

Cytotoxic Activities of *Cymbopogon citratus* Extracts against Three Different Human Cancer Cell Lines: Lung Carcinoma (A549), Breast Cancer (MCF-7) and Hepatocellular Carcinoma (Hep G2)

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Abstract

Cymbopogon citratus, commonly known as Lemon grass is a small herbaceous plant of the *Poaceae* family. It is used as traditional medicine for the treatment of numerous diseases such as cancer, fever, sore throats, cough, laryngitis, bronchitis, body ache, headache, digestive problems, etc. This study deals with the cytotoxic activities of *C. citratus* against three different human cancer cells A549, HepG2, and MCF-7. The chemical fractionation of the plant material using ethanol, ethyl acetate, and hexane was prepared and analyzed for bioactivity. Ethyl acetate extract showed the highest anticancer activity against A549, HepG2, and MCF-7 human cancer cells with cell viability percentage (64.09±1.11, 41.22± 0.34, and 66.24± 1.50%, respectively) with IC₅₀ values (147, 65, and 155µg/ml, respectively). Results of this study show that the ethyl acetate fraction of *C. citratus* is a rich source of phenolics and other compounds that can play a significant role in preventing the progression of cancer diseases.

Introduction

Lemon grass (*Cymbopogon citratus*) is commonly used in folk alternative medicine to treat a number of ailments. Citral, an acyclic monoterpene aldehyde, has been discovered as one of the bioactive compounds found in *C. citratus*. Citral has been discovered as the plant's most important bioactive component, accounting for the majority of the plant's bioactivity. [1] such as antimutagenic [2], antiproliferative [3], antinociceptive [4], and antiparasitic effects against leishmaniasis properties [5, 6]. This plant's aqueous drink, known in Portuguese as "abafado," was supposed to have potent anti-neurological and anti-gastrointestinal properties [7]. It's also known to be a good scavenger of free radicals and reactive oxygen species (ROS). Because of its high ability for free radical scavenging and antioxidation, researchers looked at the effects of *C. citratus* ethanolic extract on the proliferation and cell development of various human cancer cell lines [8].

Cancer is the leading cause of death in the majority of countries around the world. Cell proliferation that is unaffected by growth factor regulation, as well as apoptosis resistance and angiogenesis, are all hallmarks of cancer, resulting in a disruption of homeostatic circumstances and carcinogenesis. Several organs, including the brain, liver, lungs, kidney, breast, bones, and ovaries, are affected by various cancers [9]. Hepatocellular carcinoma (HCC), also known as malignant hepatoma, is a genetically and phenotypically heterogeneous tumour that shows the etiological factors that lead to the development of HCC [10]. An oxidative stress environment is created by cancer. Antioxidants have been found to reduce

stress by scavenging reactive oxygen species (ROS) and activating the DNA repair process[11].

After liver cancer, lung carcinoma is the second most fatal malignancy. [12], As well as being the leading cause of cancer-related deaths. Every year, 22,000 people in Vietnam are diagnosed with lung cancer, with 19,500 people dying as a result. Carcinomas or tumours formed from epithelial lineage altered cells account for about 98 percent of lung malignancies. There are about four dozen distinct histological variants of lung cancer that have lately been discovered. The two most frequent types of lung cancer, small cell lung cancer and non-small cell lung cancer, account for around 85 percent of all lung cancer cases. Natural commodities have an unequalled chemical diversity, making them excellent for the development of novel medicines. Folk medicine is utilised by more than 80% of the world's population for primary healthcare, according to the World Health Organization. Medicinal plants have been discovered to have novel anti-cancer drugs. Several herbal drugs have been used for cancer treatment for thousands of years because of their traditional acceptability and minimal adverse effects [13].

Breast cancer is described as the uncontrolled spread of malignant tissue within the breast that must be surgically removed because if it spreads too far, it can lead to death. Breast tumours are characterised as channel, lobular, or inflammatory structures depending on where they originate. Several early indications of breast cancer include persistent nipple discharge and inversion of the nipples. Natural-derived chemicals are increasingly the focus of scientific research since they have

fewer hazardous side effects than existing treatments like chemotherapy [14].

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and one of the most widely distributed malignancies worldwide, with an ever-increasing prevalence over the last decade. [15]. HCC is a preventative disease rather than a curable one, because to the lack of appropriate treatment. Future study should focus on the usage of several plants that are thought to be rich sources of physiologically active chemicals [16]. The purpose of this study was to assess the cytotoxic activities of several *C. citratus* plant fractions.

