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Evaluation of Anti-oxidant and Anti-cancer Activity of Cultivated Yemeni Curcuma Longa L. Extracts on Skin (A431) and Lung (A549) Cancer Cell Lines

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Abstract

Background: Turmeric (Hurud) is one of the cultivated medical plants in Yemen in various geographical areas mountainous highlands such as Mohaweet, where the plant of this study was collected. This plant is used in alternative medicine in Yemen as preparation for skin cosmetics. This study was designed to evaluate the chemical composition, antioxidant and anticancer activities of Yemeni Curcuma fractions against two human cancer cell lines were used (A431 skin cancer and A549 lung cancer cell line).

Methods: Tumeric fractions were tested for their chemical analysis using Fourier Transform Infrared (FTIR) and Spectral analysis (Ultra violate analysis).

The antioxidant activity of all selected extracts was evaluated by DPPH and FBAAC methods. The cytotoxicity of the fractions was determined on two human cancer cell lines were used (A431 skin cancer and A549 lung cancer cell line). and noncancerous using the tetrazolium-based colorimetric (MTT) assay. Vinblastine, a broad-spectrum anticancer drug was used as a positive control to assess the cytotoxic activity of extract and oils. The potential mechanism of action of the active fractions was apoptosis.

Results: The cells viability results of this study showed that the use of ethyl acetate fraction of local cultivated Yemeni Curcuma longa extract has high activity on the viability of A431skin cancer and A 549 lung cancer cell line compared with untreated cells and the activity was high whenever the concentration is high, followed by acetone extract, whereas crude turmeric had the lowest activity. The morphological examination using crystal violates stain results in an adverse relationship between the extract concentration and the number of cancer cells compared with control.

Conclusion: based on their good antioxidant, the ethyl acetate, acetone, and aqueous Turmeric fractions represent the promising alternative natural origin of compounds effective against lung and skin cancer.

Keywords: Cultivated Curcuma longa; Skin (A431); Lung (A549) cancer cell line; Antioxidant; Viability; Cytotoxic activity

Introduction

Cancer represents a group of diseases characterized by uncontrolled growth of cells, which spreads from the original sites to other parts of the body, resulting in the destruction and dysfunction of those areas [1]. Skin cancer is one of the most common malignancies in dermatology. With the rapid development of industrialization and changes in people's living environment, the incidence of skin cancer has been increasing year by year, which has gradually developed into a worldwide public health problem [2]. Early specificity of diagnosis and treatment is still lacking; the current common treatment methods are surgery, radiation/chemotherapy, laser, and cryotherapy [3]. However, these methods are symptomatic treatments, and poor patient compliance and prognosis. Therefore, further study of its pathogenesis and looking for a new and specific target of treatment has become an important issue in clinical research. Lung cancer is the most common cancer throughout the world and it accounts for 14% of all cancers and 28% of all cancer-related deaths worldwide. Chemotherapy is the standard treatment for lung cancer patients, but despite its ability to improve the symptoms and the quality of life of patients with lung cancer, only a minimal increase in survival rate can be achieved. Along with palliative care, many cancer patients tend to use alternative medicines, among which herbal therapies are more common [4]. Antioxidants are the compounds that prevent biomolecules from undergoing oxidative damage through free radical-mediated reactions [5]. Turmeric (*Curcuma longa L.*) is belonged to the family Zingiberaceae and is used widely in the food industries as a coloring agent and an additive flavor in curries. Turmeric is a rich source of many important bioactive compounds like antioxidants, polyphenols, and flavonoids, which may be a substitute for antibiotics used in food and food products [6]. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons [7].

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Materials and Methods

Plant material

Freshly harvested local cultivated turmeric rhizomes were collected from Al Mahweet Governorate (Jun 2014 and authenticated by Dr. Hasan Ibraheem, Assistant prof of Taxonomy, Biology Department, Sana'a University. Turmeric rhizomes were cleaned under running tap water to remove adherent sand and clay particles then rinsed with distilled water. Afterward turmeric rhizomes were cut into small pieces and shade dried at room temperature before being pulverized with an electric grinder into powder according to Nisar T, et al. [8].

Chemicals

Curcumin is an orange-yellow crystalline substance water-insoluble. It was purchased from Sigma-Aldrich (India).

Dimethyl sulfoxide (DMSO), crystal violet (0.5% (w/v) dissolved in 50% methanol then made up with ddH_2O and filtered through a Whatman No.1 filter paper.

Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L- glutamine, gentamycin, and 0.25% Trypsin- EDTA were purchased from Lonza (Bornem, Belgium). Ascorbic acid was purchased from India, ADWIC, 8010057.

Preparation of the samples

Turmeric fractions were prepared using five solvents (ethanol, acetone, ethyl acetate, petroleum ether, and distilled water) using the soxhlet apparatus for 2h. According to Morakinyo AO, et al. [9] curcumin and powder turmeric were not extracted.

The other extracts were filtered through What man No.1 filter paper and then concentrated through a rotary evaporator (Eyela, Japan) and then dried in the freeze dryer (LABCONCO, Freezone 4.5, USA) in the University of Sciences and Technology Hospital. The dried fractions were collected, weighed, and packed in dark glass containers then stored at -20°C in the refrigerator at the Sciences and Technology Hospital until used Chemical Composition of the fractions Fourier Transform Infrared (FTIR) analysis of turmeric fractions. Each *Curcuma long a* fraction (10 mg) was mixed uniformly with 100 mg of spectroscopic grade potassium bromide powder (1% w/w) to prepare translucent sample discs and then pressed into a disc before spectroscopical analysis. The spectra measurement of all samples was performed using an FT-IR spectrometer (FITR- 410, Jasco, Japan). The IR absorption spectra of all the samples were recorded from 4000 to 400 cm^{-1}. An air background spectrum was recorded before the analysis FT-IR spectral [10] (Figures 1-7).

Spectral analysis (Ultra violate analysis) of turmeric fraction: As shown in Figures 8-15 which placed in the yellow color spectrum, all turmeric extracts absorption spectra showed their maximum absorption wavelength at 413 nm, which was close to the spectrum of the Standard curcumin. Each turmeric fraction (10mg) was accurately weighed and transferred in a volumetric flask containing 100ml petroleum ether. The spectra measurement of all samples was performed using a UV spectrometer. The UV absorption spectra of all samples were recorded from 200 to 800 nm. Petroleum ether was used as blank. Wavelength corresponding to maximum absorbance of 420 nm [11].

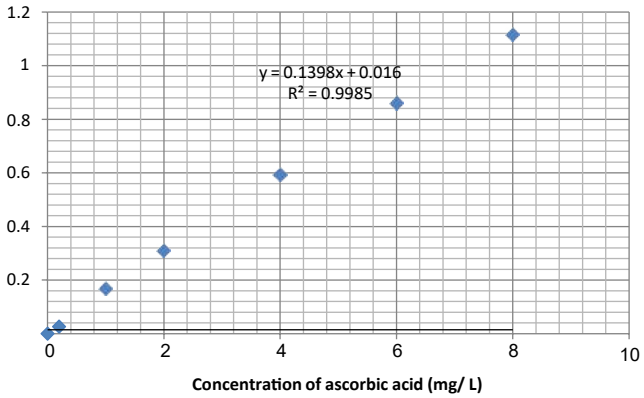

Figure 1: Calibration curve of ascorbic acid with Fe-B.p complex (Fe^{+3} (10-2M) and B.p (10-2M) iacetate buffer pH=4, after 10 min at room temperature 1:3 Fe-B.pcomplex with λmax = 535nm).

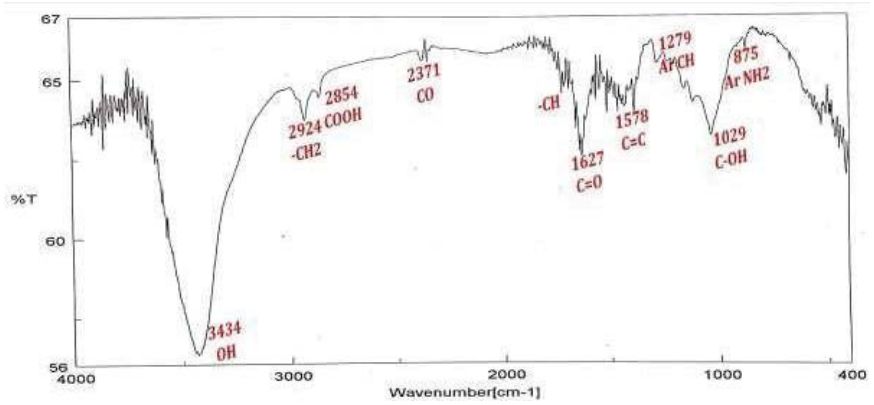

Figure 2: FT-IR spectra of standard curcumin. (˙Fourier Transform infrared (FTIR) analysis of turmeric extracts).

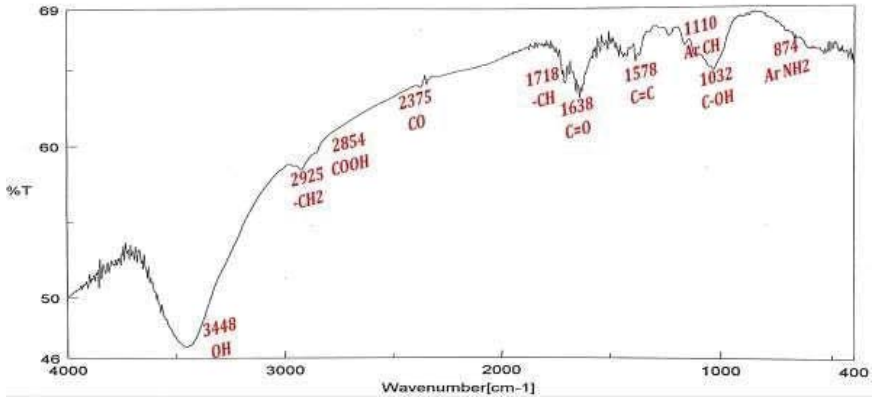

Figure 3: FT-IR spectra of crude turmeric.

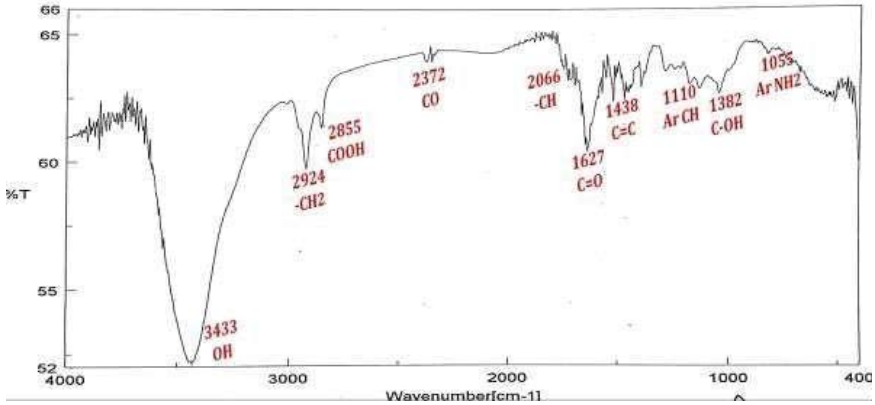

Figure 4: FT-IR spectra of ethanol fraction.

