ASSESMENT OF ANTIOXIDANT AND CYTOTOXIC ACTIVIY OF ESSENTIAL OIL EXTRACTED FROM *LAVANDULA ANGUSTIFOLIA* CALLUS LEAVES

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ABSTRUCT

This study was aimed to estimate the effect of essential oil extracted from callus of *Lavandula angustifolia* leaves as antioxidant and cytotoxic activity (*in vitro*). Different concentrations of essentail oil were selected, including 20, 40, 60, 80 and 100 µg/ml in determining antioxidant activity by using Free radical 1,1 Dyphenyl-2-picrylhydrazyl radical (DPPH). The results showed the efficacy of essentail oil as an antioxidant and the highest effect at 100 µg/ml reaching 97% and 87% at 80 µg/ml respectively. Different concentrations of essential oil (12.5, 25, 50,75 and 100) µg/ml were select to determine the effect of oil on human cervical cancer (HeLa) cancer cell line and a breast epithelial cell line (HBL) normal cell line after 72 hours from exposure time, and then antiproliferative activity of this oil was studied using MTT assay. The results showed the oil of *L. angustifolia* callus have high antioxidant influence (97%) in 100 µg/ml concentration followed by (87%) in concentration 80 µg/ml. The inhibition effect of extract on cell proliferation in HeLa cell line at highest concentration which reached 77%. The study confirmed the possibility of using essential oil from *L. angustifolia* callus could be used for medical application and treatment various types of cancer disease.

Key wards: *Lavandula angustifolia*, plant tissue culture, oil, cancer cell line.

تقدير الفعالية المضادة للاكسدة للزيت الطيار المستخلص من كالس اوراق نبات اللافندر وتقييم فعاليته السمية

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المستخلص

هدفت الدراسة الى تقدير تاثير الزيت الطيار المستخلص من كالس اوراق نبات اللافندر (***Lavandula angustifolia***) كمضاد للاكسدة وللخطوط السرطانية (خارج الحسم الحي). انتخبت تراكيز مختلفة من الزيت الطيار شملت 20، 40، 60، 80، 100 مايكروغرام/ مللتر في تحديد الفعالية المضادة للاكسدة باستخدام مادة DPPH –2– Dyphenyl 1,1 picrylhydrazyl radical. اظهرت النتائج كفاءة الزيت الطيار كمضاد للاكسدة وكانت اعلى فعالية عند التركيز 100 مايكروغرام/ مل حيث وصلت الى 97% ثم 87% عند التركيز 80 مايكروغرام/ مل على التوالي. كما انتخبت تراكيز من الزيت الطيار (12.5، 25، 50، 75، 100) مايكروغرام/ مل لتحديد تاثير الزيت على خطوط خلايا HeLa السرطانية و HBL الطبيعية بعد 72 ساعة من التعريض وبينت النتائج ان الزيت المستعمل له تاثير مثبط على خط الخلايا السرطانية HeLa حيث اعطى اعلى فعالية سمية (77)% عند التركيز 100 مايكروغرا/ مل. اكدت الدراسة الى امكانية استعمال الزيت الطيار المستخلص من كالس نبات اللافندر في التطبيقات الطبية وفي معالجة انواع مختلفة من السرطان.

الكلمات المفتاحية: نبات اللافندر، زراعة الانسجة النباتية، الزيت، الخطوط السرطانية

