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Essential Oils Chromatograph Analysis and Potency in Breast Cancer Therapy

Iqbal Harbi Mohammed AL_Zaidi[1], Shurook M.K. Saadedin[2], Taif Abedulhussein[3]
[1] *Research Department, Horticulture Office, Ministry of Agriculture, Iraq*
[2] *University of Baghdad, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Iraq*
[3] *Baghdad Al-Russafa Health Directorate/ Kamal Al-Samarrai Specialist Hospital, Iraq*

[1] *Corresponding author: E-mail*: ssaadedin@gmail.com

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ABSTRACT

Due to the cases of multidrug resistance, cancer chemotherapy treatment has no longer effective. Therefore, there is a requirement to find better replacements new natural medicines for cancer. Essential oils are made up of a complex combination of low molecular weight with very well-known biological and pharmacological characteristic. In this research, the cytotoxic and anti-Breast cancer properties of Tea tree, Absinthe, Frankincense and Lemongrass, essential oils were estimated. The essential oils were qualitatively and quantitatively characterized using Gas Chromatography-Mass Spectroscopy (GC–MS) to identify their components. Depending on the GC-MS analysis, the main ingredients of the essential were in Tea tree (L-4-terpineneol 33.31%), Frankincense α-pinene (35.1%), Lemongrass Citral (45.43%) and Absinthe Sabinyl propionate (25.04%), Lemongrass and Tea tree essential oils significantly reduced the viability of both MCF-7 and WRL68 cells in a concentration dependent manner. However, Frankincense and Absinthe oils showed less inhibitory effects against both cell lines. The Lemongrass essential oil was more cytotoxic than Tea tree oils. Lemongrass shows a significant decrease in the survival rate of MCF-7 cells, 75% cell death rate at 400 µg/mL the IC_{50} determined at 64.10 µg /mL, while shows weak cytotoxicity in normal cell line WRL-68 and the IC_{50} was 125.2 µg /mL. comparison with DOX drug the IC_{50} was at 23.86 µg /mL .While had very weak cytotoxicity on WRL-68 the IC_{50} 1863 µg/ mL. Therefore, Lemongrass might have a good potential as an active anti-breast cancer drugs candidates with the highest efficiency and least side effects.

التحليل الكروماتوغرافيا لبعض الزيوت العطرية واختبار إمكاناتها في علاج سرطان الثدي

اقبال حربي محمد [1], شروق محمد كاظم سعد الدين [2], طيف عبد الحسين [3]
1 قسم البحوث والدراسات , دائرة البستنة ,وزارة الزراعة, بغداد, العراق
2 معهد الهندسة الوراثية والتقنيات االحيائية- جامعة بغداد, العراق
3 بغداد الرصافة, وزارة الصحة, مستشفى كمال السامرائي, العراق

