38.9	30.8
6.25	45.8	41.5	59.2	51.7
3.125	57.6	58.4	73.6	62.4
1.56	71.2	69.1	95.3	74.0
EO of *C. sinensis*				
100	33.7	38.0	41.3	29.8
50	45.9	52.1	58.1	41.7
25	59.1	65.3	63.7	56.2
12.5	68.2	71.4	79.4	73.4
6.25	91.3	94.2	92.6	86.0
3.125	100	100	100	98.3
1.56	100	100	100	100

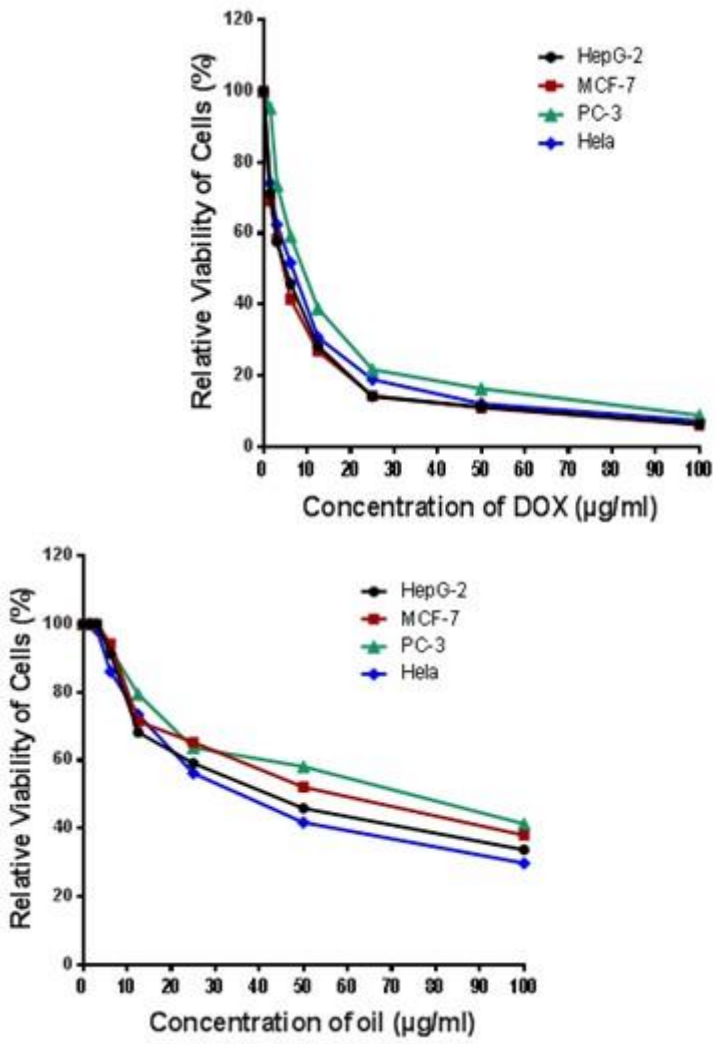

Figure 2. Relative viability cells (%) using different concentrations of Doxorubicin and EO of *C. sinensis* against four human tumor cells.

4. CONCLUSION

The essential oil extracted from the fresh orange peels of *Citrus sinensis* exhibited strong anticancer activity against three human tumor cell lines, which may be attributed to the presence of single major component viz., D-limonene (40.45%) or via the synergistic effect (Co-activity) between the overall constituents viz., D-limonene (40.45%), α-Terpineol (25.26%), γ-terpinene (16.28%), Geranyl acetate (5.80%) and Linalool (5.60%), therefore, EO from *C. sinensis* could be used as a natural source of anticancer agents.

5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests in this manuscript.

