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Study of the cytotoxic effects of Iraqi *Ocimum Basilicum L.* extracts on breast cancer cell line

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Abstract

Ocimum Basilcum L. (Basil) is a popular herb used in many countries of the world, including Iraq, where it is grown with high quality and used as a popular diet. Because of it is health benefits, anti-oxidant and anti-proliferative contents, it was important to study the basil contents and shed light on its.

Extract effects on the most common types of cancer (breast cancer) using two cell lines (MCF7 and MDA). Before applying this effect, Basil (leaves and waste of the fresh and dry plant) was extracted using Soxhlet apparatus with Ethanol as a solvent (two different percentage: absolute ethanol 99% and ethanol 70%), then Gas chromatography mass spectroscopy (GC-MS) was used to identify the chemical composition. Depending on dry basil leaves extract (DG_L) composition using absolute ethanol as a solvent; it was selected for study of basil effect on the two used cell lines.

To reach the best effect, three different concentrations (5, 10 and 20 µg) of (DG_L) were used for the three different period times (24, 48 and 72 hours).

Comparing the results for MCF7 and MDA, it was observed that 20 µg decreased the growth inhibition percentage at (24 and 48, 24hr and 72hr) for MCF7 and MDA, respectively and 5 µg at 72hr for MCF7 and 10 µg at 48hr for MDA.

The overall conclusion of the current study was that the antioxidant and proliferative contents of (DG_L) had significant effects on the two breast cancer cell lines by decreasing the rate of growth inhibition, meanwhile the results added more healthy benefit of this important plant.

Keywords: Basil extract, Gas Chromatography Mass spectroscopy and breast cancer cell line.

1- Introduction

Ocimum Basilicum L. (Basil) also known genus Ocimum belongs to the Lamiaceae family. There are about 150 species of Ocimum[1]. The genus Ocimum has a number of species that are used to treat different types of ailments from ancient time, especially the species basil [2]. Otherwise it is known as sweet basil, also it is a universally cultivated herbaceous, perennial plant [3]. In addition, it is a popular herb used in Italian and Southeast cuisines of Thailand and Vietnam [4]. in addition, the Iraqi gardens cultured high quantity of basil, hence, it is an important to study basil contain because it is popular diet in Iraqi market [5]. Furthermore, it has numerous potent activities due to the metabolites present in it. As a consequence of its virulent metabolites, it is used in traditional medicine [6] and also as an ornamental plant [7]. O. basilicum is describing as numerously in areas related to agriculture, food, and pharmacology. In fact, the recent study approved the antiproliferative effect of basil [8].

Mainly, breast cancer is that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, newly-inverted nipple and red or scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin [9].

Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time. Mutations that can lead to breast cancer have been experimentally

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linked to estrogen exposure [10]. Additionally, G-protein coupled estrogen receptors have been associated with various cancers of the female reproductive system including breast cancer. Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth [11]. In breast adipose tissue, overexpression of leptin leads to increased cell proliferation and cancer [12].

Hence, the current study focuses on the chemical component of Iraqi Basil that may give its antiproliferative effect on breast cancer cell line (MCF7 and MDA).

2- Experimental

2.1- Plant collection

Basil was pooled freshly from Dyala Government of Iraq at October 2020; all extraction process was done at Biochemistry laboratory/Collage of Science/Biochemistry laboratory of university of Baghdad.

After pooling, basil leaves were separated from their stems, and then each part washed. So there were the following groups:

A- Dry group (DG):

The plant was dried (on air in shade place [13]), and stored until being analyzed. The used basil was grinded to a powder just before the extraction for two weeks to dry at room temperature with purified conditions

i. Dry Group (DG_L):

Cutting dry basil leaves only,

ii. Dry Group waste (DG_w):

Collect all part of the dry basil except leaves.

B- Fresh group (FG):

That directly extract after collected.

i. Fresh Group leaves (FG_L):

Cutting fresh basil leaves only,

ii. Fresh Group waste (FG_w):

Collect all parts of fresh basil except leaves.

2.2- Extraction and GC-MS

Both main groups (dry & fresh) of Basil were extracted by Soxhlet apparatus at 90 Ċ for 8 hours using ethanol solvent with two different concentrations (absolute ethanol (99%) and ethanol 70%).

After extraction, the solvent is removed typically by using a rotary evaporator, yielding the extracted compound; the non-soluble portion of the extracted solid remains in the thimble [14], [15].