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INTRODUCTION

Lavandula angustifolia, the English lavender, is plant species that native to the southern Europe and Mediterranean. It's belonged to family Lamiaceae which contain 25-30 different species (5, 16). Plant grows as a small shrub up to 60 cm high, the bottom of stem was woody while the top was green with linear leaves and blue- violet flowers arranged in circle at the top of stem (9,15). Medicinal properties of lavender were known since ancient time, Romans used it as additive for bath and use oil in the middle ages in perfumes and soap. *L. angustifolia* was used in cosmetics, shampoo, food processing, tea and traditional medicine as sedative, antispasmodic, antidiabetic, aromatherapy and mantihypertensive against flu and colic, treat rheumatic diseases and nephrotic syndroms. In Arabic medicine it was used to treat kidney and stomach pain (10,20 ,11). *Lavandula angustifolia* considered most important species due to high quality of its essential oil. The oil of its flowers and leaves are colorless or light yellow having bitter test. (8). Phytochemical studies of *L. angustifolia* revealed that the major constituents of its essential oil are 1-8-cineol, camphor, linalyl acetate, lavandulol, linalool, limonene, terpenes and terpenoids. Essential oil may vary depending on growing location, climate, genotype, stage of growth plant and extraction methods (7, 19). These constituents in the oil have valuable pharmacological properties such as antioxidant, anti-inflammatory and antimicrobial (19). Some studies have shown that some constituents of lavender essential oil have anticancer and antimutagenic properties. Components of essential oil can be encapsulated in polymers as targeted drug delivery system and local administrated to prostate cancer, lung and liver cancer. Sixty percent of anticancer compounds derived from plants due to their low side effect relative to chemical drug (6, 17). Bozin *et al* (6) mentioned that aryl-benzofuran compounds isolated *L. angustifolia* showed significant growth inhibition of A549 (alveolar basal epithelial carcinoma), NB4 (leukemia), PC3 (prostate cancer) and MCF7 (breast adenocarcinoma) cell lines, and some constituents isolated from ethyl acetate extract of lavender show stronger anti-oxidant activity than vitamin C. The antitumor activity essential oils related to linalyl acetate, camphor and alpha-terpineol which caused inhibition of growth of human cancer cell line. Antioxidant substances in essential oils prevent oxidative damage of cell and reactive oxygen species (ROS) reacting with free radical, scavenging free radicals. The aim of this study was to determine the antioxidant activity of essential oil extracted from *L. angustifolia* callus of leaves and evaluate the cytotoxic activity.

MATERIALS AND METHODS

The callus of *L. angustifolia* were collect from callus cultures which initiated from leaves of *L. angustifolia* growing on Murashige and Skoog (MS) medium supplemented with (10 mg/l benzyl adenine) and 3 mg/l naphthalene acetic acid, extraction and analysis were done as mentioned in (4).

Antioxidant activity

Oil of callus was assessed by quantifying the scavenging ability to the free radical by using 1,1 Dyphenyl-2-picrylhydrazyl (DPPH). Equal volumes (0.5 ml) of DPPH solution (0.4 µM) and 1ml of each concentration (20, 40, 60, 80 and 100 µg/ ml) from extract were mixed and allowed to stand for 30 min at room temperature in the dark. The absorbance of samples was recorded at 518 nm, by a spectrophotometer. Ascorbic acid was used as standard. The scavenging activity was calculated according to the formula:

$$\boldsymbol{Scavenging\ activity}\ \% = \frac{\boldsymbol{A518\ of\ control - A518\ of\ sample}}{\boldsymbol{A518\ control}}\ \boldsymbol{x\ 100}$$

Where A518 of control is the absorbance of DPPH prepared in methanol; A518 of sample is the absorbance of DPPH with sample of extracted oil or compound. This experimental repeated triplicate for callus extract (4).

Cytotoxic activity

Maintenance of cell culture: The cell lines used in this study were supplied by tissue culture unit/ Iraqi Centre Cancer. One cancer cell lines were used in this search (HeLa cell line). It was derived from cervical cancer cells taken on February 8, 1951 from Henrietta Lacks, a patient who died of her cancer. The (HBL) was used as normal cells. HeLa cancer cell line and HBL normal cell line were maintained in RPMI-1640 supplemented with

10% fetal bovine serum, 100 units/ ml penicillin, and 100 µg/ml streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week and incubated at 37°C (2, 3).