REFERENCES

[1] Ghareeb, M.A., Shoeb, H.A., Madkour, H.M.F., Refahy, L.A., Mohamed, M.A., Saad, A.M.., "Radical scavenging potential and cytotoxic activity of phenolic compounds from *Tectona grandis* (Linn.). *Global Journal of Pharmacology* 7, 486-497, 2013.
[2] Ghareeb M.A., Shoeb, H.A., Madkour, H.M.F., Refahy, L.A., Mohamed, M.A., Saad, A.M., "Antioxidant and cytotoxic activities of flavonoidal compounds from *Gmelina arborea* (Roxb.) ,"*Global Journal of Pharmacology*, 8, 87-97, 2014.
[3] Yang C, Chen H, Chen H, Zhong B, Luo X, Chun J. Antioxidant and anticancer activities of essential oil from Gannan Navel orange peel. Molecules. 2017; 22: 1391.
[4] Ghareeb, M.A., Refahy, L.A., Saad. A.M., Ahmed, W.S., "Chemical composition, antioxidant and anticancer activities of the essential oil from *Eucalyptus citriodora* (Hook.) leaves," *Der Pharma Chemica*, 8, 192-200, 2016
[5] Saad, A.M., Mohammed, M.M.D., Ghareeb, M.A., Ahmed, W.S., Farid, M.A., "Chemical composition and antimicrobial activity of the essential oil of the leaves of *Cupressus macrocarpa* Hartweg. ex Gordon," *Journal of Applied Pharmaceutical Science*, 7, 207-212, 2017.
[6] Anwar F, Naseer R, Bhanger MI, Ashraf S, Talpur FN, Aladedunye FA. Physico-chemical characteristics of *Citrus* seeds and seed oils from Pakistan. Journal of American Oil Chemist Society. 2008; 85: 321-330.
[7] Kamal GM, Anwar F, Hussain AI, Sarri N, Ashraf MY. Yield and chemical composition of *Citrus* essential oils as affected by drying pretreatment of peels International Food Research Journal. 2011; 18(4): 1275-1282.
[8] Saidani M, Dhifi W, Marzouk B. Lipid evaluation of some Tunisian *Citrus* seeds. Journal of Food Lipids. 2004; 11: 242-250.
[9] Saad AM, Mohammed MMD, Ghareeb MA, Ahmed WS, Farid MA. Chemical composition and antimicrobial activity of the essential oil of the leaves of *Cupressus macrocarpa* Hartweg. ex Gordon. Journal of Applied Pharmaceutical Science. 2017; 7: 207-212.
[10] Randhaw MA, Ahmad I, Javed MS, Sajid MW, Khan AA. Volatile flavor components of orange juice obtained from major citrus producing cities of Punjab. Pakistan Journal of Food Sciences. 2012; 22(1): 40-44.
[11] Bourgou S, Rahali FZ, Ourghemmi I, Tounsi MS. Changes of peel essential oil composition of four Tunisian Citrus during fruit maturation. The Scientific World Journal. 2012; 1-10.
[12] Adams RP. (1989). Identification of the essential oils by ion trap mass spectrometry. Academic press, INC, London, pp. 1-310.
[13] Adams RP. Identification of essential oils by Gas Chromatography Quadrupole Mass Spectrometry. Carol Stream, Allured Publishing Co., Illinois, USA, 2001.
[14] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 1983; 65: 55-63.
[15] Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods. 1986; 22: 271-277.
[16] Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science. 2006; 311: 808-811.
[17] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Toxicol. 2008; 46: 446-475.
[18] Lin C, Sheu S, Hsu S, Tsai Y. Determination of bactericidal efficacy of essential oil extracted from orange peel on the food contact surfaces. Food Control. 2010; 21; 1710-1715.
[19] Egharevba HO, Oladosun P, Izebe KS. Chemical composition and anti-tubercular activity of the essential oil of orange (*Citrus sinensis* L.) peel from North Central Nigeria. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(1): 91-94.
[20] Sikdar DC, Menon R, Duseja K, Kumar P, Swami P. Extraction of citrus oil from orange (*citrus sinensis*) peels by steam distillation and its characterizations. International Journal of Technical Research and Applications. 2016; 4(3): 341-346.
[21] Das DR, Sachan AK, Shuaib M, Imtiyaz M. Chemical characterization of volatile oil components of *citrus reticulata* by GC-MS analysis. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(7): 1197-1204.
[22] Colecio-Juárez MC, Rubio-Núñez RE, Botello-Álvarez JE, Martínez-González GM, Navarrete-Bolaños JL, Jiménez-Islas H. Characterization of volatile compounds in the essential oil of sweet lime (*Citrus limetta* Risso). Chilean Journal of Agricultural Research. 2012; 72(2): 275-280.
[23] Saad NY, Muller CD, Lobstein A. Major bioactivities and mechanism of action of essential oils and their components. Flavour Fragr J. 2013; 28: 269-279.

[24] Monajemi R, Oryan S, Haeri-Roohani A, Ghannadi A, Jafarian A. Cytotoxic effects of essential oils of some Iranian *Citrus* peels. Iranian Journal of Pharmaceutical Research. 2005; 3:183-187.

[25] Gautam N, Mantha AK, Mittal S. Essential oils and their constituents as anticancer agents: A mechanistic view. BioMed Research International. 2014: 1-23.