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Bioguided screening for cytotoxic active constituents of *Cuminum cyminum* volatile oil

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Abstract

Volatile oils hold tremendous potential for the production of high quality plant based medicines. The aim of this study was to evaluate the cytotoxic activity of cumin volatile oil (CVO) against hepatocellular carcinoma (Hep-G_2). In MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, CVO showed potent cytotoxic activity against Hep-G_2 cell line with IC_{50}=2.4 µg/ml. Bioguided fractionation of CVO using silica gel column chromatography afforded two active fractions(VO-1and VO-2) with IC_{50}= 0.6 and 0.9 µg/ml, respectively. GC-MS analysis was carried out for CVO and its two active fractions. Crystallization of active fraction VO-1 lead to the isolation of cuminic acid with IC_{50}=6 µg/ml. The structure of cuminic acid was established using IR, MS, ^{1}H-NMR and ^{13}C-NMR.

Keywords: Cumin volatile oil, GC-MS, hepatocellular carcinoma (Hep-G_2).

INTRODUCTION

Active components of many volatile oils impressively exhibit amazing potential medicinal benefits. For instance, α-pinene showed cytotoxic activity against Hep-G_2, lung carcinoma,human ovarian adenocarcinoma, mammary adenocarcinoma, human cervical carcinoma and gastric carcinoma (Marianna et al., 2014); β-pinene showed cytotoxic activity against Hep-G_2, lung carcinoma, human colorectal adenocarcinoma, breast carcinoma and human melanoma (Marianna et al., 2014); γ-Terpinene showed cytotoxic activity against Hep-G_2, mouse leukemia , erythromyeloblastoid leukemia and melanoma (Marianna et al., 2014); myrcene showed cytotoxic activity against Hep-G_2, human cervical carcinoma, human lung carcinoma, human colon adenocarcinoma, crown gall tumors, breast carcinoma, mouse leukemia and melanoma (Marianna et al., 2014); p-cymene showed cytotoxic activity against lung carcinoma and colorectal adenocarcinoma (Marianna et al., 2014) and cumin aldehyde possessed cytotoxic activity against human colorectal adenocarcinoma (Kuen-daw et al., 2016). It was reported that, cumin seed decreased the incidence of stomach and liver tumors and prevented the growth of breast and colon cancer cells (Daljeet et al., 2012). CVO showed dose-dependent antioxidant activity which is responsible for its cytotoxic activity (Allahghadri et al., 2010).

Cumin (*Cuminum cyminum*), is a small annual herbaceous plant that belongs to family Umbelliferae. It is indigenous to Eastern Mediterranean countries and South Asia (Uma et al., 2017). It is noteworthy that there are considerable qualitative and quantitative differences of the CVO as reported by previous studies (Nicola et al., 2005; Latif et al., 2007; EL-Kamali et al., 2009; El-Ghorab et al., 2010; Nisha et al., 2014; Rasha et al., 2014). The variation in the chemical composition of CVO according to geographical source can be summarized in table (1).

Table 1: Effect of geographical source on chemical composition of CVO

Geographical origin	Chemical composition
Italy	*p*-Mentha-1,4-dien-7-al, cumin aldehyde, *γ*-terpinene, and *α*-pinene (Nicola et al., 2005).
Iran	*α*-Pinene (29.1), 1,8-cineole (17.9) ,linalool (10.4) and limonene (21.5%) (Latif et al., 2007).
Central Sudan	Cuminaldehyde (32.70), 2-caren-10-al (20.30), fenylglycol (15.76) and *γ*-terpinene (11.72%) (EL-Kamali et al., 2009).
Pakistan	Cuminal, *γ*-terpinene and pinocarveol (El-Ghorab et al., 2010).
India	Trans-dihydrocarvone (31.11), *γ*-terpinene (23.22), p-cymene (15.8), *α*- phellandrene (12.01), p-menth-2-en-7-ol (3.48) and cuminlaldehyde constituted only 0.58% of the volatile oil (Nisha et al., 2014).
Egypt	*γ*- Terpinene (22.7), *β*-pinene (19.2), cuminaldehyde (18.0) and *p*-cymene (11.5%) (Rasha et al., 2014).
Current study (Egypt)	Cuminaldehyde (34.72) and cuminic acid (16.72%).