الخلاصة

نظرا للمقاومة المتعدده للادوية اصبح العلاج الكيمياوي للسرطان غير فعال. لذلك فان هناك حاجة لايجاد بديل طبيعي كدواء لعلاج السرطان. الزيوت الأساسية هي مزيج معقد من مكونات ذات وزن جزيئي منخفض وهي ذات خصائص بيولوجية وصيدلانية معروفة جدًا. في هذه الدراسة، تم تقييم التأثيرات السامة للخلايا ومضادة للأورام (المضادة لسرطان الثدي) للزيوت الاساسية لشجرة الشاي والأفسنتين واللبان وعشب الليمون . والتي تم تشخيصها نوعياً وكمياً باستخدام كروماتوغرافيا الغاز – التحليل الطيفي الكتلي (GC – MS) للتعرف على مكونات كل الزيت. . اعتمادًا على تحليل GC-MS، كانت المكونات الرئيسية الأساسية في زيت شجرة الشاي (L-4-terpineneol 33.31%)، وفي اللبان α-pinene (35.1%)، وفي عشبة الليمون Citral (45.43%) وفي الأفسنتين Sabinyl propionate (25.04%). قللت الزيوت العطرية لعشبة الليمون وشجرة الشاي بشكل كبير من حيوية خلايا MCF-7 وWRL68 وذتك اعتمادا على التركيز. امازيوت اللبان والأفسنتين فقد اظهرت تأثيرات مثبطة أقل ضد كلا الخطين الخلويين. وكان زيت عشبة الليمون العطري أكثر سمية للخلايا من زيوت شجرة الشاي. يُظهر عشب الليمون انخفاضًا ملحوظًا في معدل البقاء على قيد الحياة لخلايا MCF-7، ومعدل موت الخلايا بنسبة 75% عند 400 ميكروجرام/مل، حيث تم تحديد IC50 عند 64.10 ميكروجرام/مل. . في حين أظهر سمية خلوية ضعيفة في خط الخلايا الطبيعي WRL-68 وكان IC50 125.2 ميكروغرام / مل. بالمقارنة مع عقار DOX كان IC50 عند 23.86 ميكروجرام / مل. بينما كان ذو سمية خلوية ضعيفة جدًا على WRL-68 و IC50 1863 ميكروجرام / مل. ولذلك، قد يكون الزيت العطري لعشبة الليمون مرشح جيدا و فعال كدواء مضادة لسرطان الثدي ذو كفاءة عالية و اثار جانبية قليلة.

الكلمات المفتاحية: الزيوت الطيارة، السمية الخلوية، الفعالية المضادة للسرطان، سرطان الثدي.

INTRODUCTION

People used herbs for illness treatment form the ancient (Djordjević,et al(2022), JS,et al,(2014) Rad,et al,(2021)) Classic method present over than thousands of years, the medicinal plant have been ethno reputedly used for the treatment of numerous diseases by many human civilizations, like Chinese, Indian, Persian Arabic, and Greek (Rad ,et al,(2022),Sharma E, et al ,(2022)), (Rad,et al,(2021)).

One of the important resources of secondary plant metabolites are Essential Oils (EOs), which play a key play in plant safeguard and occupy a notable place in popular medicine worldwide with their variety of biological activities, including antibacterial, antiviral, antioxidant, and anticancer features, which predestine them to be used exclusively or in combination with artificial drugs against various diseases, including cancer, and they extensive used in the food, pharmaceutical, agricultural, and cosmetic manufacturing (Rad J ,et al,(2021) , D ,et al (2022) C.A,et al (2018) , A,et al,(2017), Bhavaniramyaet al,(2019).

Human are interested in EOs due to their pleasant aroma(Maurya,et al,(2021)) Their use as anti-tumor is a very promising field (ER,et al,(2016))Many research referred to their anti-cancer activity and treatment(J.et al (2022) Six different EOs from Portugal designated for their cytotoxic properties against tumor cancer cell (Tavirani,et al,(2013).

In 2005, Warnke *et al.*,(Beeby E,et al,(2020) pointed out that the inflammation and the tumor smell in patients with cancer has drop due to the use of tea tree and eucalyptus EOs. The EOs of *Rosa damascena* raise cell proliferation on SW742 when beyond dosage were used and confirmed that similar effects were conceivable in human normal fibroblasts, for that the author suggest that the EO of this plant could catalyse cell growth (PH ,et al,(2005).

Researchers have suggested that natural monoterpenes for example, D-limonene are a possible new class of potential anticancer agents. They showed anticancer activity against many rodent solid tumor types by cancer-causing agent detoxification (MN,et al,(1997), PL,et al,(1994). Aims of this study were to analysis, Tea tree, Absinthe, Frankincense and Lemongrass, constituent and to evaluate the anti-Breast Cancer activity of those essential oils.

MATERIALS AND METHODS

Essential oils Collection and analysis

The EOs, Tea tree, Absinthe were imported from Vetiver Aromatics (USA), Frankincense, Lemongrass, were imported from Bristol Botanicals LTD (UK).

