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In Vitro Cytotoxicity Activity of Phytochemicals Isolated from Coriandrum sativum Linn in Selected Cell Lines

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Abstract: *Coriandrum sativum Linn (Dhanyaka; Apiaceae) is a widely used medicinal plant throughout India and popular in various Indigenous System of Medicine like Ayurveda, and Siddha. Plants have been used for medical purposes since the beginning of human history and are the basis of modern medicine. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives. Isolation of Umbelliferone belongs to sesquiterpene coumarin and β-Ionone to monoterpenoids are the first time record from this species.The main objective of the study was designed to isolate of Umbelliferone($C_9H_6O_3$) and Beta – Ionone($C_{13}H_{20}O$) from aerial part of the Coriandrum Sativum Linn by Column chromatography and structure of the compounds were identified by UV ,IR,^{1}H-NMR ,^{13}C-NMR and HMBC techniques respectively by direct comparison of the spectra with those reported in literature The MTT assay results for 24 hrs and 72 hrs incubation of RR 2386(Umbelliferone) and RR2387(β-Ionone) samples at the concentrations 1000,500,250,125 and 62.5% showed increased A-549 (Human Small Lung Cacinoma), HT-29 (Human Colon Carcinoma), HeLa (Human Cervical Carcinoma) RPMI (Human–Nasal Septum Cacinoma) and HEp G2 (Human Liver Carcinoma) Cells viability as the concentration got diluted. In the present study, the treatment with the 2386 (Umbelliferone) and RR2387(β-Ionone) suppressed the cell viability up to 62.5% at 1000 and 500 µg/ml concentrations when compared to the untreated cells. The results of our study showed that β-Ionone (73.50±3.5 HEpG2 cell line) has extremely high % cytotoxicity than Umbelliferone (44.34±5.2 against HeLa cell line)*

Keywords: *Beta – Ionone, Cell lines , Coriandrum sativum Linn, Cyctotoxicity, Umbelliferone.*

I. Introduction

Fruits and vegetables are natural medicines and have been used in our daily diet. Phytochemicals present in the dietary fruits and vegetables have anticancer properties [1].Plants have formed the basis of sophisticated traditional medicine systems that have been inexistence for thousands of years – such extensive dependence of human being on "Mother Nature" has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses. Plants have economic and environmental uses, depending on the natural characteristics. Some are consumed in human diet, while other species have medicinal values and still other species are good resource of minerals and vitamins[2].

Coriandrum sativum Linn. of family Apiaceae(Umbelliferae) is an annual herbaceous plant and is cultivated all over the world for its use not only in the indigenous medicines but also as one of the ingredients of all spicy foods especially of Pakistan and India.The plant is a rich source of essential oil and many of the researchers have almost concentrated on extraction,composition, biological activities, and use against various diseases of its crude extracts and essential oils. The different parts of this plant contain oleanane[3], α-pinene, limpnene, γ-terpinene, p-cymene, borneol, citronellol, camphor, geraniol, coriandrin, dihydrocoriandrin, coriandronsA-E, β-sitosterol, triacontane, triacontanol, tricosanol, psoralen, angelicin, β-sitosterol glucoside, butyl phthalides-neoenidilide, Z-ligustilide[4-8], and essential oils. Various parts of this plant such as seed, leaves, flower and fruit, possess Diuretic, Antioxidant Activity, Ant-diabetic Anti-convulsant activity, Sedative Hypnotic Activity, Anti-microbial Activity, Antimutagenic, Anthelmintic activity. Many of today's synthetic drugs originate from the plant kingdom. Herbal drugs are proved as effective as synthetic drugs with lesser side effects Coriander is one of a few savory plants, a potential source of phenolic compounds having biological activities.

Plants have been used for medical purposes since the beginning of human history and are the basis of modern medicine. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives. The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [9]. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year [10]Today, despite considerable efforts, cancer still

remains an aggressive killer worldwide. Moreover, during the last decade, novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development.Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs [11]. From the dawn of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. Natural products have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents [12, 13].