Materials and Methods

2.1. Plant sample collection and preparation of extracts

The leaf of *C. citratus* was collected from the experimental farm, Fac. of Agric. Minia University. The fresh and healthy leaf was cleaned carefully and rinsed well with running tap water and air dried, away from the sun, at ambient condition for 4 weeks and ground with an electric grinder to a fine powder. The powdered leaf was kept in a sealed container at ambient conditions. One hundred grams of powdered sample was transferred into the round bottom flask (1000 mL) mixed with a magnetic stirrer and 700 mL solvents (80% ethanol, ethyl acetate and hexane) added with constant stirring for 6 hrs. at ambient conditions. The extract was then collected and filtered off using Whatman No.1 filter paper. The supernatant was dried using a rotary evaporator and speed vacuum. Then stored at 4°C. 10 mg/ml dimethyl sulfoxide (DMSO) was dissolved for further analysis modified [17].

2.2. Cell culture and reagents

Breast cancer (MCF-7) cell, Lung carcinoma (A549) and Hepatocellular carcinoma (HepG2) were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO₂ atmosphere at 37 °C.

2.3. Cytotoxicity assay

Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5×10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with 100 µL media containing plant extracts at numerous concentrations. After 72 h of adding extracts, cells were fixed by replacing media with 150 µL of 10% TCA and incubated at 4°C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µL of TRIS (10 mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany) [18,19].

3. Results and Discussion

3.1. Cytotoxic activity of *Cymbopogon citratus* extracts against HepG2, A549 and MCF-7 human cancer cells.

Cytotoxicity of *Cymbopogon citratus* extracts was tested on pulmonary cancer (A549), hepatocellular carcinoma (HepG2), and Breast cancer (MCF-7) cells. All the extracts (ethanol, ethyl acetate, and hexane) of *C. citratus* exhibited significant ($p < 0.005$) cytotoxicity against the cancer cells tested. Ethanol extract consistently showed higher cytotoxicity levels in all cells when compared to the other extracts.

3.1.1 Effect of *C. citratus* on HepG2 Cell Viability.

Liver cancer (Hep G2) cell viability was determined by using sulforhodamine B (SRB) assay and compared with untreated cells as a control. The results showed that in (table 1) the viability of HepG2 cells significantly decreased by increasing the ethyl acetate extract of *C. citratus* concentrations with IC₅₀ 65 µg/ml compared to other fractions (Fig. 1). The viability percentage of *C. citratus* ethanol 80%, ethyl acetate and hexane on liver cancer (HepG2) cell lines were 89.80% , 86.73% and 89.64% at 10 µg/ml, respectively. While the viability percentage of *C. citratus* ethanol 80%, ethyl acetate and hexane on liver cancer (HepG2) cell lines were 86.55% 41.22% and 71.72% at 100µg/ml, respectively. But the viability percentage of *C. citratus* ethanol 80%, ethyl acetate and hexane on liver cancer (HepG2) cell lines were 2.09 % , 3.86% and 12.03% at 1000 µg/ml, respectively.

Table (1): Percentage Cell Viability and IC₅₀ of Ethanol 80%, Ethyl acetate, and Hexane extracts of *C. citratus* against HepG2 cell line.

Plant extract	Concentration (µg/ml)	Cell viability %	IC ₅₀ (µg/ml)
Ethanol 80%	10	89.80±0.28	215
	100	86.55± 0.57	
	1000	2.09 ± 0.80	
Ethyl acetate	10	86.73± 0.20	65
	100	41.22± 0.34	
	1000	3.86± 0.83	
Hexane	10	89.64± 0.35	218
	100	71.72± 1.04	
	1000	12.03±1.00	

The data were expressed as mean ± SD of separated triplicate experiments. IC₅₀: the half-maximal inhibitory concentration is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. Ethanol, Ethyl acetate and Hexane.

3.1.2 Effect of *C. citratus* on A549 cell viability.

Lung cancer (A549) cell viability was determined by using SRB assay and compared with untreated cells as a control. The results showed that in (table 2) the viability of A549 cells significantly decreased by increasing the ethyl acetate extract of *C. citratus* concentrations with IC₅₀ 147 µg/ml compared to

other fractions (Fig2). The viability percentage of *C. citratus* ethanol 80%, ethyl acetate and hexane on lung cancer (A549) cell lines were 95.26% ,97.3% and 94.51% at 10 µg/ml, respectively. While the viability percentage of *C. citratus* ethanol 80% , ethyl acetate and hexane on lung cancer (A549) cell lines were 72.34% , 64.09% and 70.58 % at 100µg/ml, respectively. But the viability percentage of *C. citratus* ethanol 80% , ethyl acetate and hexane on lung cancer (A549) cell lines were 1.84 % , 12.47 % and 33.44% at 1000 µg/ml, respectively