Cytotoxicity assays

To determine the cytotoxic effect of essential oil, the MTT cell viability assay was done using 96-well plates. Cell lines were seeded at $1x10^4$ cells/ well. After 24 hours or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration. Cell viability was measured after 72 hours of treatment by removing the medium, adding 28 µL of 2 mg/ml solution of MTT and incubating the cells for 2.5 hours at 37°C. After removing the MTT solution, the crystals remaining in the wells is solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by incubation at 37 °C for 15 min with shaking. The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

Cytotoxicity = A-B/A *100

Where A and B are the optical density of control and the optical density of test respecting. For visualize the shape of cells under inverted microscope, 200 µL of cell suspensions were seeded in 96-well micro-titration plates at density $1x10^4$ cells mL^{-1} and incubated for 48 hours at 37 °C. Then the medium removed and added essential oil after 24 hours, the plates were stained with 50 µL with crystal violet and incubated at 37°C for 15 min, the stain was washed with tap water until the dye removed. The cell observed under inverted microscope at 100x magnification microscope filed and photographed with digital camera (14).

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference- LSD test was used to significant compared between means in this study.

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity was expressed in term percentage of inhibition, the value of standard of ascorbic acid obtained and compared with antioxidant activity. The results in Table 1 show the essential oil concentrations (20-100) µg/ml have response and varied of antioxidant activity from (31- 97) %. The highest activity of essential oil is a reflection of its chemical compositions. The high content of linoleic acid as mentioned by (4) was contributed to the antioxidant activity of the oil from *L. angustifolia* and can be regarded as a source of antioxidant with high value. Hamad *et al* Showed that extract of *L. angustifolia* has superior antioxidant capacity due to high content of linalool. The differences in constituents of lavender essential oil due to environmental, genetic factor and source of essential oil.

Table 1. Antioxidant activity of *L. angustifolia* essential oil of callus initiation from leaf explant compared with ascorbic acid

Concentration µg/ml	Means ± SE		LSD value
	Anti-Oxidation	Ascorbic acid (Control)	
20	31.39 ± 0.95 e	58.60 ± 0.63 c	3.887 **
40	50.41 ± 0.71 d	81.69 ± 0.92 b	5.136 **
60	79.33 ± 1.09 c	84.60 ± 0.78 b	2.809 **
80	87.71 ± 1.21 b	97.20 ± 1.04 a	3.061 **
100	97.85 ± 0.49 a	98.60 ± 1.37 a	2.677 NS
LSD value	2.931 **	4.027 **	---

Means having with the different letters in same column differed significantly, ** (P≤0.01).

Cytotoxic activity

The cytotoxic activity was determined by using five different concentrations of *L. angustifolia* essential oil on one cancer cell line and one normal cell line after 72 hours exposure time. The results show significant inhibition of cell proliferation in HeLa cell line after 72 hours. The cell proliferation was significantly lower when compared to untreated control cell. After 72 hours of

treatment these cells with *L. angustifolia* essential oil at concentrations 12.5, 25,50, 75 and 100 µg/ml, the cytotoxicity of essential oil showed good cytotoxic effect. The results indicate that *L. angustifolia* essential oil is considered to be particularly valuable source of anti-proliferative and cytotoxic substances. Apoptogenic property was investigated through morphological changes Hela cell line using inverted phase contrast microscope. As seen in Figure 1, the control (untreated) showed that cells maintained their original morphology form that most of the` control cells adherent to the tissue culture dishes. In contrast, Hela cells treated with *L. angustefolia* essential oil exhibit high efficiency on proliferation and morphology.