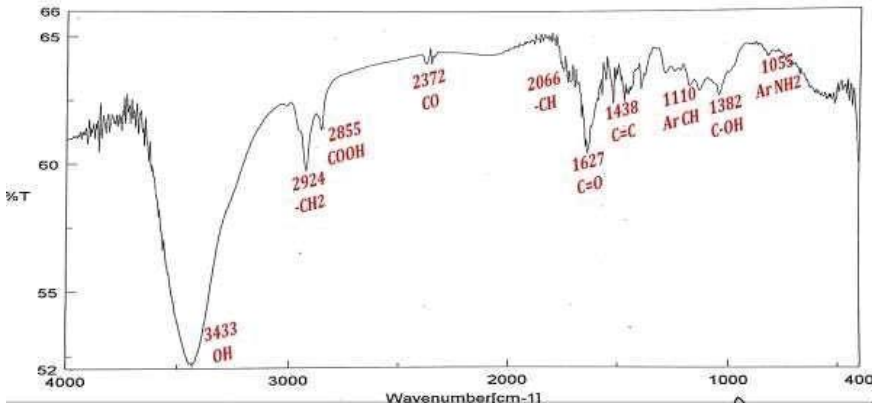

Figure 5: FT-IR spectra of ethyl acetate fraction.

Biological Activity Antioxidant assay (Free radical scavenging activity DPPH assay): Antioxidant activity was carried out according to the method described by Rao KVK [12]. The free radical scavenging activity of *Curcuma longa* rhizomes is based on its scavenging activity against the stable 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) free radical. Different extracts of *Curcuma longa* (0.1gm) were added to 3 ml of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30min, and the percentage inhibition activity was calculated from the equation: % of radical scavenging activity = (Abs control - Abs sample/ Abs control) × 100. Samples were diluted by methanol with the percentage of diluted 1:100, respectively.

Ferric–Bipyridine assay of antioxidant capacity (FBAAC): Calibration curve of ascorbic acid with Bipyridine A series of solutions were prepared by mixing 1 mL of Bipyridine (1×10^{-2} M) solution and 1 mL of FeCl3.6H2O (1×10^{-2} M) with different volumes of ascorbic acid (0.001M).

Two mL of acetate buffer pH=4 was added to every mixture then the volume was made up to 10 mL with deionized water. All measurements were carried out at room temperature, with λ max = 535 nm. The samples were treated the same way above [13].

Cell lines: Mammalian cell lines A-549 (Lung carcinoma) and A-431(Skin carcinoma) were obtained from VACSERA Tissue Culture Unit, Al-Azhar University which obtained them from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% heat-inactivated

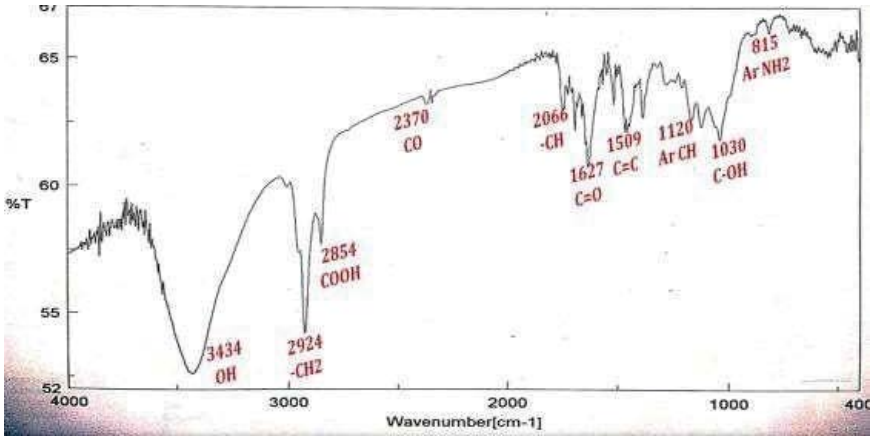

Figure 6: FT-IR spectra of acetone fraction.

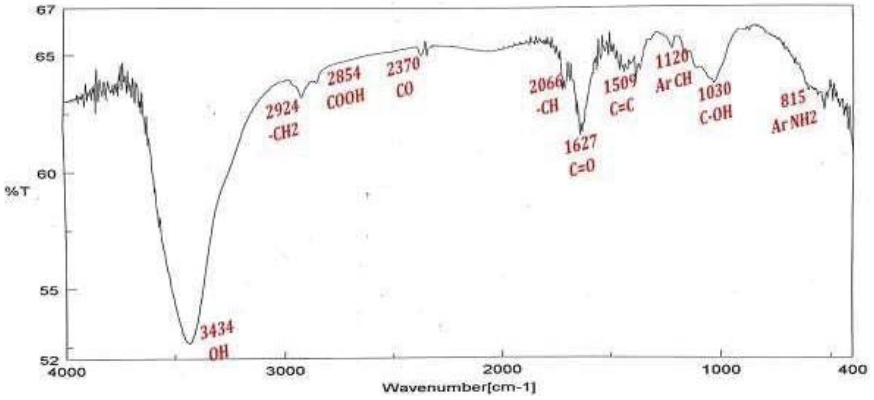

Figure 7: FT-IR spectra of aqueous extract.

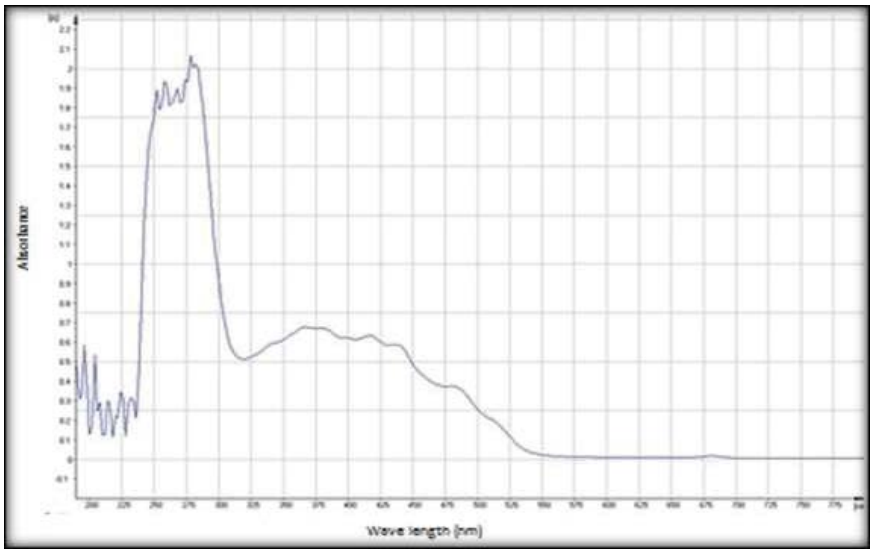

Figure 8: UV spectra of standard curcumin. (*Spectral analysis (Ultra violate analysis) of turmeric fraction).

fetal calf serum, 1% L-glutamine, and 50 µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with a 5% CO_2 incubator (Shel lab 2406, USA) and were sub-cultured two to three times a week.

***In-Vitro* Antiproliferative Activity Testing:** The tested compounds in this study were tested against tumor cells using 3- (4, 5-dimethylthiazol-2- yl)-2, 5- diphenyl tetrazolium bromide viability assay as described before (Mosmann, 1983; Wilson, 2000). The tumor cells were seeded in 96-well plate, flat-bottomed microtiter plates (Falcon, NJ, USA), in 100µl of growth medium at a cell concentration of 1×104 cells per well. After 24 h of seeding, the confluent cell monolayers dispensed into a 96-well plate were then washed with sterile phosphate- buffered saline (0.01 M pH 7.2), and simultaneously the

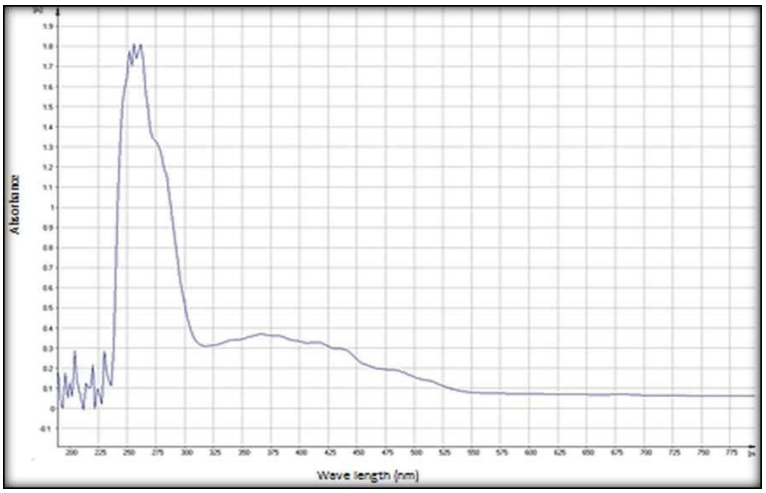

Figure 9: UV spectra of crude turmeric.

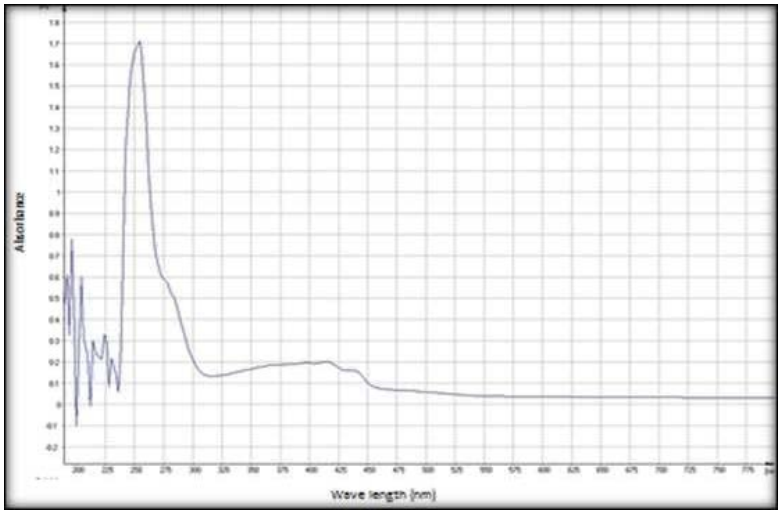

Figure 10: UV spectra of ethanolic fraction.

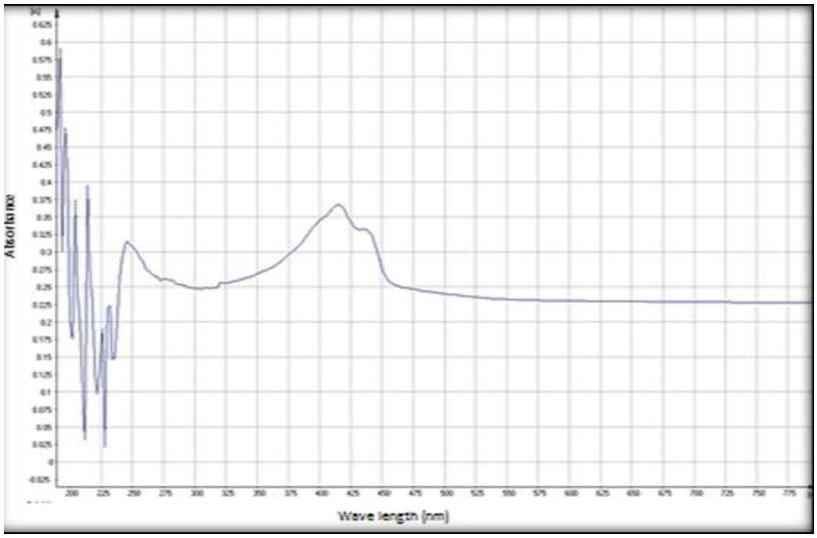

Figure 11: UV spectra of ethyl acetate fraction.