2. MATERIALS and METHODS

2.1. Plant material

The fruits were purchased from the registered famous Harraz stores in Cairo and kindly identified by Dr. Abd-Elhalim Abdel-Mogly, Prof. of Taxonomy, Flora Department, Agricultural Research Institute, Ministry of Agriculture, Cairo, Egypt. Voucher specimens were kept in the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

2.2. GC-MS analysis

GC-MS were carried on Shimadzu GC/MS-QP505-A, software:class 5000, searched library: Wiley Mass Spectral Data Base, column: DB 5.25m; 0.53mm ID:1.5um Film (J&W SCIENTIFIC), carrier gas: He, ionization mode: EI, ionization voltage: 70eV, temperature program: initial temperature was 40^{o}C(30 sec), then was increased to 150 ^{o}C (1min) at rate 7.5 ^{o}C/min, until reached 250 ^{o}C (5min) at rate 7 ^{o}C/min, detector temperature : 280 ^{o}C, injector temperature : 280 ^{o}C. Identification of the components of the CVO was performed by comparing their determined retention index (RI) with the reference of a homologous series of n-alkanes (C_8-C_{24}) as well as comparing their mass spectra and retention time with published data and Wiley Mass Spectral Data Base (Davies, 1990; Adams, 2007).

2.3. Isolation of cuminic acid

About 30 g of CVO were dissolved in the least amount of petroleum ether and adsorbed on 40 g silica gel to prepare initial zone and placed on the top of a silica column (4x60cm, 300g) packed with petroleum ether. The column was eluted in a gradient elution technique using petroleum ether then methylene chloride and methanol, respectively. Fractions (200 ml each) were collected, concentrated, examined by silica gel TLC plates and similar fractions were combined. The first active fraction (VO-1) that eluted with 25% methylene chloride in petroleum ether was crystallized from petroleum ether afforded 1.2 gm cuminic acid as white needle crystals. It has m.p 115-118 ^{0}C and R_f value 0.65 using silica gel TLC plates eluted with petroleum ether: ethyl acetate (6:4). It gives pink arch shaped

spot upon visualization with anisaldehyde /sulphuric acid. *O*-cumenol, methyl palmiate , 3-(6-hydroxyhexyloxy)propyl cuminate and 3-(4-(4-(4-butoxybutoxy) butoxy) butoxy) propyl cuminate were detected by GC-MS as shown in table (3); Cuminaldehyde, myrtanol (cis), myrtenol (trans), cryptone (4-hydroxy), butylated hydroxytoluene, daucol, deca-2,4-dienyl cuminate, undeca-2,4-dienyl cuminate, 3-(6-hydroxy hexyloxy) propyl cuminate and 3-(4-(4-(4-butoxybutoxy) butoxy) butoxy)propyl cuminate were detected by GC-MS as shown in table (4).

2.4. Biological Study

Human hepatocellular carcinoma cell line Hep-G_2 (ATCC, USA), were used to evaluate the cytotoxic effect of the tested oil fractions. Cytotoxicity was measured against Hep-G_2 cells using the MTT Cell Viability Assay (Hansen et al., 1989).

3. RESULTS

GC-MS of the cumin oil showed 23 components constituting 99.28 % of the oil content. Monoterpene hydrocarbons represented about 14.32% while oxygenated compounds represented about 84.96 % of the oil content. Aldehyde percentage accounting for 39.34 % of the oxygenated compounds. The major components was cuminaldehyde (34.72%) as shown in Table (2). CVO exhibited potent cytotoxic activity against Hep-G_2 cell line with IC_{50} 2.4 µg/ml.

GC-MS of the active fraction (VO-1) showed 5 components constituting 99.19 % of the volatile oil content which are only oxygenated compounds. The major component was cuminic acid (97.9 %) as shown in Table (3). VO-1 had cytotoxic activity against Hep-G_2 cell line with IC_{50} = 0.6 µg/ml.