The extraction of groups was done in the same laboratory conditions and that was in the following step:

- Weight teen grams of each part of plant (fresh or dry)
- Put the plant in the thumble of Soxhlet
- Use the solvent
  I. Absolute Ethanol (99%)
  II. Ethanol (70%)
- Start the heater with 90 Ċ for 8 hours
- Take the extract solution and leave it for 15 minutes to Alcohol evaporation with room temperature
- Use a rotary evaporator to remove the remain of solvent [14]
- Finely GC-MS test and record the results.

This cycle allowed repeating many times, over eight hours.

While the GC-MS analysis of the plant extract (basil) was Agilent 7890 an instrument under computer control at 70 eV. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries [15].

2.3- Cell line treatment

The experiments of cell line were completed in Technology Research Center/ Molecular and Medical Biotechnology department of Al-Nahrin University. In the recent study, basil extract with different concertation (5, 10, 20 µg) was applied in two breast cancer cell line (MCF7 and MDA) in compassion with control cell line (REF) all process of the tissue culture was highly sterilize condition.

2.4- Viable Cells Count

Tissue culture flasks which contain cancer cells are received, and growth medium is decanted off, then the flasks are washed twice using 1mL of trypsin-versene EDTA (Ethylene Diamine Tetra Acetic) solution. After washing, 1mL of trypsin versene is added to the flask and incubated 2-5 min. for cells detachment, then 4-5mL of PRMI growth medium (contains 10% FBS) is added to the flask. Then the cells are counted using counting chamber by inverted microscope (400x) to detect the viable cells [16].

2.5- Cytotoxicity Assay

For tissue culture, the microtiterplate of 96 (12×8) well is used, each well is used to seed 10,000 cancer cell to be incubated at 37° C for 24 hours to generate

a monolayer (inverted microscope is used to confirm monolayer generation). The wells of microtiterplate are grouped virtually (four wells in each group) to be ready 12 wells are exposed to basil extract by different concertation (5µg, 10µg and 20µg). Also 36 wells are measured as control, and then re-incubated at 37º C, for different three intervals, 24, 48, and 72 hrs. After incubation, Crystal violet staining is added in the third attempt, in order to stain cancer cells for the purpose of capturing, then incubated for 20 min. at 37º C, after that, the microtiterplate is washed. Then assay analyzer device is used to analyze the described staining, to calculate the percentage of the cancer live cells (inhibition rate), then the mean value is computed for each group. The calculated mean of inhibition percentages of 24, 48, and 72hrs are recorded separately for results analysis.

3- Results and discussion

Medicinal herbs are known as sources of active compounds that are widely sought after worldwide for their natural properties. They have been used since ancient times as sources of flavorings and for their pharmaceutical properties [17]. Phytochemicals may be effective in combating or preventing disease (breast cancer) due to their antioxidant effect[18]. A great number of organizations and scientists turn their attention to traditional therapies in order to find and conserve important resources and up to 80% of the population relies on traditional medicines or folk remedies for primary health care needs[15].

During each cycle (In soxhlet), a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. Gas chromatography and mass spectroscopy analysis For the 4 extracts (DG_L, FG_L, DG_W, FG_W) using Absolute Ethanol (99%) as a solvent was carried out as summarized in Tables (1-4)

Table 1: GC-MS results of the main compounds found in the dry basil leaves extract (Using ethanol (99%) with its benefit.

RT	Area%	Compound Name	Main Benefit
22.60	11.76	Estragole	Anticancer, antioxidant and antimicrobial activities[19][7]
52.46	22.22	Linalool	antibacterial and anticancer activity[7] [20]
49.2	3.36	Methyleugenol	Antioxidant activity and hepatoprotective effect[21]
49.83	3.00	Trans-anethole	antimicrobial, anti-inflammatory, anticancer and antioxidant properties[22]
48.36	4.98	n-Hexadecanoic acid Octadecanoic acid	essential for the synthesis of various hormones[23]
56.89	2.26	heptadecene-(8)-carbonic acid	Antioxidant[24]
57.68	4.75	Cycloheptane	hydrocarbon pneumonitis[25]
58.01	23.35	Oleic Acid	reduce the cardiovascular risk[26]
58.17	16.65	2-octyl-Cyclopropaneoctanal	immune boosters[27]
58.73	7.66	1,8-Cineole	Induce apoptosis [22]

Table 2: GC-MS results of the main compounds found in the dry basil waste extract (Using ethanol (99%) with its benefit.