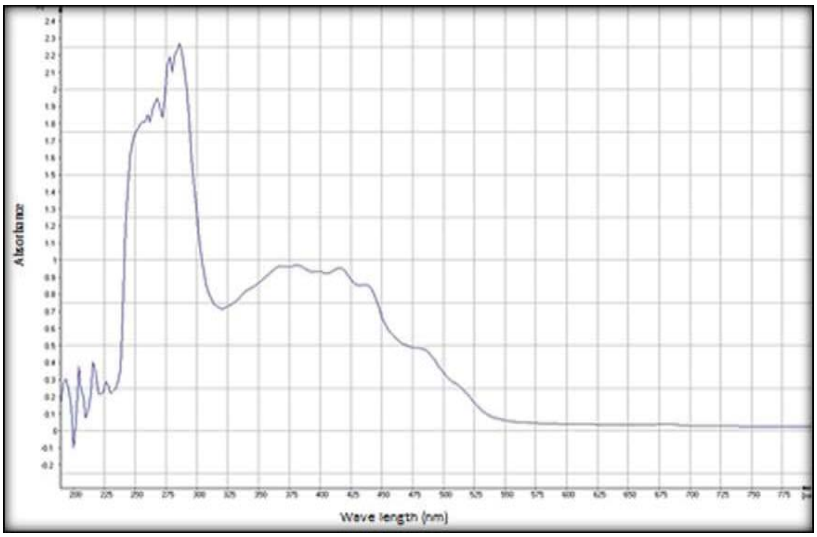

Figure12: UV spectra of acetone fraction.

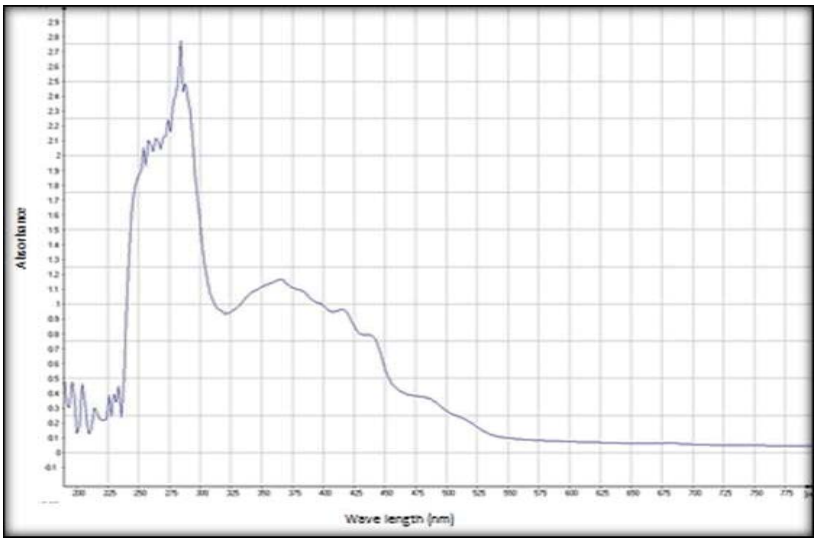

Figure 13: UV spectra of petroleum ether fraction.

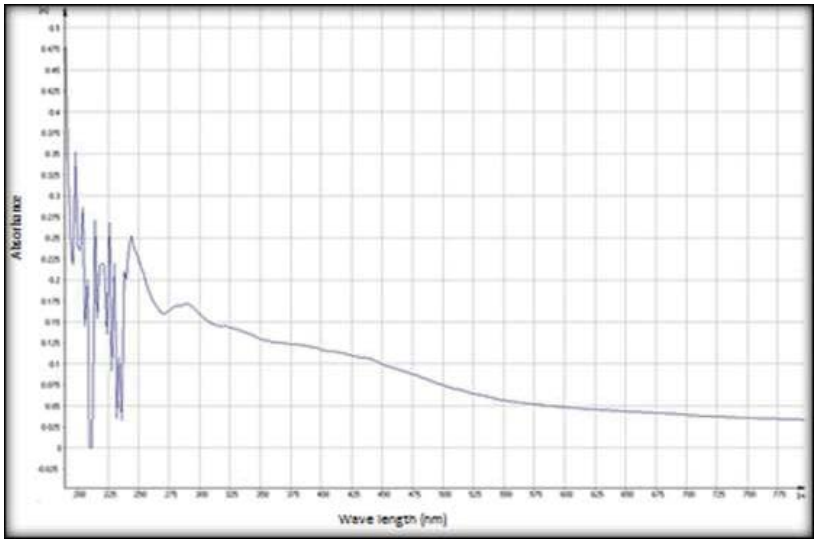

Figure 14: UV spectra of aqueous fraction.

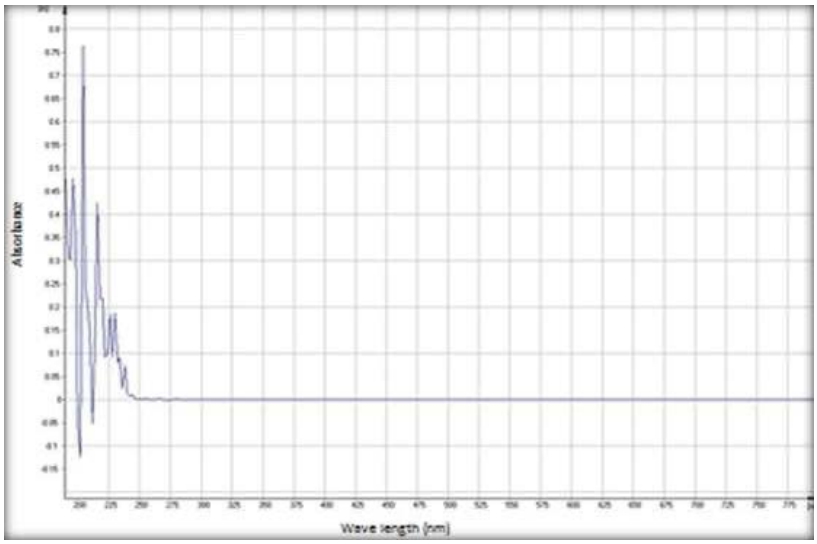

Figure 15: UV spectra of petroleum ether.

cells were treated with 100 µl from the tested sample (at different doses) in a fresh maintenance medium. Serial two-fold dilutions of each tested compound (500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 2 and 1 µg/ml) were added to confluent cell monolayers using a multichannel pipette in a humidified incubator with 5% CO_2 at 37°C for a period of 24h. Untreated cells were served as negative controls and vinblastine sulfate (chemotherapy) was used as a positive control. Two independent experiments were performed each containing three replicates for each sample. After incubating for 24h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT (Sigma) stock solution (5 mg of MTT in 1mL of phosphate-buffered saline) was added to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85µl aliquot of the media was removed from the wells, and 50µl of DMSO was added, to dissolve insoluble formation crystal to each well and mixed thoroughly with the pipette and incubated at 37°C for 10min. Then, the optical density was measured at 590 nm with the microplate reader (Sunrise, TECAN, Inc, USA) to determine the number of viable cells. The relation between surviving cells and plant extracts concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified extract. The 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose-response curve for each conc. The percentage of cell viability was calculated using Microsoft Excel, according to the following equation:

The percentage of cell viability = (ODt /ODI) × 100%

where it is the mean optical density of wells treated with the tested extract and ODI is the mean optical density of untreated cells.

Inhibition% = 100-cell viability%

The test extracts were also compared using the IC_{50} value, i.e., the concentration of individual extracts leading to 50% cell death that was estimated from graphical plots of surviving cells vs extract concentrations [14].

Cytotoxicity evaluation on normal cell line: For cytotoxicity assay, normal fibroblast cell lines were seeded in 96- well plate at a cell concentration of 1×10^4 cells per well in 100 µl of growth medium. After incubation of the cells for 24h at 37°C, various concentrations of the extracts were added, and the incubation was continued for 24h and viable cells yield was determined by a colorimetric method as previously reported [15]. In brief, after the end of the incubation period, the MTT solution (25 µl) was added to each well, and plates were incubated again in 5% CO_2 at 37°C for 4h. Acid- isopropanol (100 µl) was added to all wells and mixed thoroughly to dissolve the formed crystals. After 20 min at room temperature to ensure that all crystals were dissolved, the plates were read on an ELISA reader, using a test wavelength of 490 nm. Treated samples were compared with the untreated control and all experiments were carried out in triplicate. The cell cytotoxic effect of each tested extract was calculated by the following formula:

Percent of cell cytotoxic effect = [(ODI - ODt /ODI)] × 100%.

Where ODc and ODt indicate the absorbance of the cell control and the test substances, respectively.

The 50% cell cytotoxic concentration (CC50) required to cause visible changes in 50% of intact cells was estimated from graphic plots.

Microscopic observation of the tumor cells treated with turmeric fractions: After the end of the treatment, the plates were inverted to remove the medium, the wells were washed three times with 100 µl of phosphate-buffered saline (pH 7.2) and then the cells were fixed to the plate with 10% formalin for 15 min at room temperature. The fixed cells were then stained with 100 µl of 0.25% crystal violet for 20 min. The stain was removed and the plates were rinsed using deionized water to remove the excess of stain then allowed to dry. The cellular morphology was observed using an inverted microscope (CKX41; Olympus, Japan) equipped with a digital microscopy camera to capture the images representing the morphological changes compared to control cells [14]. The cytopathic effects (morphological alterations) were microscopically detected at 100X magnification and summarized semi-quantitatively of the alterations score as the following (0=No alterations 0%, +=Mild alterations 20-40%, ++=Moderate alterations 45-75%, +++=Severe alterations up to 90%).

Statistical analysis: Data were analyzed using SPSS v23. Data were summarized as means ± SD as the data was normally distributed. An independent t-test was used to conduct the significance of association between extracts, curcumin, and chemotherapy at different concentrations using SPSS program version 23. One-way ANOVA and post-hoc LSD tests were used to identify the significance of association

between the independent variables. Differences were considered significant at P values of less than 0.05.

Results and Discussion

Lung cancer is the most common cause of cancer-related death worldwide and is classified as either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). NSCLC accounts for 75–80% of patients with primary lung cancer. Compared with NSCLC, SCLC shows an aggressive behavior with rapid tumor growth and early spread to distant sites; its malignancy is the highest of all types of lung cancer.1, 2, 3 Surgery is the most effective therapeutic modality, but the postoperative prognosis remains poor. In addition, most patients present with advanced-stage disease, and chemotherapy with or without radiotherapy is recommended [16,10] Skin cancer is one of the most common malignancies in dermatology. With the rapid development of industrialization and changes in people's living environment, the incidence of skin cancer has been increasing year by year, which has gradually developed into a worldwide public health problem [2]. Early specificity of diagnosis and treatment is still lacking; the current common treatment methods are surgery, radiation/chemotherapy, laser, and cryotherapy [3]. From this point of view, the screening for anticancer activity of some natural extracts with lower adverse effects is an interesting issue

Antioxidant activities of Yemeni Cultivated Curcumalonga L. Extracts on Skin (A431) and Lung (A549) Cancer Cell Line was investigated using a 3-(4,5-dimethylthiazole-2yl)-2,5-diphenyl tetrazolium bromide assay on two human cancer cell lines and the results were shown in Table 1. A mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan and the amount of formazan produced is directly proportional to the number of viable cells vinblastine, a broad-spectrum antitumor agent is widely used in the treatment of several cancers. This drug was used as a positive control in this study (Table 2).