Gas Chromatography/ Mass Spectroscopy (GC/MS) Analysis

The four EOs samples were inquiries via GC/MS (Hewlett Packard 5890) machine detected the essential oils substances after dilution with diethyl ether. Gas chromatography column properties and injection conditions corresponds (Elshamy,et al, (2020)).

In Vitro Cytotoxic Activity of Essential Oils

Cell Line

MCF7 Cell Line

Completed culture medium comprised of Dulbecco's modified Eagle's medium with L-glutamine and phenol red (DMEM; Sigma-Aldrich, D5796), completed with 10% heat/inactivated fetal bovine serum (FBS; Biochrom GmbH, S0615), 1% penicillin-streptomycin mixture (Sigma-Aldrich, P4333), and 1% Amphotericin B (Sigma-Aldrich, A2942), were used to grow Cultures of human breast adenocarcinoma MCF7 cell line (ATCC® HTB-22™) then cultures cell were incubated at 37°C in 5% CO_2. The confluent, cells were washed three times with DPBS, separated using TrypLE Express™ (Gibco Invitrogen, INV 12605028, 4 to 5 ml/175 cm^2 tissue culture flask), and seeded at 4×10^4 cells/ml in 3.3 cm^2 diameter tissue culture dishes which enclosed 3 ml of complemented DMEM and were so further incubated for 24 hrs to permit the cell for attachment. Then were examined at (passage 9).

Cell Line (WRL 68)

The human liver cell line (WRL 68) displays morphology the same as that of hepatocytes and liver Primary culture. Cells have been shown to discharge albumin and alpha/fetoprotein and express liver-specific enzymes, such as alanine aminotransferase (B.S,et al,(2020))

In vitro Anticancer Activity Assay by Methyl Thiazol Tetrazolium (MTT) Method:

MTT test is a widely admitted, credible, rapid, and sensitive method which is not only used to screen for anticancer compounds but has also found application in medicine such as in the selection of active chemotherapy in patients with acute leukemia (Prasad,et al,(2009))

The MTT assay was carried out to determine the potential anti proliferative and cytotoxic properties of the EOs from Tea tree , Lemongrass ,Absinthe and Frankincense oils against human cancer cell lines MCF-7 Human breast adenocarcinoma and hepatic human cell line (WRL-68 cells) compared with Doxorubicin (DOX) as a positive control.

To detect the cytotoxicity of the four EOs and DOX the anti-cancer drug, MCF-7 (5.0×10^3 cells/well), were planted into a 96/well plate then incubated overnight at 37˚C with 5% CO2 the cells after that, treated with 100 µL of each two/fold serially diluted EOs (concentrations ranging from 400 to 6.250 µg/mL) , next 15 µL of MTT dye solution (5 mg/mL) (Sigma-Aldrich, St. Louis, MO, USA) was added and incubated for 4 h.

At the beginning the formed formazan crystals were liquefied in 200 µL of DMSO (Sigma-Aldrich, St. Louis, MO, USA); then, ELISA plate reader (Metertech, Taipei, Taiwan) at 575 nm with a repertoire wavelength of 630 nm were used to measure the optical density. The equation:

Cell viability (%) = (At / Ac) x 100

Where At is implies absorbance in the EOs well

Ac is the mean absorbance in the control well.

Were used to calculate the surviving cells percentage by the absorbance values of the four EOs and the control (Doxorubicin drug).The percentage rates of surviving cells at each concentration were the average of a three replicate, were graphed as a dose-response curve. IC_{50} declared as the lowest concentration of the test EOs that inhibited cell growth by 50% compared to the untreated control (He Y,et al,(2016))

RESULTS

GC/MS Analysis

GC- MS analysis (Table 1-4) are shown that all EOs have their own certain chemical fingerprint. The major oil represents (%) were L-4- terpineneol (33.31) and ɣ.-Terpinene (16.17) in Tea tree, while in Frankincense α-pinene (35.10) and Limonene (10.02) were the main compounds. In Lemongrass β – Citral (45.43) and Citral (31.78) were the major components. Sabinyl propionate (25.04) and iso-3-Thujone (24.61) were the main compounds in the Absinthe.