RT	Area%	Compound Name	Main Benefit
50.3	46.15	Benzo[b]naphtho[2,1-d]thiophene	Thiophene analog[28]
52.5	16.81	n-Hexadecanoic acid	essential for the synthesis of various hormones[23]
54.51	16.19	Ethyl 9,12-hexadecadienoate	act as plasticizer in many products, such as intravenous (IV) bags and tubing[19]
56.11	72.99	9-octadecenoic acid	An emollient[29]
56.68	84.51	Cycloundecanecarboxylic acid	Water treatment[30]
56.90	51.30	Octadecanenitrile	
58.02	26.29	Octadec-9-enoic acid	Replace saturated fats in the diet.[31]
58.16	18.61	Oleic Acid	replace saturated fats in the diet[1]
58.73	7.74	Octadecanoic acid	essential for the synthesis of various hormones[23]
59.50	6.63	(methylsulfanyl)({[4-(4-pentylcyclohexyl)cyclohexyl]methoxy})methane thione	an important antioxidant[32]
59.77	2.77	(Z)- 9,17-Octadecadienal(Z,Z)- 9,12-Octadecadienoic acid	essential for the synthesis of various hormones[23]
56.90	51.30	Octadecanenitrile	
58.02	26.29	Octadec-9-enoic acid	Replace saturated fats in the diet.[31]
58.16	18.61	Oleic Acid	replace saturated fats in the diet[1]
58.73	7.74	Octadecanoic acid	essential for the synthesis of various hormones[23]
59.50	6.63	(methylsulfanyl)({[4-(4-pentylcyclohexyl)cyclohexyl]methoxy})methane thione	an important antioxidant[32]

Table 3: GC-MS results of the main compounds found in the fresh basil leaves extract (Using ethanol (99%) with its benefit.

RT	Area%	Compound Name	Main Benefit
4.4	98.03	Acetic acid ethyl ester	act biologically as antimicrobial effect
4.78	0.18	Propanedioic acid	act biologically as antimicrobial effect
5.33	0.39	2,2-dimethyl(methylthio) methyl ester	act biologically as antimicrobial effect [16]
48.62	0.84	1H-Indole, 2-methyl-3-phenyl Hexanedioic acid, bis(2-ethylhexyl) ester	act as plasticizer in many products, such as intravenous (IV) bags and tubing[19]
56.19	0.31	Cyclotrisiloxane	antiproliferative and antioxidant effect
58.84	0.04	Tetrasiloxane,decamethyl-Methyltris (trimethylsiloxy) silane	antiproliferative and antioxidant effect [7]

Table 4:
GC-MS results of the main compounds found in the fresh basil leaves extract (Using ethanol (99%) with its benefit.

RT	Area%	Compound Name	Main Benefit
4.4	98.63	Acetic acid ethyl ester	act biologically as antimicrobial effect[33]
5.33	0.40	Propanoic acid, ethyl ester	a solvent, flavoring agent and fragrance[34]
48.63	0.87	Hexanedioic acid, bis(2-ethylhexyl) ester	antiproliferative and antioxidant effect[35]
58.59	0.10	hexamethyl-Cyclotrisiloxane	antiproliferative and antioxidant effect[24]

Tables (5-8) shows the GC-MASS For the 4 extracts (DG_L, FG_L, DG_W, FG_W) using (70%) as a solvent

Table 5: GC-MS results of the main compounds found in the dry basil leaves extract (Using ethanol (70%) with its benefit

RT	Area%	Compound Name	Main Benefit
4.4	98.03	Acetic acid ethyl ester	act biologically as antimicrobial effect[33]
4.78	0.18	2,2-dimethyl-Propanedioic acid (methylthio)methyl ester	act biologically as antimicrobial effect[22]
4.78	0.18	3-chloro-2-Propenenitrile	antioxidants[36]
5.33	0.39	Propanoic acid, ethyl ester	a solvent, flavoring agent and fragrance[34]
48.62	0.84	2-methyl-3-phenyl-1H-Indole	act as plasticizer in many products, such as intravenous (IV) bags and tubing[23]
56.19	0.31	Hexanedioic acid, dioctyl ester	antiproliferative and antioxidant effect[35]
56.19	0.31	hexamethyl-Cyclotrisiloxane	antiproliferative and antioxidant effect[37]
58.60	0.21	Tris(tert-butyldimethylsilyloxy) arsane	antiproliferative and antioxidant effect[38]
58.84	0.04	decamethyl-Tetrasiloxane	antiproliferative and antioxidant effect[37]

Table 6: GC-MS results of the main compounds found in the dry basil waste extract (Using ethanol (70%) with its benefit