The cytotoxic effect of seven tumuric fractions on A549 cell lines was examined at different concentrations ranging from 1 to 500 µg/mL, respectively. As shown in Tables 3-5 and Figures 16-21, there was a reversible relationship between the extract concentration and the cell viability. At the high concentration (500 µg/ml), ethyl acetate turmeric extract had the highest effect on the viability of normal cell line (7.80%) followed by aqueous extract (10.57%) then acetone extract (11.80%) $p<0.01$, $p<0.001$, $p<0.01$, respectively, while crude turmeric had the lowest effect on the viability of this type of cells (33.17%) $p<0.01$. At the low concentration (1 µg/ml), ethyl acetate turmeric extract had the most effect on the viability of the control cell line (99.29%) $p<0.01$, while all other extracts had the lowest effect on the viability of this type of cells (100.0%) $p<0.05$. At the high concentration (500 µg/ml), ethyl acetate turmeric extract had the highest effect on the viability of the A431 skin cancer cell line (4.80%) followed by acetone extract (7.17%) than aqueous extract (7.39%) $p<0.05$, $p<0.001$, $p<0.01$, respectively, while crude turmeric had the lowest effect on the viability of this type of cells (25.90%) $p<0.001$. At the low concentration (1 µg/ml), ethyl acetate turmeric extract had the most effect on the viability of normal cell line (99.92%) $p<0.01$, while all other extracts had the lowest effect on the viability of this type of cells (100.0) $p<0.01$. Turmeric ethyl acetate fraction in our study represented the highest effect on the viability and inhibition of skin and lung cancer cell lines with IC50 of 13.7 and 7.27, respectively. This agrees with Hansen HH [17] who recorded a significant inhibitory effect on the colony- forming ability of the highly metastatic prostate cancer cell line (PC-3M). They refer to the cause of the presence of three main curcuminoids namely curcumin, demethoxycurcumin, and bisdemethoxycurcumin in ethyl acetate fraction [12]. Apoptosis or programmed cell death (PCD) is critically important for normal physiological development, which maintains by a homeostatic balance between cell division and cell death. In general, it is characterized by distinct morphological characteristics (including cell shrinkage, membrane blebbing, nuclear fragmentation, and chromatin condensation) and energy-dependent biochemical mechanisms [18]. Consequently, the cytotoxic activity of ethyl acetate and other fractions were evaluated microscopically. The results revealed that the mode of cytotoxic activity is due to the apoptosis mechanism at tested doses from (3.9 to 500 µg/mL) towards skin and lung cancer cell lines as shown in Plates 1-14. Moreover, severity of each loss of cellularity/intercellular adhesions, pyknotic chromatin, karyorrhexis/chromatolysis and apoptotic bodies beside membrane blubbing were summarized in Tables 6 and 7 as a semi-quantitative alteration scores, which revealed that for the activities as anticancer for lung cell lines were as follows: (strongest to weakest effect) ethyl acetate fraction being strongest then aqueous enthanol, acetone pet.ether and crude turmeric. As well as for skin cell line , the effectiveness differs from lung cell line , as the most effective was : Ethyl acetate , then acetone , aqueous , ethanal , pet.ether and crude turmeric. The selectivity degree of extracts compounds can be expressed by their selectivity index (SI) values and calculated from the ratio of CC50 value normal cell line, over the IC_{50} values against cancerous cells lines, the higher SI value, the more selective it is. An SI value less than 2 indicates the general toxicity of the compound [19, 20, 13]. According to this, the SI values indicated that crude Turmeric has high selectivity against A431 cancer cell lines compared to vinblastine and curcumin as it shows (SI = 296). Meanwhile, vinblastine showed the lowest selectivity index thus higher cytotoxicity towards normal cell lines. (SI= 0.4) The selectivity of a cytotoxic agent to cancer cells is one of the most essential features which should be considered in the discovery and screening of the novel anticancer agents [13]. Since is more selective towards cancer cells than a normal cell, it can be considered as a promising anticancer agent. Techniques such as UV and FT-IR are applied to assess the quality control of turmeric as shown in Figures 2-15. Analysis results concluded that the different turmeric extracts have functional groups

Table 1: Free radical scavenging activity of the different turmeric fractions, standard (curcumin) and ascorbic acid.

Tested matters	DPPH (%)
Standard (Curcumin)	89%↑
Crude turmeric	15%↓
Ethanolic extract	61%
Ethyl acetate extract	60%
Acetone extract	81%↑
Petroleum ether extract	30%
Aqueous extract	10%↓
Ascorbic acid	91%

Table 2: Ferric-Bipyridine assay of antioxidant capacity (FBAAC) of the different turmeric fractionsand standard (curcumin).

Tested matters	Antioxidant capacity (mg/l)
Standard (Curcumin)	2.94×10^{-5}
Crude turmeric	6.20×10^{-6}
Ethanolic extract	2.38×10^{-5}
Ethyl acetate extract	2.26×10^{-5}
Acetone extract	4.75×10^{-5}
Petroleum ether extract	7.36×10^{-6}
Aqueous extract	7.00×10^{-6}

Table 3: Cytotoxicity activity of extracts against A-549 cell line.

Tumor cell line A-549	**Mean of variability ± S.D.#**							
Sample concentration(µg)	**Vinblastine Sulfate***	**Curcumin**	**Crude turmeric**	**Ethanol**	**Ethyl acetate**	**Acetone**	**Petroleum ether**	**Aqueous fraction**
500 µg	4.87±0.29	2.89±0.15^{A}	17.02±1.12^{A,b}	6.78±0.68^{C,a}	3.32±0.68^{C}	5.63±0.98^{c}	10.32±0.59^{A,a}	6.24±0.78^{C,b}
250 µg	7.99±1.15	5.18±0.26^{C}	30.98±3.36^{A,b}	13.82±1.33^{B,a}	6.81±1.34	10.84±1.05^{C,a}	18.22±0.33^{A,a}	11.05±1.61^{a}
125 µg	14.06±1.30	9.52±0.80^{B}	43.03±3.58^{A,a}	21.80±1.86^{B,a}	12.72±1.13^{c}	21.07±1.72^{B,a}	27.24±1.28^{A,a}	17.96±1.67^{C,b}
62.5 µg	19.85±1.78	15.19±0.62^{C}	63.37±3.41^{A,a}	29.59±2.21^{B,a}	19.38±1.10^{b}	31.65±3.14^{B,a}	39.29±1.90^{A,a}	27.35±1.45^{B,a}
31.25 µg	23.67±1.63	19.29±1.31^{C}	78.27±3.12^{A,a}	39.49±2.48^{A,a}	26.98±1.38^{b}	41.09±1.59^{A,a}	48.70±3.57^{A,a}	37.61±2.99^{B,a}
15.6 µg	30.69±1.69	27.53±1.06	91.11±2.98^{A,a}	56.91±4.82^{A,a}	36.12±1.14^{B,a}	57.21±3.07^{A,a}	65.83±3.76^{A,a}	51.68±2.69^{A,a}
7.8 µg	38.95±2.00	34.87±0.60^{C}	97.61±1.02^{A,a}	69.91±4.93^{A,b}	47.99±2.81^{C,b}	75.73±3.28^{A,a}	81.74±2.71^{A,a}	72.18±1.79^{A,a}
3.9 µg	47.30±4.27	46.10±3.42	99.88±0.21^{B,b}	85.89±3.24^{A,a}	62.81±3.33^{B,b}	89.28±2.40^{A,a}	93.83±2.26^{A,a}	87.04±3.31^{A,a}
2 µg	60.40±2.13	56.29±1.29^{C}	100.0±0.0^{A,a}	94.72±3.20^{A,a}	80.71±2.20^{A,a}	96.33±1.85^{A,a}	99.29±0.98^{A,a}	95.21±2.94^{A,a}
1 µg	71.72±2.38	68.75±4.84	100.0±0.0^{B,b}	99.30±0.63^{A,b}	91.86±2.06^{A,b}	99.30±0.65^{A,b}	100.0±0.0^{B,b}	99.71±0.30^{B,b}
IC50 (µg)	**3.58 ±0.48**	**3.23±0.41**	**103.5±5.96**	**21.7±2.86**	**7.27± 0.65**	**22.5±1.91**	**30.0± 2.84**	**17.4± 2.34**

^{A,B,C} are *p* –values compared to Vinblastine Sulfate and ^{a,b,c} are *p* –values compared to Curcumin.
^{A,a} *p* value <0.001, ^{B,b} *p* value <0.01, ^{C,c} *p* value <0.05 compared to reference drug. #Mean of variability % ±standard deviation.

Table 4: Cytotoxicity activity of extracts against WI-38 cell line.

Normal cell line WI-38	**Mean of variability ± S.D.#**							
Sample concentration(µg)	**Vinblastine Sulfate***	**Curcumin**	**Crude turmeric**	**Ethanol**	**Ethyl acetate**	**Acetone**	**Petroleum ether**	**Aqueous extract**
500 µg	5.36±0.44	5.55±1.03	33.17±3.44^{B,a}	16.64±2.39^{B,b}	7.80±0.46^{B,c}	11.80±1.48^{B,b}	16.19±1.60^{A,a}	10.57±0.73^{A,b}
250 µg	8.30±0.67	10.47±0.74^{C}	46.07±3.40^{A,a}	29.70±2.93^{A,a}	18.10±2.36^{B,b}	23.22±1.59^{A,a}	30.57±1.14^{A,a}	20.83±2.38^{A,b}
125 µg	13.77±1.23	17.26±0.71^{C}	70.78±1.43^{A,a}	41.61±1.21^{A,a}	25.59±3.46^{B,c}	33.68±2.81^{A,a}	41.71±1.83^{A,a}	36.03±1.20^{A,a}
62.5 µg	24.74±2.79	25.68±2.28	86.05±2.28^{A,a}	48.83±2.18^{A,a}	33.97±2.92^{C,c}	43.49±1.85^{A,a}	57.62±2.84^{A,a}	44.35±2.18^{A,a}
31.25 µg	32.80±2.13	34.89±1.98	95.25±2.06^{A,a}	69.03±3.38^{A,a}	41.77±3.09^{C,c}	52.49±4.07^{B,b}	71.34±3.38^{A,a}	54.62±2.20^{A,a}
15.6 µg	40.06±1.25	44.91±3.72	99.51±0.45^{A,b}	85.25±2.89^{A,a}	53.08±3.64^{B}	73.86±5.86^{A,b}	84.78±1.67^{A,a}	71.44±3.31^{A,a}
7.8 µg	48.72±2.28	56.18±4.63	100.0±0.0^{A,b}	93.86±2.46^{A,a}	72.49±2.55^{A,b}	86.54±3.04^{A,a}	92.81±1.20^{A,a}	87.06±2.92^{A,a}
3.9 µg	63.81±2.29	70.80±7.66	100.0±0.0^{B,c}	98.71±0.80^{A,c}	87.95±2.97^{A,c}	95.91±2.81^{A,b}	98.60±1.03^{A,c}	95.89±3.00^{A,b}
2 µg	78.74±3.48	83.89±3.14^{B}	100.0±0.0^{B,c}	100.0±0.0^{B,c}	96.24±1.59^{B,b}	99.24±0.98^{A,b}	100.0±0.0^{B,c}	99.59±0.72^{A,b}
1 µg	91.38±2.12	91.12±3.31	100.0±0.0^{C,c}	100.0±0.0^{C,c}	99.29±1.01^{B,c}	100.0±0.0^{C,c}	100.0±0.0^{C,c}	100.0±0.0^{C,c}
CC50 (µg)	**7.47 ±0.49**	**12.1± 2.35**	**230 ± 12.4**	**60.7±2.96**	**19.8 ± 3.92**	**39.9 ±9.45**	**92.4 ±6.86**	**45.3±4.79**

^{A,B,C} are *p* –values compared to Vinblastine Sulfate and ^{a,b,c} are *p* –values compared to Curcumin.
^{A,a} *p* value <0.001, ^{B,b} *p* value <0.01, ^{C,c} *p* value <0.05 compared to reference drug. #Mean of variability % ±standard deviation.