Table 1. The main components of Tea tree essential oil identified by GC-MC

No	Compound	R. Time	Area%
1	α Terpinene	8:48	11.61
2	p-Cymene	8:75	9.05
3	ɣ.-Terpinene	9:70	16.17
4	α-Terpinolene	10:27	4.28
5	L-4- terpineneol	13:28	33.31

Table 2. The main components of Frankincense essential oil identified by GC-MC

No	Compound	R. Time	Area%
1	α-Thujene	9:01	7.11
2	α-pinene	9:10	35.10
3	Sabinene	9:50	2.70
4	β-Pinene	9:57	3:21
5	Myrcene	9:66	3:92
6	Limonene	10:02	9:98

Table 3. The main components of Lemongrass essential oil identified by GC-MC

No	Compound	R. Time	Area%
1	D-Limonene	8:26	1.57
2	Linalool	10:37	1.55
3	Isoneral or Isogeranial	12:46	3.15
4	Citral	14:57	31.78
5	β -Citral	15:56	45.43
6	Geranyl isobutyrate	17:72	3.40
7	Caryophyllene	18:77	2.34

Table 4. The main components of Absinthe essential oil identified by GC-MC

No	Compound	R. Time	Area%
1	Sabinene (β-Thujene)	7:08	3.50

2	iso-3-Thujone	11:39	24.61
3	Thujone	11:64	15.17
4	Sabinyl propionate	16:03	25.04
5	Caryophyllene	18:86	2.10
6	Isobutyric acid	20:36	2.44

Viability Assay Methyl Thiazol Tetrazolium (MTT):

The *in vitro* cytotoxic action of the EOs (Tea tree, Frankincense, Absinthe and Lemongrass oils) were demonstrated against one cancer cells (MCF-7) and one normal cells (WRL-68).

Cytotoxicity of different doses of the tested EO_S against cell lines (MCF-7) versus normal WRL-68cells in comparison with DOX drug and the IC_{50} values are shown in Table 5. and figure 1 to 4 . The EO_S, IC_{50} values ranged from 64.10 to147.1µg/mL for the cancer cell lines MCF-7. When analyzing cytotoxic activity in non-cancerous cells (WRL-68), EOs exhibited IC_{50} values that ranged from 125.2 to 253.9 µg/mL.

Table 5. Methyl Thiazol Tetrazolium (MTT) Assay, Essential oils and their IC_{50} values of the cancer cells (MCF-7) and non-cancerous cells (WRL-68).

Cell line/ oils µg/ ml	Tea tree	Frankincense	Lemongrass	Absinthe	DOX drug
MCF-7	117.3	142.6	64.10	147.1	23.86
WRL68.	179.0	212.2	125.2	253.9	1863

The MCF-7 was treated with various concentrations of the essential oils ranging from 6.250 to 400 µg /mL for 48 hour incubation period, and cell validity was measured by MTT assay. Well, showed that raised concentration of the oil decreased the cell validity of the cells in a concentration dependent manner. Tea tree oil resulted in a significant decrease in the survival rate of MCF-7 cells in dose accreditation ($P < 0.0001$) and 75% cell death rate at 400 µg/mL, while IC_{50} was at 117.3 µg /mL Figure (1).