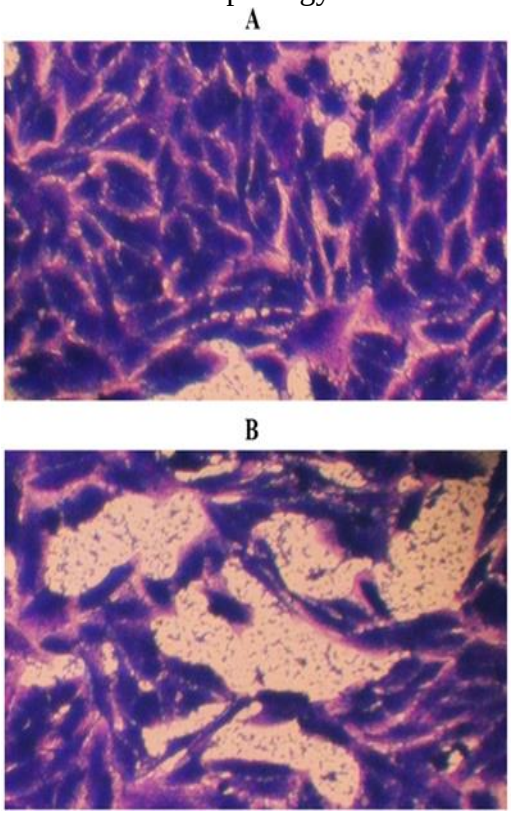

Figure1. Antiproliferative activity of *L. angustifolia* essential oil of callus initiated from leaf explant against HeLa cells (A: untreated cell (control), B treated cell

Figure 2 revealed that essential oil inhibit cell growth at highest concentration which reached 77% while reduced at lower concentration gave 8%.

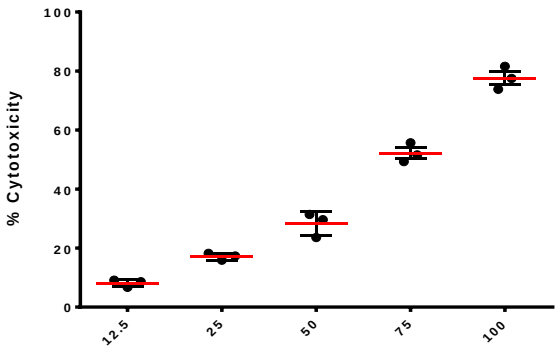

Figure 2. Cytotoxicity of *L. angustifolia* essential oil of callus initiated from leaf explant against HeLa cells

These concentrations were not significant on HBL cell line, or in another meaning the essential oil has no effect on normal cell (Figure 3).

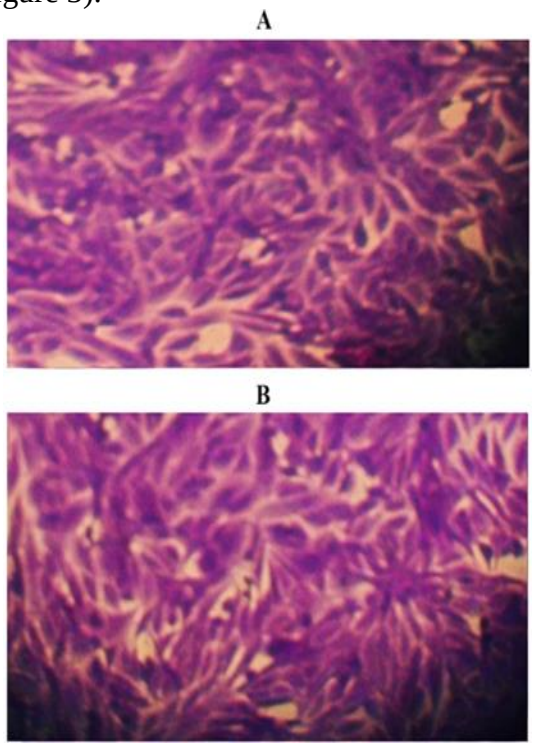

Figure 3. Antiproliferative activity of *L. angustifolia* essential oil of callus initiated from leaf explant against HBL cells (A: untreated cell (control), B treated cell

This study was indicated that components present in *L. angustifolia* essential oil associated with molecular mechanisms. The mixture of these compounds induced apoptosis and inhibited cell proliferation in Hela cells. It has been shown that more components in this oil have anticancer properties. Natural products play important role in drug discovery and studied these compounds have led to development many potent anticancer agents. Plant essential oils became the focus of

phytomedicine researcher, *L. angustifolia* essential oil display some variation (1). Zhao *et al* (21) investigated that linalool and linalyl acetate are the major constituents in *L,. angustifolia* essential oil have therapeutic effect on human prostate cancer cells. Results agree with many researchers who mentioned that essential oil of *L. angustifolia* exhibited remarkable anticancer and antiproliferative activity against cancer cells, it observed that cell growth and viability in cancer cell line were inhibited (12).

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