Table 5: Cytotoxicity activity of extracts against A-431 cell line.

Tumor cell line A-431	**Mean of variability ± S.D.#**							
Sample concentration(µg)	**Vinblastine Sulfate***	**Curcumin**	**Crude turmeric**	**Ethanol**	**Ethyl acetate**	**Acetone**	**Petroleum ether**	**Aqueous extract**
500 µg	3.23±0.66	3.26±0.37	25.90±3.17^{A,b}	9.99±0.21^{A,a}	4.80±0.43^{C,b}	7.39±0.90^{B,b}	13.13±1.38^{A,a}	7.17±0.94^{B,b}
250 µg	4.87±0.38	5.81±0.14^{C}	36.76±1.67^{A,a}	18.32±1.56^{A,b}	8.65±2.14^{C}	13.52±0.93^{A,b}	21.59±0.89^{A,b}	12.13±1.29^{A,c}
125 µg	9.20±1.30	11.22±1.02	60.03±2.40^{A,a}	26.13±1.14^{A,a}	16.30±1.77^{B,c}	28.42±1.76^{A,a}	30.47±1.54^{A,a}	20.70±1.17^{A,a}
62.5 µg	16.15±1.55	17.77±0.88	77.23±2.87^{A,a}	34.94±3.20^{A,a}	23.21±2.02^{B,c}	34.75±1.89^{A,a}	43.69±2.37^{A,a}	30.36±1.57^{A,a}
31.25 µg	24.96±1.59	22.88±1.80	91.41±2.45^{A,a}	46.12±2.85^{A,a}	30.91±1.76^{C,b}	43.30±1.55^{A,a}	59.80±3.19^{A,a}	43.44±1.46^{A,a}
15.6 µg	33.41±2.24	29.52±2.42	98.29±1.74^{A,a}	75.51±2.93^{A,a}	43.13±1.54^{B,b}	61.20±3.14^{A,a}	77.12±3.45^{A,a}	62.93±2.14^{A,a}
7.8 µg	42.33±1.43	38.60±1.07^{C}	99.91±0.16^{A,a}	89.55±1.04^{A,a}	70.77±2.44^{A,a}	79.17±4.61^{A,a}	89.09±2.06^{A,a}	80.15±1.65^{A,a}
3.9 µg	50.66±1.79	52.84±1.76	100.0±0.0^{A,a}	96.53±1.04^{A,a}	86.71±1.75^{A,a}	91.50±1.52^{A,a}	97.32±1.28^{A,a}	93.46±2.81^{A,a}
2 µg	68.00±2.81	64.53±2.71	100.0±0.0^{B,b}	99.37±0.68^{A,a}	97.41±1.32^{A,a}	98.72±0.71^{A,a}	99.83±0.30^{B,b}	99.28±1.01^{A,a}
1 µg	78.96±1.93	77.27±1.86	100.0±0.0^{B,b}	100.0±0.0^{B,b}	99.92±0.14^{B,b}	100.0±0.0^{B,b}	100.0±0.0^{B,b}	100.0±0.0^{B,b}
IC50 (µg)	4.21 ±0.66	**4.68±0.63**	179.0± 4.02	29.1±1.2	**13.7 ± 0.37**	25.4 ± 0.87	50.3 ± 4.31	25.9 ±1.10

^{A,B,C} are *p* –values compared to Vinblastine Sulfate and ^{a,b,c} are *p* –values compared to Curcumin.
^{A,a} *p* value <0.001, ^{B,b} *p* value <0.01, ^{C,c} *p* value <0.05 compared to reference drug. #Mean of variability % ±standard deviation.

such as alkanes, carbonyl, alkenes, primary amines, nitro compounds, aromatics, aromatic amines which detected the curcuminoids, natural phenols that cause the yellow color of turmeric. All turmeric extracts have similar peaks that may be due to the similar chemical compounds contained. However, there is a bit of difference in terms of peak absorbance caused by different concentrations of components present in all extracts of C. longa. This result agrees with [21-23]. Further, The antioxidant DPPH assay of this study is shown in Table 1 revealed that acetone extract had the highest antioxidant activity (81%) followed by the ethanolic extract (61%), while the aqueous extract had a lower antioxidant activity (10%), all compared with ascorbic acid and standard curcumin. The present study is in agreement with that of [24] who

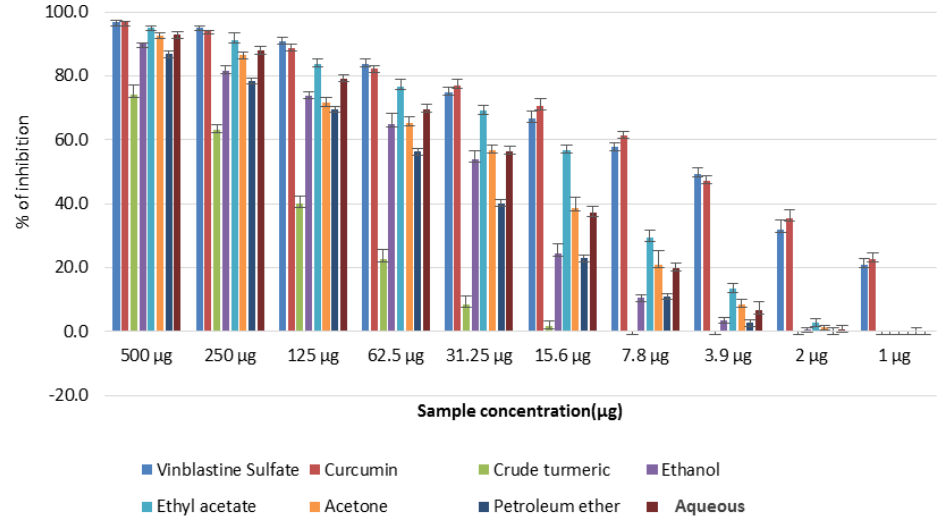

Figure 16: Percentage of inhibition of turmeric fractions on A549 cell line.

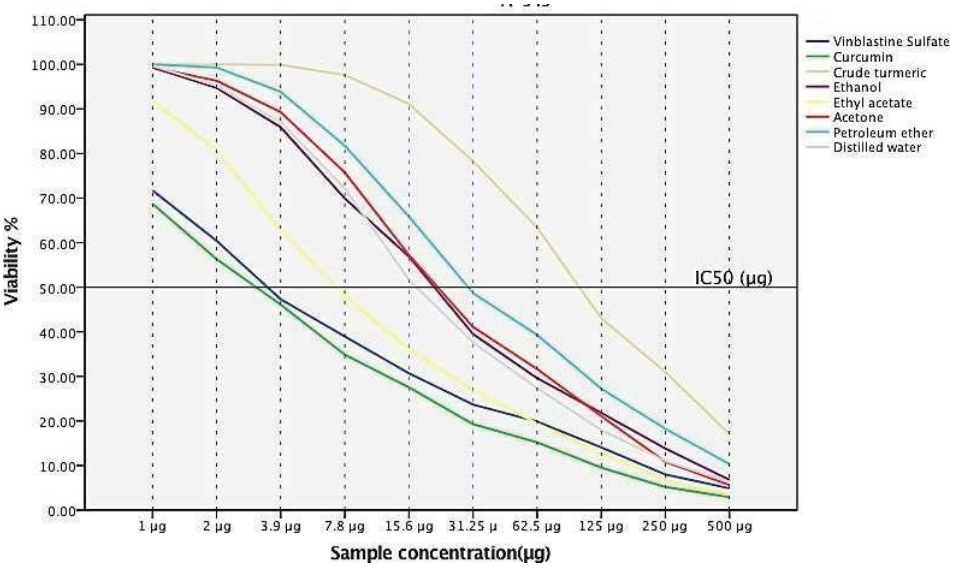

Figure 17: Percentage of viability of A549 lung cancer cell line against turmeric fractions.

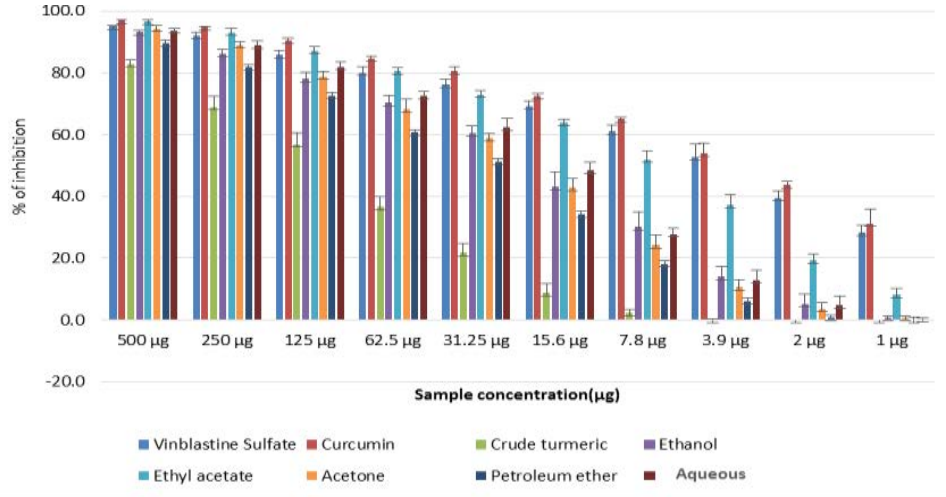

Figure 18: Percentage of inhibition of extracts against normal cell line.