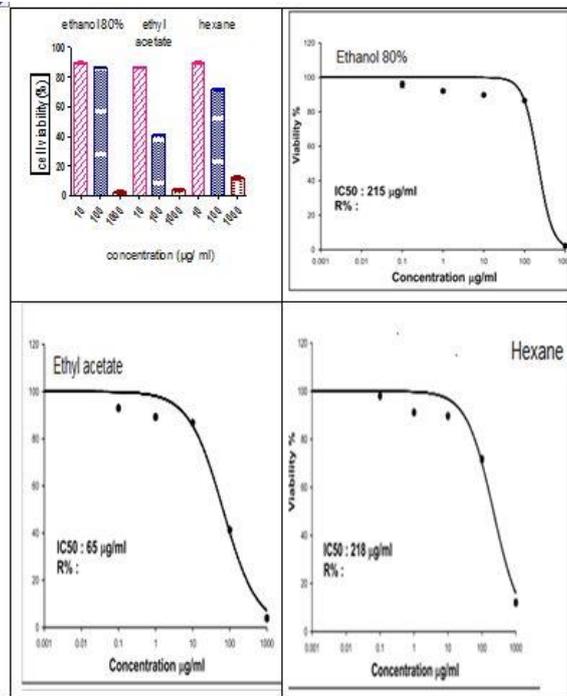


Figure 1: Effect of *C. citratus* extracts (Ethanol, Ethyl acetate and Hexane) on human liver cancer (HepG2) cell viability by the SRB assay. The data were expressed as Mean ± SD of separated triplicate experiments.

Table (2): Percentage Cell Viability and IC₅₀ of Ethanol 80%, Ethyl acetate, and Hexane extracts of *C. citratus* against A549 cell line.

Plant extract	Concentration (µg/ml)	Cell viability %	IC ₅₀ (µg/ml)
Ethanol 80%	10	95.26±0.32	162
	100	72.34± 0.86	
	1000	1.84 ± 0.26	
Ethyl acetate	10	97.3±0.19	147
	100	64.09± 1.11	
	1000	12.47 ± 0.43	
Hexane	10	94.51± 0.13	367
	100	70.58± 1.23	
	1000	33.44±1.33	

The data were expressed as mean ± SD of separated triplicate experiments. IC₅₀: the half-maximal inhibitory concentration is a

measure of the effectiveness of a compound in inhibiting biological or biochemical function. Ethanol, Ethyl acetate and Hexane.

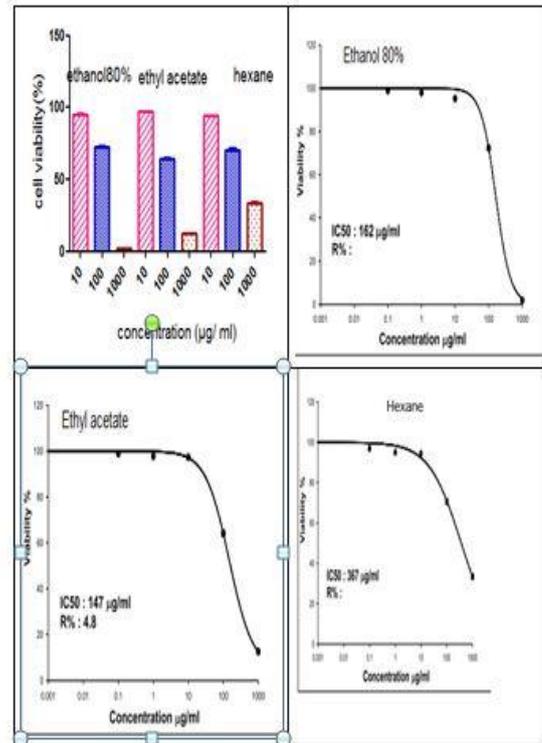


Figure 2: Effect of *C. citratus* extracts (Ethanol, Ethyl acetate and Hexane) on Human lung cancer(A549) cell viability by the SRB assay. The data were expressed as Mean ± SD of separated triplicate experiments