RT	Area%	Compound Name	Main Benefit
4.33	97.08	Hexane	Solvent [39]
4.33	97.08	3-methyl-Pentane	Improve athletic performance, and improve brain function[40]
4.58	1.58	methyl-Cyclopentane	hydrocarbon pneumonitis[25]
5.33	0.21	Propanoic acid ethyl ester	a solvent, flavoring agent and fragrance[34]
48.70	0.51	Hexanedioic acid bis(2-ethylhexyl) ester	antiproliferative and antioxidant effect[35]
52.29	0.10	Phthalic acid, 1-cyclopentylethyl dodecyl ester	Plasticizers[41]
57.28	0.52	hexamethyl-Cyclotrisiloxane,	antiproliferative and antioxidant[37]

Table 7: GC-MS results of the main compounds found in the fresh basil leaves extract (Using ethanol (70%) with its benefit

RT	Area%	Compound Name	Main Benefit
4.4	97.89	Acetic acid ethyl ester	act biologically as antimicrobial effect[33]
4.78	0.19	Butanoic acid	flavouring agents[42]
4.78	0.19	4-(methylthio)-Acetic acid ethyl ester	flavouring agents[42]
4.78	0.19	Ethanol, 2-(2-ethoxyethoxy)acetate	Solvent [42]
5.33	0.39	Propanoic acid	inhibits growth of mold and various bacteria and is used as a preservative for food[43]
48.69	0.88	Diisooctyl adipate	antiproliferative and antioxidant effect[35]
48.68	0.88	Hexanedioic acid	antiproliferative and antioxidant effect[35]
48.68	0.88	dioctyl ester	antiproliferative and antioxidant effect[35]
48.68	0.88	bis(2-ethylhexyl) ester	antiproliferative and antioxidant effect[35]
58.07	0.66	Cyclotrisiloxane	antiproliferative and antioxidant effect[37]
58.07	0.66	Tetrasiloxane	antiproliferative and antioxidant effect[37]

Table 8: GC-MS results of the main compounds found in the fresh basil waste extract (Using ethanol (70%) with its benefit.

RT	Area%	Compound Name	Main Benefit
4.4	98.03	Acetic acid ethyl ester	act biologically as antimicrobial effect[33]
4.78	0.18	Propanedioic acid,	act biologically as antimicrobial effect[33]
4.78	0.18	2,2-dimethyl-(methylthio)methyl ester	act biologically as antimicrobial effect[33]
5.33	0.39	Propanoic acid, ethyl ester	a solvent, flavoring agent and fragrance[34]
48.62	0.84	1H-Indole, 2-methyl-3-phenyl-Hexanedioic acid, bis(2-ethylhexyl) ester	act as plasticizer in many products, especially in medical devices, such as intravenous (IV) bags and tubing,[44]
48.63	0.84	Hexanedioic acid, dioctyl ester	antiproliferative and antioxidant effect[38]
56.2	0.31	Cyclotrisiloxane	antiproliferative and antioxidant effect[38]
58.60	0.21	Tris(tert-butyldimethylsilyloxy)arsane	antiproliferative and antioxidant effect[38]
58.84	0.04	Tetrasiloxane, decamethyl-Methyltris(trimethylsiloxy)silane	antiproliferative and antioxidant effect[38]

The extraction basil in this study was absolute ethanol 99% as a solvent show highly result than ethanol 70%, that may be because penetration of organic (less polarity) solvent to cell wall of extract is rather than organic (more polarity) solvent.

As mentioned above, basil extract showed content that effect as anti-oxidant and anti-proliferative such as [Estragole (11.76%), Linalool (22.22%), heptadecene-(8)-carbonic acid (7.66%), 1,8-Cineole(2.26%), Trans-anethole(3.00%) and Methyleugenol (3.36%)], The chemical components of sweet basil have been described in several reports and main ingredient of basil present in this study was compatible with several recent studies [45], [46].

However, the molecules that act as antioxidant and antiproliferative for example [Estragole (11.76%), Linalool (22.22%)] [47] was presented in all part of basil extract results and both solvents, but the part of dry basil leaves (DG_L) group the highest group % than FG_L, DG_W and FG_W, and this fact proven with R. Tahira study[48], so that this group of extract was selected to apply on cell line culture.

Basil extract was significantly effect on all cell lines (MCF7, MDA and REF) growth inhibition % in Figures (1-3, respectively) and Tables (9-11) illustrate the mean and standard division of each parameter that approved with Aburjai study[49], as well as that mean the basil extract (dry basil leaves) was effective as antiproliferative on MCF7 and MDA cell line and this part of study was first trial on mentioned cell line, while REF cell line that gives result less growth inhibition % and that mean basil extract (dry basil DG_L group) was less harmful on normal cells.