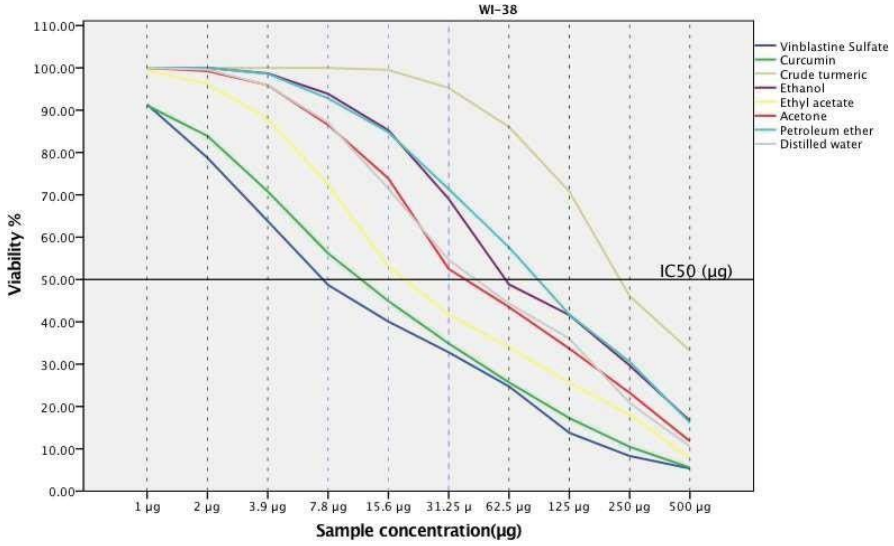

Figure 19: Percentage of viability of normal cell line against turmeric extracts.

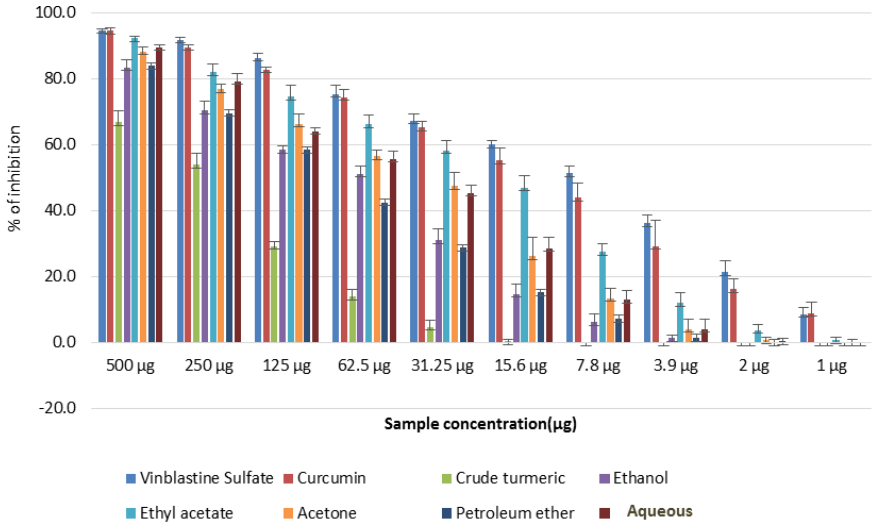

Figure 20: Percentage of inhibition of turmeric fractions on A431 cell line.

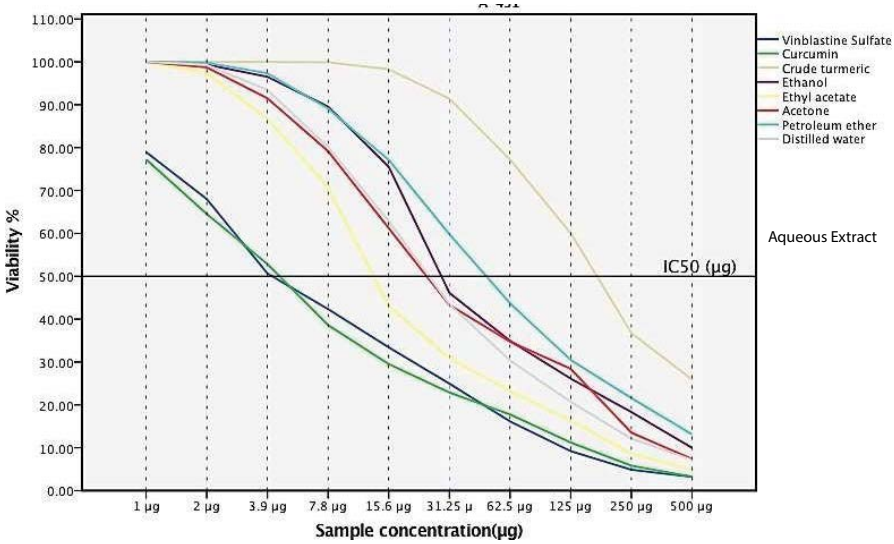

Figure 21: Percentage of viability of A431 skin cancer cell line against turmeric fraction.

Table 6: Score of the alterations among different concentration tumeric fractions.

Types	µg	Loss of Cellularity	loss of Adhesions	Chromatin Condensation (pyknotic)	Chromatin Fragments (karyorrhexis or lysis)	Apoptotic Bodies	Membrane Blebbing
Ethyl acetate extract	0	0	0	0	0	0	0
	3.9	++	++	+++	++	+	+
	7.8	++	+++	+	+	++	+
	15.6	++	++	+	++	++	++
	31.25	+++	+++	+++	+++	+++	++
	62.5	+++	+++	+++	+++	+++	+++
	125	+++	+++	+++	+++	+++	+++
	250	+++	+++	+++	+++	+++	+++
	500	+++	+++	+++	+++	+++	+++
Crude	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	0	0	0	0	0	0
	15.6	0	0	0	0	0	0
	31.25	0	0	0	0	0	0
	62.5	0	+	+	+	+	0
	125	+	+	+	+	+	0
	250	++	++	+	+	+	+
	500	++	++	+++	+++	++	+
Ethanol extract	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	+	+	+	0	0	0
	15.6	0	0	+++	+	0	0
	31.25	++	++	++	+	+	++
	62.5	++	++	+	+	+	++
	125	+++	+++	++	++	+	+
	250	+++	+++	++	++	++	+
	500	+++	+++	++	+++	+++	++
acetone extract	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	0	+	0	+	0	0
	15.6	+	+	+	+	0	0
	31.25	+	+	+	+	+	+
	62.5	+	+	+	+	+	+
	125	+++	+++	++	++	++	+++
	250	+++	+++	++	+++	+++	+++
	500	+++	+++	+++	+++	+++	+++
Curcumin	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	+	+	++	+	+	0
	15.6	++	++	++	++	++	+++
	31.25	+++	+++	+++	+++	+++	+++
	62.5	++	++	++	+++	+++	+++
	125	+++	+++	+++	+++	+++	+++
	250	+++	+++	+++	+++	+++	+++
	500	+++	+++	+++	+++	+++	+++
Petroleum ether extract	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	0	0	0	0	0	0
	15.6	+	+	+	0	0	0
	31.25	+	+	+	+	+	0
	62.5	+	+	+	+	+	0
	125	++	++	++	++	+	+
	250	++	++	++	++	+	+
	500	++	++	+++	+++	++	++
Aqueous extract	0	0	0	0	0	0	0
	3.9	+	+	+	+	+	0
	7.8	+	+	+	+	+	+
	15.6	+	+	+	+	+	+
	31.25	+	+	+	+	+	++
	62.5	++	++	++	++	+	+++
	125	++	++	++	++	+++	+++
	250	++	++	++	++	+++	+++
	500	++	++	++	++	+++	+++
Score system designed as the following (0=No alterations 0%, +=Mild alterations 20-40%, ++=Moderate alterations 45-75%, +++=Severe alterations up to 90%).							

Table 7: Score of the alterations among different concentration extracts.

Types	µg	Loss of Cellularity	loss of Adhesions	Chromatin Condensation (pyknotic)	Chromatin Fragments (karyorrhexis or lysis)	Apoptotic Bodies	Membrane Blebbing
Ethyl acetate extract	0	0	0	0	0	0	0
	3.9	++	++	+++	++	+	+
	7.8	++	+++	+	+	++	+
	15.6	++	++	+	++	++	++
	31.25	+++	+++	+++	+++	+++	++
	62.5	+++	+++	+++	+++	+++	+++
	125	+++	+++	+++	+++	+++	+++
	250	+++	+++	+++	+++	+++	+++
	500	+++	+++	+++	+++	+++	+++
Crude	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	0	0	0	0	0	0
	15.6	0	0	0	0	0	0
	31.25	0	0	0	0	0	0
	62.5	+	+	++	+	+	+
	125	++	++	+	+	+	+
	250	++	++	+	+	+	+
	500	++	++	+++	+++	++	+
Ethanol extract	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	+	+	+	0	0	0
	15.6	+	+	++	+	+	+
	31.25	++	++	++	+	+	++
	62.5	++	++	+	+	+	++
	125	+++	+++	++	++	+	+
	250	+++	+++	++	++	++	+
	500	+++	+++	++	+++	+++	++
acetone extract	0	0	0	0	0	0	0
	3.9	0	0	0	0	+	0
	7.8	+	+	++	+	+	+
	15.6	++	++	++	++	+	+
	31.25	+++	+++	++	++	++	++
	62.5	+++	+++	++	++	++	++
	125	+++	+++	++	++	++	+++
	250	+++	+++	++	+++	+++	+++
	500	+++	+++	+	+++	++	+++
Curcumin	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	+	+	++	+	+	+
	15.6	++	++	+++	+++	++	++
	31.25	++	++	+++	+++	++	++
	62.5	++	++	++	+++	++	+++
	125	+++	+++	+++	++	+++	+++
	250	+++	+++	+++	+++	+++	+++
	500	+++	+++	+++	+++	+++	+++
Petroleum ether extract	0	0	0	0	0	0	0
	3.9	0	0	0	0	0	0
	7.8	0	0	0	0	0	0
	15.6	+	+	+	0	0	0
	31.25	+	+	++	++	++	+
	62.5	++	++	+++	+	+	++
	125	++	++	++	++	+++	++
	250	+++	+++	+++	+++	+++	+
	500	+++	+++	+++	+++	+++	+++
Aqueous extract	0	0	0	0	0	0	0
	3.9	+	++	+	+	+	0
	7.8	+	+	+	+	+	+
	15.6	+	+	+	+ +	+	+
	31.25	+	+	+	+ +	+	++
	62.5	++	++	++	+++	+	+++
	125	+++	+++	+++	+++	+++	+++
	250	+++	+++	+++	+++	+++	+++
	500	+++	+++	+++	+++	+++	+++

Score system designed as the following (0=No alterations 0%, +=Mild alterations 20-40%, ++=Moderate alterations 45-75%, +++=Severe alterations up to 90%).

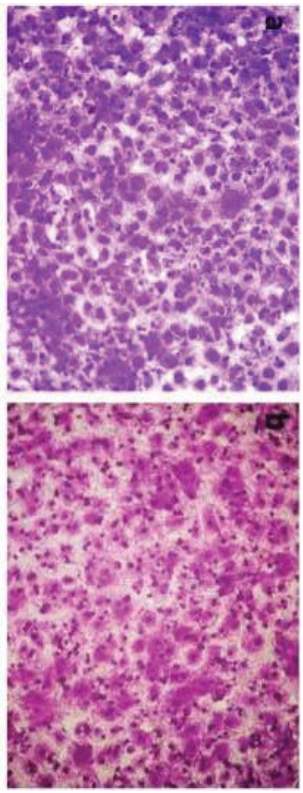

Plate 1: Control group without treatment of the A431 epidermal cell carcinoma showing Continuous attached sheet with typical neoplastic characterizations (polymorphisms, large vascular cells with abundant chromatin) *Skin: Ethyl acetate, acetone, aqueous, ethanol, pet either, crude.