GC-MS of the active fraction (VO-2) showed 10 components constituting 80.2 % of the volatile oil content which are only oxygenated compounds. The major component was 3-(6-hydroxyhexyloxy) propyl cuminate (32.02 %) as shown in Table (4). VO-2 had cytotoxic activity against Hep-G_2 cell line with IC_{50} = 0.9 µg/ml.

Crystallization of active fraction VO-1 lead to the isolation of pure compound with IC_{50}=6 µg/ml identified as cuminic acid. The structure of this compound was established using IR, mass, ^{1}H-NMR and ^{13}C-NMR.

4.DISCUSSION

CVO of the current study has qualitative and quantitative differences regarding chemical composition. This could be attributed to several factors among them is the geographical impact.

GC-MS analysis for CVO and both active fractions (VO-1 & VO-2) were carried out to determine their chemical composition as shown in tables (1, 2 and 3). CVO, VO-1 & VO-2 exhibited potent cytotoxic activity against Hep-G_2 cell line with IC_{50} 2.4, 0.6 and 0.9 µg/ml, respectively.

The structure of cuminic acid was established using IR, mass, ^{1}H-NMR and ^{13}C-NMR in comparison with published data [16] as follows:

IR spectrum indicates the presence of hydroxyl group at 3421 cm^{-1} in addition to carboxylic carbonyl group at 1685 cm^{-1}. Other bands at 3063 (C=C-H), 2954 (CH-stretching) and1608 cm^{-1} (C=C) revealed the occurrence of an aromatic ring together with C-O stretching at 1423 cm^{-1} [15]. MS spectrum showed molecular ion peak at m/z 164 suggesting molecular formula $C_{10}H_{12}O_2$ and base peak at 149 (M^+ - CH_3). Other fragmentation peaks at 131, 105, 91, 77 [16].^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed signals characteristic for benzyl

Table 2: Results of GC-MS analysis of the identified CVO components.

No.	Compound	M.W	RI	Relative %
1	*α*-Thujene	136	924	0.31
2	*α*-Pinene	136	932	0.67
3	*β*-Pinene	136	974	3.87
4	Myrecene	136	988	0.63
5	*δ*-3-Carene	136	1008	0.05
6	*α*-Terpinene	136	1014	0.04
7	*p*-Cymene	136	1020	2.04
8	*γ*-Terpinene	136	1054	6.71
9	*O*-Cumenol	136	1196	0.02
10	Cumin aldehyde	148	1238	34.72
11	Car-3-en-2-one	150	1244	4.48
12	Myrtanol (cis)	154	1250	0.04
13	Myrtanol (trans)	154	1258	0.1
14	*γ*-Terpinen-7-al	150	1290	4.62
15	Cryptone(4-hydroxy)	154	1314	1.36
16	Cuminic acid	164	1417	16.72
17	Butylated hydroxytoluene	220	1514	0.98
18	Daucol	238	1641	6.33
19	Methyl palmiate	270	1848	0.01
20	Deca-2,4-dienyl cuminate	304	1891	1.48
21	Undeca-2,4-dienyl cuminate	318	1930	3.92
22	3-(6-hydroxyhexyloxy) Propyl cuminate	324	1947	7.41
23	3-(4-(4-(4-butoxybutoxy)butoxy)butoxy) Propylcuminate	502	2137	2.77
Total				99.28

Table 3: Results of GC-MS analysis for the identified components of active fraction (VO-1).

No.	Compound	M.W	RI	Relative %
1	*O*-Cumenol	136	1196	0.02
2	Cuminic acid	164	1417	97.9
3	Methyl palmiate	270	1848	0.11
4	3-(6-hydroxyhexyloxy) Propyl cuminate	324	1947	0.94
5	3-(4-(4-(4-butoxybutoxy) butoxy) butoxy) Propyl cuminate	502	2137	0.22
Total				99.19

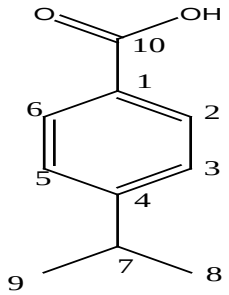
Table 4: Results of GC-MS analysis for the identified components of active fraction (VO-2).