As stated above, this study was taking three cell lines; every cell line was cultured for three periods (24, 48 and 72 hours).

The result of MCF7 as illustrate in (Figure 1) and (Table 9), on 24 hours period was the growth inhibition highest percent at 20µg of basil extract concentration, also the same concentration was a chief growth inhibition percent at 48 hours period, while 5µg concentration of basil extract was top growth inhibition percent at 72 hours period, all that results were first trial on this cell lines.

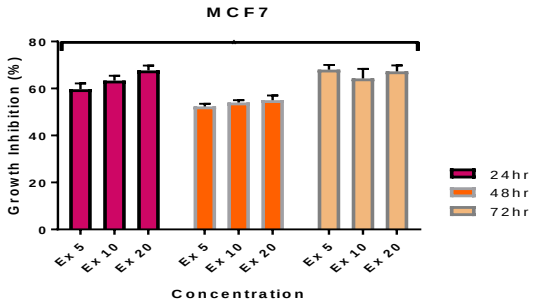

Figure 1: Growth inhibition percent of MCF7 [* : significant effect]

Table 9: Mean and standard division of MCF7 parameters.

#	٢٤hr	48hr	72hr
Ex 5µg	43.3±1.527	42.3±2.3	42.3±2.3
Ex 10µg	32.6±6.1	37.3±5.03	37.3±5.03
Ex 20µg	30.3±14.01	35±5	35±5

Also, the outcome data of MDA as explain in (Figure 2) and (table 10), on 24 hours period was the growth inhibition highest percent at 20µg of basil extract concentration, also the same concentration was a chief growth inhibition percent at 72 hours period, while 10µg concentration of basil extract was top growth inhibition percent at 48 hours period.

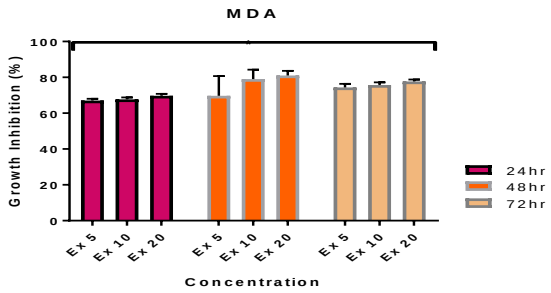

Figure 2: Growth inhibition percent of MDA [* : significant effect]

Table 10: Mean and standard division of MDA

#	٢٤hr	48hr	72hr
Ex 5µg	43.3±1.527	42.3±2.3	42.3±2.3
Ex 10µg	32.6±6.1	37.3±5.03	37.3±5.03
Ex 20µg	30.3±14.01	35±5	35±5

Finely, the conclusion data of REF as illuminate in (figure 3) and (table 11), on 24, 48 and 72 hours periods was the growth inhibition percent decrease with increase the concentration, so that 5 µg concentration give highest growth inhibition highest percent in all culture periods.

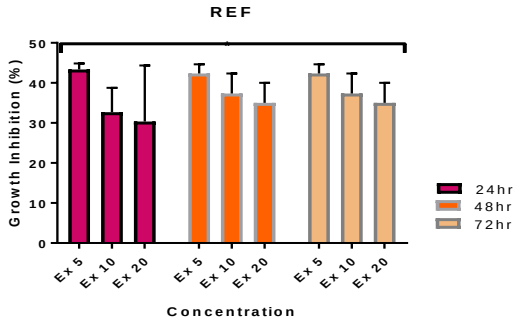

Figure 3: Growth inhibition percent of REF [* : significant effect]

Table 11: Mean and standard division of REF

#	٢٤hr	48hr	72hr
Ex 5µg	43.3±1.5	42.3±2.3	41.3±2.3
Ex 10µg	32.7±6.11	37.3±5.03	36.3±5.02
Ex 20µg	30.3±14.01	35±5	35.2±5.02

In addition, many researchers agreed with basil anticancer activity on MCF7 and MDA cell line [50],[47].

4- Conclusion

In this study, DGL fragment of basil was highly antioxidant and antiproliferative content, also absolute ethanol (99%) give highly result of basil extract identified with GC-MS.

In addition, DGL part of basil showed significant effect as antiproliferative with MCF7 and MDA cell line, while presented significant effect as less growth inhibition % with REF cell line.

Finally, the effects of this study deliver data on phytochemical features of basil; the essential is innate plant of Iraq. It comprises chemical which may be useful for various herbal formulation as anticancer (in breast cancer as proved by the cell line treatment of several researchers as showed above).

5- Conflicts of interest

"There are no conflicts to declare"

6- Acknowledgments

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