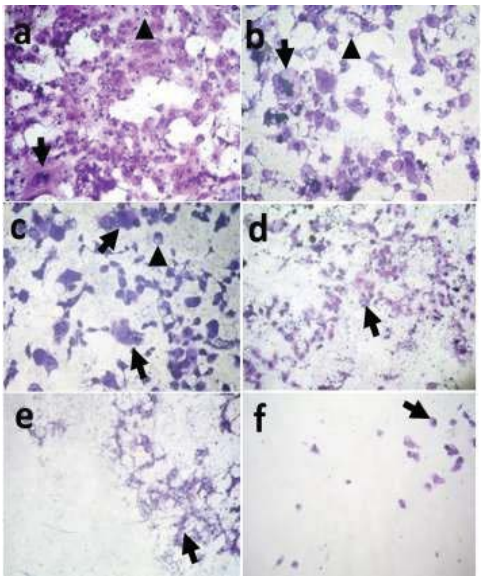

Plate 2: Photomicrograph (400X) of the different concentration Ethyl acetate extract treatment A431 epidermal cell carcinoma showing a) a few cells have early apoptosis (arrowhead), moderate loss of the cell adhesions beside large bazaars cell (arrow) in 3.9µg, b) Marked loss of the cellularity and cell adhesions with the still presence of bazaars cell (arrow) and commonly condense chromatins and cell shrinkage with the presence of apoptotic bodies (arrowhead) in both 7.8µg. c) marked loss of cellularity with destructed and lysis bazaars cells (arrows) besides numerous cytoplasmic blubbing (arrowhead) in 15.6 µg. d) marked lysis of cells with prominent pyknotic chromatin (arrow) in 31,25µg. e,f) completely absence of cell details due to cytolysis with still numerous pyknotic chromatin (arrows) with increases of cellular debris in each 62.5, 125, 250&500µg.

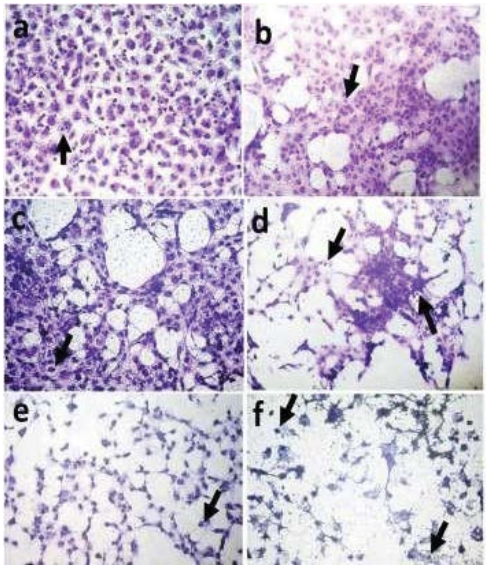

Plate 3: Photomicrograph (400X) of the different concentration acetone extract treatment A431 epidermal cell carcinoma showing a) continuous uniform shape of neoplastic cells without any improvement effects except numerous early apoptotic cells (arrow) in 3.9µg, b,c) mild to moderate loss of the cellularity and cell adhesions in each 7.8 and 15.6 µg, d) severe loss of cellularity and cells adhesions with still mitotic index (arrow) in 31.25 µg, d) Severe loss of cell adhesions with prominent apoptotic features and cytoplasmic blubbing (arrows) in 62.5 µg, e,f) diffuse de-attachment of the cell sheet with found cytoplasmic blebbing and karyorrhexis chromatin (arrows) in each 125,250 and 500µg.

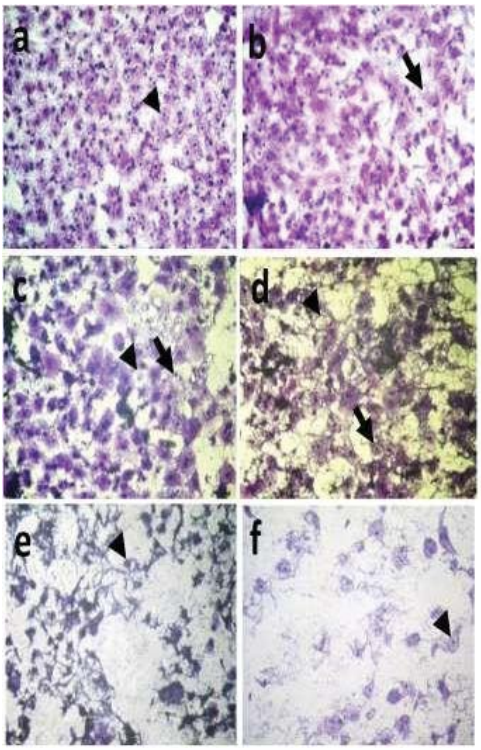

Plate 4: Photomicrograph (400X) of the different concentration Aqueous extract treatment A431 epidermal cell carcinoma showing a) neoplastic features with abundant apoptotic cells (arrowhead) and partial loss of cell adhesions in 3.9 and 7.8µg, b) severe apoptosis and pyknosis, membranous blubbing (arrow) Slight loss of adhesions in 15.6µg, c) moderate membranous blubbing, prominent of apoptotic bodies (arrow) beside moderate to a severe loss of adhesions (arrowhead) in 31.25µg, d) severe loss of adhesions with cytolysis, individuals pyknotic nucleoli (arrow) besides vacuolation cytoplasm's in 62.50µg (arrowhead), e and f) severe loss of cellularity and its adhesions with actuality stages of chromatin destruction from pyknotic chromatolysis (arrowheads) gradually showed in each 125, 250 and 500µg.

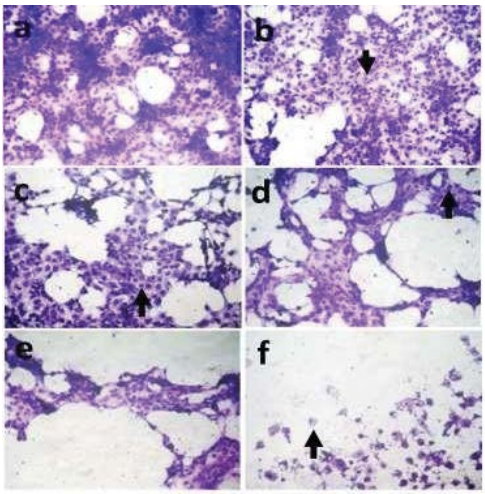

Plate 5: Photomicrograph (400X) of the different concentration Ethanol extract treatment A431 epidermal cell carcinoma showing a,b) mild lossof cellularity besides decrease in cells sizes with an unbroken sheet as well as mild apoptosis (arrow) in 15.6 µg marked than 3.9 and 7.8 µg, c,)gradually moderate loss of the cellularity and cell adhesions (50%) with marked condense chromatins (arrow) which surrounded by empty spaces in both 31.25µg. d,e) Severe loss of cellularity and cell adhesions(up to 70%) with marked apoptotic bodies (arrow) respectively In 62.5 and 125µg. f) completely loses of cell adhesions, massive cytoplasmic blubbing, and prominent pyknotic chromatin (arrow) gradually in 250 and 500 µg.

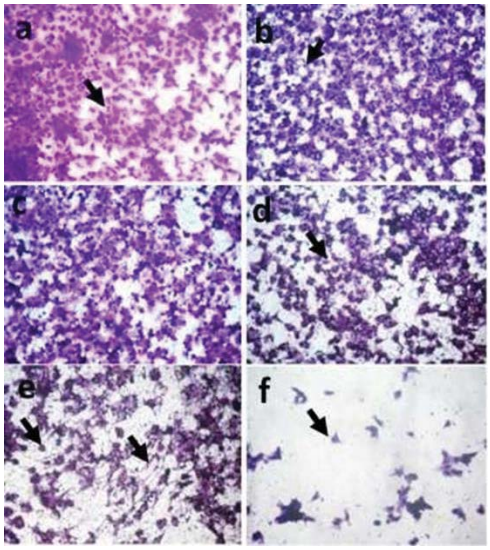

Plate 6: Photomicrograph (400X) of the different concentrations of Petroleum either treatment A431 epidermal cell carcinoma showing a,b) sheets of non- continuous neoplastic cells without any changes except the mild loss of cellularity, apoptotic and vacuolar cytoplasms in a few cells (arrows) 3.9, 7.8 and 15.6µg. c) Marked pyknotic chromatin and increased the loss of cellularity and cytoplasmic blubbing in 31.25. d) up to have of cells suffered from loss of adhesions with prominent blubbing cytoplasmic membrane (arrow) 62.25µg. e) loss of cellularity and appears destructed intercellular attachment bundles (arrow) 125 and 250 µg. f) complete loss of cellularity and still presents of remnant destructed cells (arrow) 500µg.

reported that acetone extract of turmeric has the greatest free radical scavenging activity followed by ethanolic extract than by the aqueous extract In this study, standard curcumin exhibits a strong antioxidant effect through free radical-scavenging activity. This result agrees with that of [25]. It has been found that curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals which are important to inhibit lipid peroxidation [26-32]. Showed [18] that the phenolic group in curcumin is the most important for scavenging radical reaction that enhances antioxidant properties to

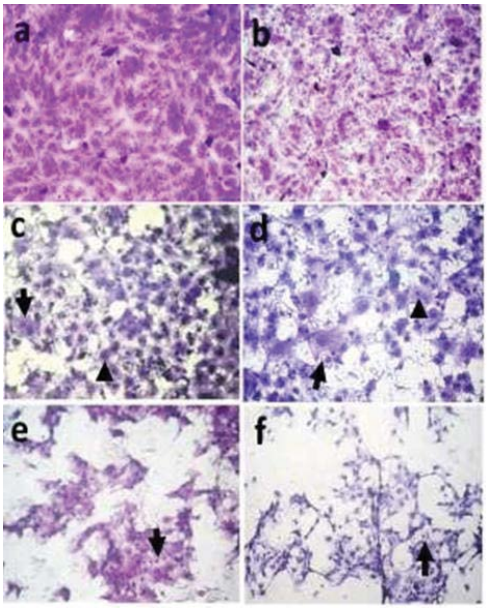

Plate 7: Photomicrograph (400X) of the differentconcentration Crude treatment A431 epidermal cell carcinoma showing a,b) contact seem like-a sized sheet with mitotic index (arrows) in each 3.9, 7.8, 15.6 and 31.25 µg, c,d) a cluster of separated, condense chromatins (arrowheads) and shrunken cells (early apoptosis) with still presence of large cells (arrows) in both 62.5 and 125 µg, e,f) Marked loss of the cellularity which characterized by small size, condense chromatins and prominent apoptotic bodies (arrows) in each 250 and 500µg.