No.	Compound	M.W	RI	Relative %
1	Cuminaldehyde	148	1238	6.98
2	Myrtanol (cis)	152	1250	1.24
3	Myrtenol (trans)	152	1258	1.64
4	Cryptone(4-hydroxy)	154	1314	5.54
5	Butylated hydroxytoluene	220	1514	3.87
6	Daucol	238	1641	10.02
7	Deca-2,4-dienyl cuminate	304	1891	2.16
8	Undeca-2,4-dienyl cuminate	318	1930	7.36
9	3-(6-hydroxyhexyloxy) Propyl cuminate	324	1947	32.02
10	3-(4-(4-(4-butoxybutoxy) butoxy) butoxy) Propyl cuminate	502	2137	9.37
Total				80.2

Conclusion

It is noteworthy that, bioguided fractionation of potent CVO (IC_{50} 2.4 µg/ml) using silica gel column chromatography afforded extremely potent two active fractions (VO-1and VO-2) with IC_{50}= 0.6 and 0.9 µg/ml, respectively. Crystallization of active fraction VO-1 lead to the isolation of cuminic acid which had moderate cytotoxic activity against Hep-G_2 cell line with IC_{50}=6 µg/ml. This indicates the synergistic effect between the volatile oil components in CVO, VO-1and VO-2, respectively. moiety by appearance of two broad doublet signals at δ 8.02 (J= 8 Hz; H-3, H-5), 7.31(J= 8 Hz; H-2, H-6). The multiplet signal at δ 2.98 (H-7) in addition to doublet signal at δ 1.27 (J= 8 Hz; 6 H) indicating the presence of isopropyl group [16]. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) revealed the presence of carbonyl carbon at δ 171.41, in addition to six carbons corresponding to the carbons of benzyl moiety: C-2 and C-6 at δ 127.07; C-3 and C-5 at δ 126.76; C-1 at δ 155.4 and C-4 at δ 130.53. Also, the signal at δ 23.83 (CH_3) in addition to a signal at δ 34.49, for 3^{ry} carbon, characteristic for isopropyl group [16].

Cuminic acid which was previously isolated from the petroleum ether extract of cumin in China.



Cuminic acid

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استجلاء الأثر المضاد للتسرطن واستخلاص المكونات ذات الأثر السام لسرطان الكبد لخلاصة الزيت الطيار للكمون

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يمكن استخدام الزيوت الطيارة للكثير من النباتات للحصول علي أدوية ذات قيمة عالية. و قد كان الهدف من هذه الدراسة هو استجلاء الأثر المضاد لتسرطن الكبد ا لخلاصة الزيت الطيار للكمون باستخدام الخلايا الكبدية (Hep-G2). أظهرت هذة الخلاصة فعالية قوية ضد تسرطن الكبد (2.4 مكجم /مل). فعن طريق كروماتوجرافيا العمود, تم تجزئة الزيت الطيار إلي ثمانية أجزاء و وجد أن لجزئين فقط هما (VO-1 و VO-2) فعالية قوية ضد تسرطن الكبد (نصف التأثير المثبط الأقصي 0.6 و 0.9 مكجم /مل) على التوالي و باستخدام كروماتوجرافيا الغاز للزيت الطيار والجزئين النشطين تم التعرف علي التركيب الكيميائي لهم كماً و نوعاً. قد تم فصل حمض الكيومنك عن طريق التبلور للجزء النشط (VO-1) و هو مركب ذو فعالية قوية (6 مكجم /مل) و قد تم إثبات بنيته الكيميائية باستخدام طرق التحليل الطيفي المختلفة (الأشعة تحت الحمراء, مطياف الكتلة و الرنين المغناطيسي للبروتون و الكربون) .