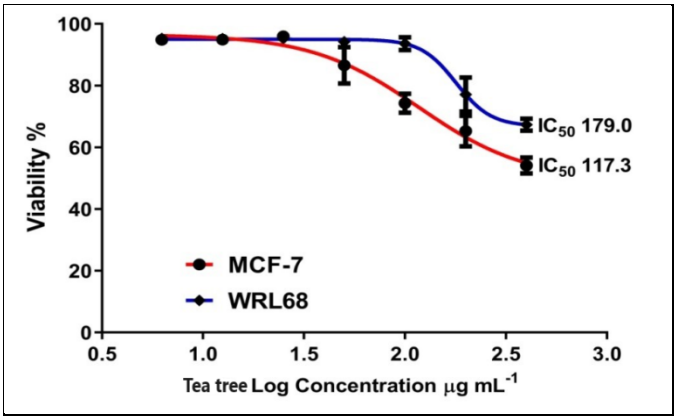

Figure 1. Cytotoxicity of Tea tree essential oil against MCF-7 and normal cell WRL68.

The same figure of normal cell line WRL-68 the Tea Tree oils shows weak and moderate cytotoxicity the IC_{50} was 179.0 µg/ m.

Frankincense essential oils figure (2) yielded in a important reduction in the presence rate of MCF-7 cells in dose dependence ($P < 0.0001$), 75% of the cell death rate at 400 µg/mL, while IC_{50} was at 142.6

μg /mL. The same figure shows that Frankincense oils had weak cytotoxicity on WRL-68 the IC_{50} (normal cell line) was at 212.2 μg/ mL.

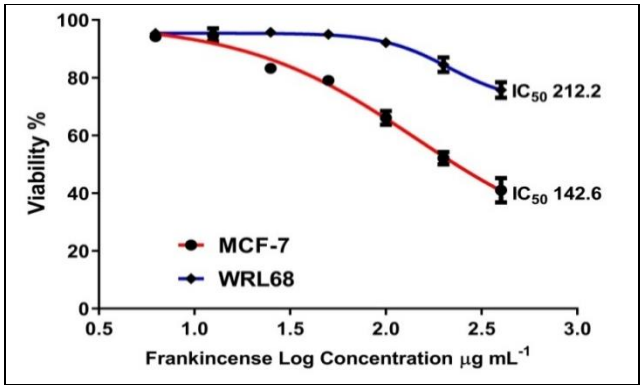

Figure 2. Cytotoxicity of Frankincense essential oil with MCF-7 and normal cell WRL68.

Lemongrass essential oil yielded in a significant decrease in the survival rate of MCF-7 cells in dose dependence ($P < 0.0001$), 75% cell death rate at 400 μg/mL, while IC50 was at 64.10 μg /mL Figure (3). The same figure shows that Lemongrass essential oil and weak cytotoxicity on WRL-68 the IC_{50} 125.2 μg/ mL.

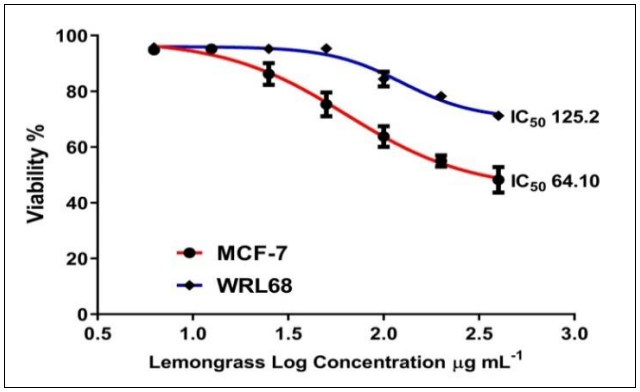

Figure 3. Cytotoxicity of Lemongrass essential oil against MCF-7 and normal cell WRL68.

Absinthe essential oil resulted in a major reduction in the existence rate of MCF-7 cells in dose accreditation ($P < 0.0001$) 75% cell dying rate at 400 μg/mL, while IC_{50} was at 147.1 μg /mL Figure (4). The same figure displays that Absinthe essential oil had very weak cytotoxicity on WRL-68 the IC50 253.9 μg/ mL.