3.1.3 Effect of *C. citratus* on MCF-7 cell viability.

Breast cancer (MCF-7) cell viability was determined by using SRB assay and compared with untreated cells as a control. The results showed that in (table 3) the viability of MCF-7 cells significantly decreased by increasing the ethyl acetate extract of *C. citratus* concentrations with IC₅₀ 155 µg/ml compared to other fractions (Fig. 3). The viability percentage of *C. citratus* ethanol 80% , ethyl acetate and hexane on breast cancer (MCF-7) cell lines were 91.01 % , 95.60 % and 96.55% at 10 µg/ml, respectively. While the viability percentage of *C. citratus* ethanol 80%, ethyl acetate and hexane on breast cancer (MCF-7) cell lines were 79.57%, 66.24% and 77.16% at 100µg/ml, respectively. But the viability percentage of *C. citratus* ethanol 80% , ethyl acetate and hexane on breast cancer (MCF-7) cell lines were 31.28 % , 5.021% and 15.14% at 1000 µg/ml, respectively.

Table (3): Percentage cell viability and IC₅₀ of Ethanol 80% , Ethyl acetate, and Hexane extracts of *C. citratus* respectively on MCF-7 cell line

Plant extract	Concentration (µg/ml)	Cell viability %	IC ₅₀ (µg/ml)
Ethanol 80%	10	91.01 ±0.89	172
	100	79.57± 0.57	

	1000	31.28 ± 0.78	
Ethyl acetate	10	95.60 ± 0.08	155
	100	66.24 ± 1.50	
	1000	5.021 ± 0.28	
Hexane	10	96.55 ± 0.11	258
	100	77.16 ± 0.43	
	1000	15.14 ± 0.49	

The data were expressed as mean ± SD of separated triplicate experiments. IC₅₀: the half-maximal inhibitory concentration is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. Ethanol, Ethyl acetate and Hexane.

The results reported that depending on extraction solvent content, extract obtained from ethyl acetate solvent showed to be more potent on hepatocarcinoma cell line (IC₅₀ = 65 µg/mL), followed by ethanol fraction (IC₅₀ = 215 µg/mL), then hexane fraction with IC₅₀ = 219 µg/mL. On the other hand, ethyl acetate extract showed a moderate potency on MCF-7 cells having an IC₅₀ of 155 µg/mL compare to untreated cells. Ethyl acetate fraction showed anticancer activity against A549 cells with IC₅₀ = 147 µg/mL. These results suggested anti-proliferative efficacy of *C. citratus* ethyl acetate extract against human cancer cell lines may be related to the number of phenolics and flavonoids present in the ethyl acetate fraction would have contributed to the cytotoxic activity of cancer cells, but the current study could not determine the exact phytochemicals responsible for this activity.

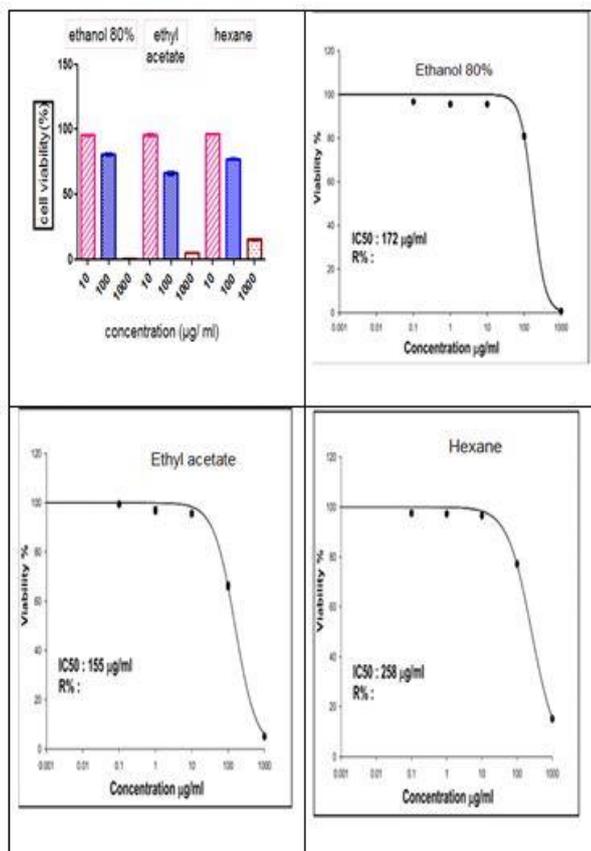


Figure (3) : Effect of *C. citratus* extracts (Ethanol, Ethyl acetate and Hexane) on Human breast cancer(MCF-7) cell viability by the SRB assay. The data were expressed as Mean ± SD of separated triplicate experiments.