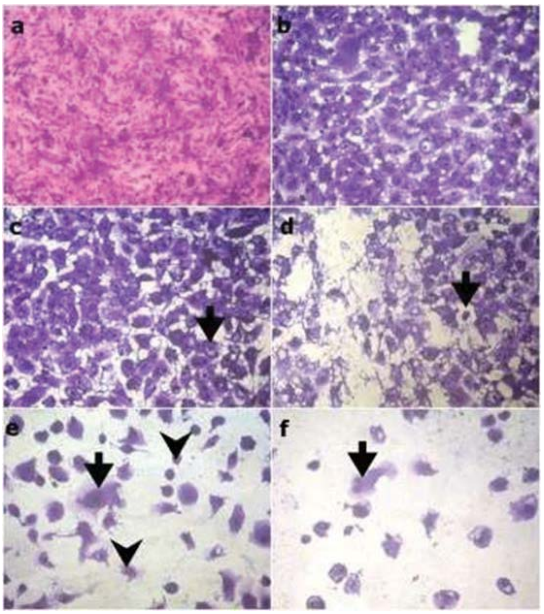

Plate 8: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration curcumin treatment showing

a) Attached continuous sheet of the carcinoma cells in the untreated control group.

b, c) Marked apoptotic features with condensation chromatins in numerous cells (arrow) and partial loss of cell adhesions increased gradually in each 1, 2, 3,9 and 7,8 µg treatment groups,

d) Marked loss of the cell adhesions and cellularity with chromatolysis (arrow) in 15.6 µg.

e, f) Severe loss of cell adhesions with still presence large cells (bezar) (arrows) besides membrane blebbing (arrow head) in both 31.25, 62.5, 125, 250 and 500 µg.

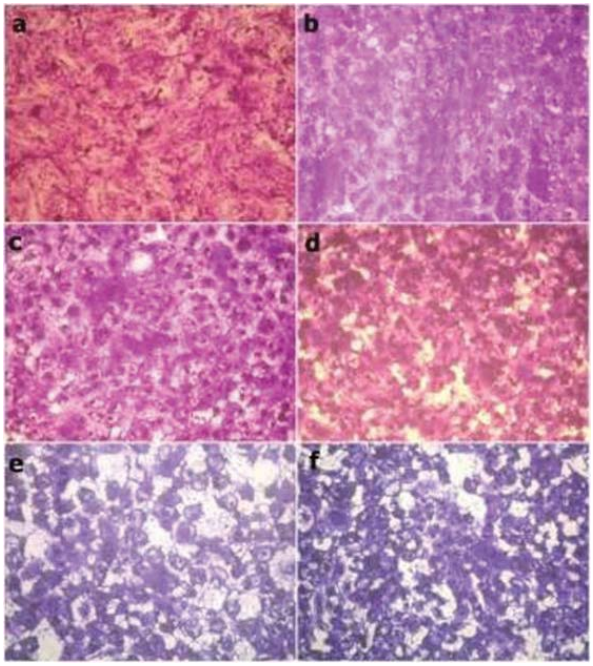

Plate 9: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration crude extract treatment showing

a) attached continuous sheet of the carcinoma cells without any apoptotic indexes in each 1, 2, 3.9, 7.8 and 15.7 µg groups.

b, c and d) early apoptotic signs in a few cells and slight loss of cell adhesions were observed in each 31.25, 62.5, 125µg treatment groups.

e, f) mild loss of the cell adhesions and vacuolation cytoplasms besides karyorrhexis in numerous cells in both 250 and 500 µg treated groups.

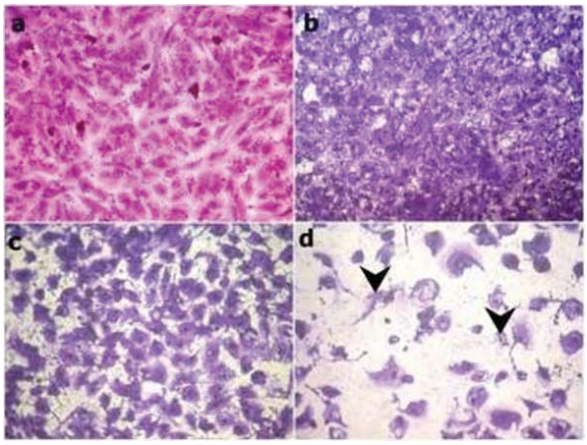

Plate 10: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration ethanol extract treatment showing

a) Continuous attached sheet of the carcinoma cells without any apoptotic indexes in each 1, 2 and 3.9 µg groups.

b) Severe pyknotic chromatins were common in majority sheet cancer cells with slight loss of cell adhesions in 15.6µg treatment group.

c) Mild to moderate loss of the cell adhesions and condense chromatins 31.25 and 62.5 µg treated groups,

d) Complete adhesions losses and marked membrane blebbing cells (arrow heads) in in 125, 250 and 500 µg treated groups.

a significant extent. Table 2 shows the antioxidant capacities of the different turmeric extracts, standard curcumin by FBAAC assay. Ethyl acetate showed the highest antioxidant capacity (2.26× 10-5), while pet. Ether showed the lowest antioxidant capacity (7.36 × 10-5).

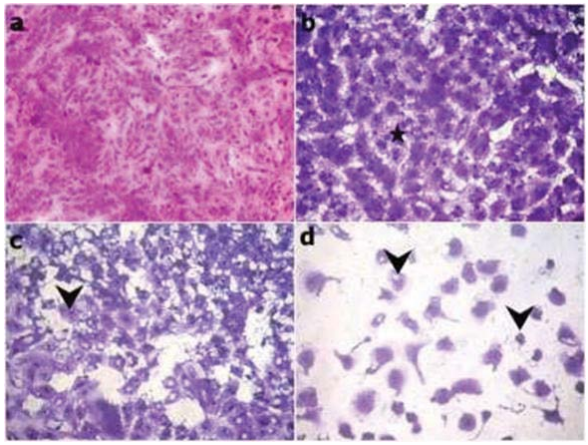

Plate 11: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration ethyl acetate extract treatment showing

a) Attached continuous sheet of the carcinoma cells without apoptotic indexes in each 1 and 2 µg groups.

b) Mild to moderate pyknotic chromatins without loss of cell adhesions in 3.9 and 7.8 µg treatment group.

c) Moderate to severe adhesion losses and apoptotic characters mainly condense and / or Karyolysis chromatins (arrow head) 15.6 and 31.25 µg treated groups.

d) Completely loss of adhesions and marked membrane blebbing cells (arrow heads) gradually in in each 62.5, 125, 250 and 500 µg treated groups.

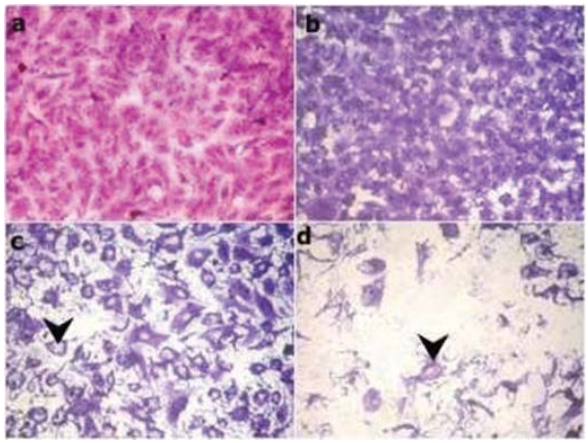

Plate 12: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration acetone treatment showing

a) Attached continuous sheet of the carcinoma cells without any apoptotic indexes in each 1, 2 and 3.9 µg groups,

b) Slight pyknotic chromatins with gradually loss of cell adhesions in each 7.8, 15.6, 31.25 and 62.5 µg treatment groups,

c) Loss of the cell adhesions and complete Karyolysis (absence chromatins) replaced by empty spaces 125.5 and 250 µg treated groups.

d) Marked destructed cells with still presence parts as a membrane blebbing (arrow head) in 500 µg treated group.

Conclusion

Our research demonstrated that extracts from the Yemeni cultivated Curcuma were cytotoxic against the skin, and lung human cell lines and induced apoptosis of the cells. The fraction ethyl acetate present in the tested extracts was probably responsible for the observed anti-tumor activity. These results rationalize the traditional medicinal application of root and rhizomes from the plants. This work presents the first report on the antiproliferative activity of the Yemeni cultivated turmeric plants in vitro.

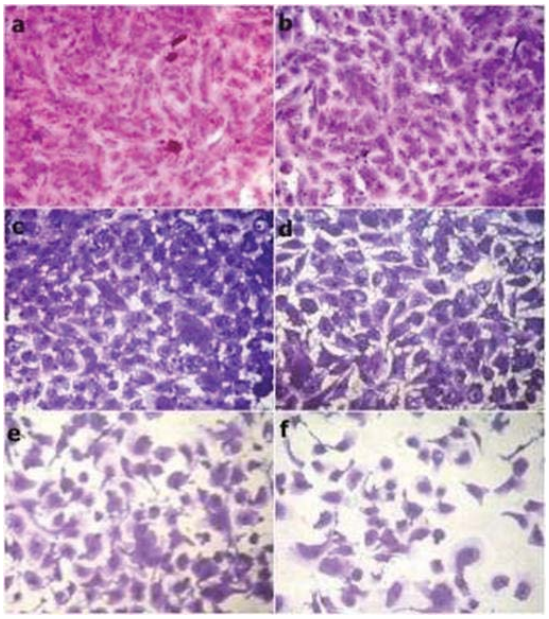

Plate 13: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration petroleum ether extract treatment showing

a,b) Attached continuous sheet of the carcinoma cells without apoptotic features in both 1, 2, 3.9 and 7.8 µg groups.

c,d) Marked pyknotic chromatins with mildly loss of cell adhesions with cytoplasmic micro- vacuolations in each 15.6, 31.25 and 62.5 µg treatment groups,

e,f) Loss of the cell adhesions in the majority examined fields and marked karyorrhexis 125.5. 250 and 500µg treated groups.

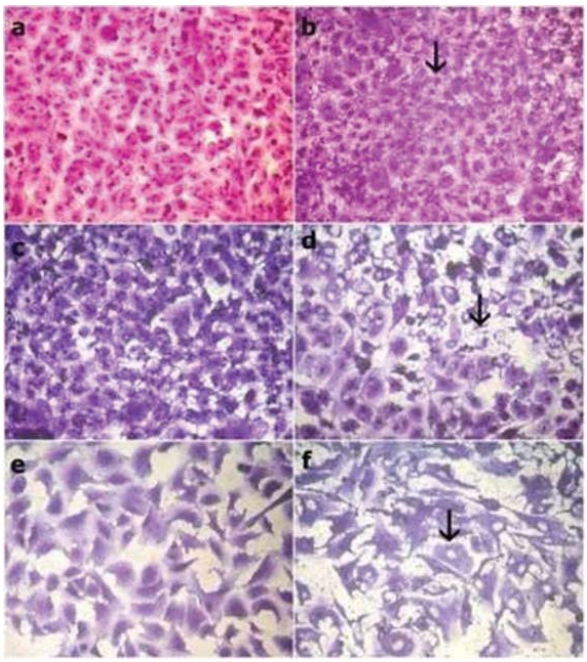

Plate 14: Representative Photomicrograph of the (400X magnification) A549 cells at different concentration aqueous extract treatment showing

a,b) Attached continuous sheet of the carcinoma cells without apoptotic features in 1and 2µg groups.

c,d) mild to moderate shrunken cells with pyknotic chromatins as well as mildly loss of cell adhesions with cytoplasmic macro and micro-vacuolations in each 3.9, 7.8, 15.6, 31.25 and 62.5 µg treatment groups,

e,f) Loss of the cell adhesions in the moderate examined fields and marked karyorrhexis with or without chromatolysis gradually in each 125.5. 250 and 500µg treated groups.

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Disclosure of conflict of interest

None.

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