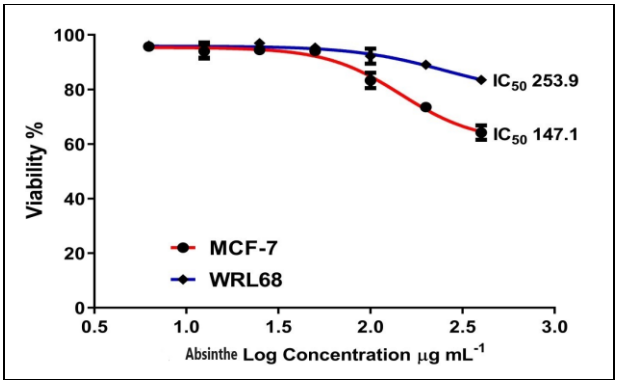

Figure 4. Cytotoxicity of Absinthe essential oil with MCF-7 against normal cell WRL68.

DOX produced a substantial reduction in the survival rate of MCF-7 cells in dose dependence ($P < 0.0001$) 100% cell death rate at 400 μg/mL, while IC_{50} was at 23.86 μg /mL Figure (5).While DOX had very weak cytotoxicity on WRL-68 the IC_{50} 1863 μg/ mL.

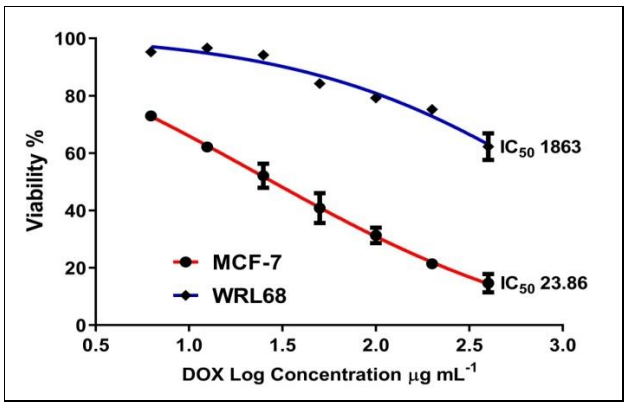

Figure 5. Cytotoxicity of DOX against MCF-7 and normal cell WRL68.

These outcome refer strong, cytotoxicity and antitumor activity of Essential oils.
Also strong antioxidants that help prohibit, free radical damage to cells, thus, they can participate to cancer suppress(Oils, (2022), Kustrin,et al,(2022)). Their cytotoxicity prohibiting tumor growth is evidenced by a wide spectrum of mechanisms of working. Also have been illustrate to have cancer cell-targeting activity and are capable to strengthening the effectiveness of commonly used chemotherapy drugs while indicating, pro-immune functions when managed to a cancer patien(Blowm,et al, (2018),National Institutes of Health,(2023)
Lemongrass is possibly a good antitumor agent. Depending on the results acquired from the Cytotoxicity assay, Lemongrass oil is the strongest extract against MCF-7 and might have the potency to inhibit cancer cell proliferation or arouse cancer cell experiencing apoptosis that lead to 26-
Blowman cell growth-inhibition. This mutually agreed with a study by(Chapuis N, et al,(2010)) showing when MCF7 cells treated with different EOs, the 5′-adenosine monophosphate-activated protein kinase (AMPK) was stimulated and mammalian objective of rapamycin (mTOR) signaling pathway was activated also, which controls cell growth, proliferation, and autophagy and is deregulated in cancer. Consequently, there was inhibition of cell viability, movement, and invasion activity. Frankincense EO was similarly active in suppress tumor growth and prompting apoptosis in human breast cancer(Ren P,et al,(2018))

CONCLUSIONS

Essential oils have been used as traditional medicine, due to the curing capacity that were confirmed in the modern studied. Various pharmacological trial have proven that they can prevent the development of cancer and deserve to be used in inhibition and even as assistants to classical chemotherapy.

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