Discussion

Cancer cells survive by several molecular signaling pathways. Therefore, chemo-preventive agents must successfully influence different signaling pathways as compared to targeting just one pathway. Cancer chemoprevention needs the cooperation of multidisciplinary groups to find target specific and less toxic agents to block, retard, or reverse the carcinogenic process. Since lemongrass is an aromatic grass that yields an essential oil used in pharmaceutical industries and treatment of different diseases as an alternative traditional medicine [1].

This result found that autophagy was significantly ($P \leq 0.05$) increased in treated HepG2 cells as compared to non-treated cells. The present data suggested that combined treatment with cinnamon and lemongrass aqueous extracts can exert an anti-cancer effect through induction of apoptosis, autophagic cell death activation, and inhibiting cell proliferation in HepG2 cells [20].

Hence, the result indicates that the concentration of phytochemicals in the different extracts plays a vital role in cytotoxicity. The previous study supports the findings of this study, that citral inhibits P388 mouse leukemia cells and oil from *Cymbopogon flexuosus* induced differential in vitro cytotoxicity in 12 human cancer cell lines and in vivo tumor growth inhibition in murine, Erlich and S-180 tumor models [21].

This result indicated that *C. citratus* essential oil (LEO) from *Cymbopogon citratus* on A549 cell line and their effect on the expression of p53 gene in lung cancer cells. The LEO was extracted and analysed using GC-MS analysis, the cytotoxicity of LEO against lung cancer cells was examined using MMT assay and finally the expression of p53 was determined via qRT-PCR. The chromatogram of GC-MS exhibited 14 compounds in ELO, the major one is citral (56.4%). The cytotoxicity of LEO was dose dependent which proliferation of the lung cells were significantly inhibited with increased concentration of essential oil ($P \leq 0.01$) as well as the IC₅₀ was 29.13 ppm. Furthermore, it showed that cell treated with IC₅₀ of LEO had a significant higher gene expression ($P \leq 0.05$) in the fold of p53 expression than untreated cells with change fold of 4.47 ± 0.7 [22].

This result found that The IC₅₀ mean values obtained by the neutral red assay were 600 and 630 µg/ml for CWE and LgWE respectively. The lower value of IC₅₀ indicates the greater antioxidant activity. Cinnamon has cytotoxic effect on HepG2 cells more than that of cinnamon more than that of lemongrass [23].

This results showed that essential oil of lemon grass possessed potential cytotoxic effect on HCT-116 and with the IC₅₀ value of 27.41 ± 4.3 µg/mL comparing to MCF-7 with IC₅₀ value of 41.90 ± 1.2 µg/mL. Significant anti angiogenic activity of the sample was observed with $99 \pm 0.8\%$ of inhibition at 100 µg/mL, the lemon grass essential oil showed promising activity on the cytotoxicity against colorectal carcinoma cell line. Thus, bioassay guided fractionation will be conducted to determine the active compound against the colorectal cancer cell line growth[24].

This result *C. citratus* showed high contents of total phenolic and total flavonoids, as well as high free radical scavenging capacity with potential as an antioxidant. *C. citratus* shows good anti-inflammatory, anti-diabetic, hypolipidemic, renoprotective and cardioprotective, as well as anticancer activities. Apart from this, *C. citratus* possesses vasorelaxant, sedative and antitussive potential. Furthermore, the compound citral is used in perfume industry as well as for cleaning wounds and treatment of skin diseases in forms of gels, or functional paper microencapsulated with essential oil, which can be used for hand hygiene [25].

Statistical analysis:

Experimental results were means \pm SD of three parallel measurements. Analysis of variance was performed by One Way ANOVA procedures. Graphpad prism $\text{\textcircled{R}}$ was used for statistical calculations Graphpad software, San Diego, CA, USA [26].

Conclusions

In this present study, we evaluated the anticancer effect of different extracts of lemongrass plants (ethanol, ethyl acetate, and hexane) on cervical cancer cell lines (A549, HepG2, and MCF-7) in vitro. Lemongrass ethyl acetate fraction treatment (24 h incubation) significantly decreased the percentage of cell viability in A549, HepG2, and MCF-7 cells. This suggested that Ethyl acetate extract showed the highest anticancer activity against A549, HepG2, and MCF-7 human cancer cells with cell viability percentage (64.09 ± 1.11 , 41.22 ± 0.34 , and $66.24 \pm 1.50\%$, respectively) with IC_{50} values (147, 65, and $155 \mu\text{g/ml}$, respectively).

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