Cancer is a disease of the cells in the body. There are many different types of cell in the body, and many different types of cancer which arise from different types of cell. What all types of cancer have in common is that the cancer cells are abnormal and multiply out of control. Each cancer is thought to first start from one abnormal cell. What seems to happen is that certain vital genes which control how cells divide and multiply are damaged or altered. This makes the cell abnormal. If the abnormal cell survives it may multiply out of control into a cancerous (malignant) tumour.We all have a risk of developing cancer. Many cancers seem to develop for no apparent reason. However, certain risk factors are known to increase the chance that one or more of our cells will become abnormal and lead to cancer. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism).Indeed, the struggle to combat cancer is one of the greatest challenges of mankind [14].This growing trend indicates efficiency in the present cancer therapies which include surgical operation, radiotherapy and chemotherapy. Since the average survival rates have remained essentially unchanged despite of such aggressive treatments, there is a critical need for anticancer agents with higher efficacy and less side effects that can be acquired at an affordable cost [15].

II. Materials and Methods

2. 1 Plant Material

The seeds, the leaves, and whole parts of the Coriandrum sativum Linn were collected from the market of Dharasuram near Kumbakonam, Tamil Nadu state, India collected at appropriate time and bioactive constituents,and bioactivity. They were identified and authenticated by Prof. N.Ramakrishnan, Head and Associate Professor and voucher specimens (Department of Botany) and voucher specimens (GACBOT-170) were deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

2.2 General Experimental Procedure

UV spectra of the isolated compounds were recorded in methanol over a scanning range of 200-400 nm and λmax of compounds were determined. Spectra were recorded with a Shimadzu double beam (UV -1700 E Pharma spec) UV-VIS spectrophotometer. IR spectra were obtained using JASCO FT IR 5300 (Japan) in TlF disc and absorption peaks in terms of wave numbers (cm^{-1}) were noted for each constituent in KBr disc method. NMR spectra were acquired on Brucker supercon multi nuclei probe spectrophotometer (West Germany) at 400 MHz (^{1}H) and 100MHz (^{13}C).Chemical shifts were recorded as δ value (ppm). The spectra were observed on $CDCl_3$ and /or DMSO.D_6.

2.3 Extraction and Isolation

The shade dried Aerial part of Coriandrum sativum Linn (2 kg) was extracted exhaustively with methanol (2.5x2 L) at the 50°C for 48 h. After extraction total filtrate was concentrated by distilling off the solvent and evaporated to dryness (304 gm). It was made aqueous with distilled water in a separating funnel and further fractionated with series of organic solvents to obtain the fractions, viz.n- hexane fraction, chloroform fraction and ethyl acetate fraction. The resulting ethyl acetate extract was subjected silica gel column(60–120 mesh) using as a n-hexane/$CHCl_3$/MeOH as eluent . At uniform interval, the eluents (each of five ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC) (silica gel G 60 F254 TLC plates of E. Merck, layer thickness 0.2mm) using solvent system chloroform: methanol (90:10) and iodine vapour as detecting agent. Fractions eluted with chloroform: methanol (95:5) and 1-5 fraction of chloroform: methanol (90:10), which showed single spot on TLC (R_f value0.35) afforded the compound -I .Fractions (12.2 g) were combined and rechromatographed on a silica gel column ($CHCl_3$/MeOH, 95:5) which yield a single fraction. The fraction was dried by vacuum distilation and crystallization using ethylacetate yielded 2.820 g of yellowish-white crystalline solid, m.p 228- 234 ^{o} C ,which has a slight solubility in hot water, but high solubility in ethanol[16]Fraction 6-10 were combined and rechromatographed on a silica gel column (n-hexane/ethylacetate 90:10) showed single spot on TLC (R_f value0.92) afforded the compound -II. The fraction was dried by distilling off the solvent and evaporated to dryness and crystallization using ethylacetate yielded 2.142 g of light yellow liquid, b.p 126- 128 ^{o} C ,which has insoluble in water, but high solubility in ethanol.

2.4 Structural Identification

2.4.1 Structure of Compound- I

UV λmax nm (EtOH): 222 , 326. IR (KBr): √ cm^{-1}: 3175,1625 , 1566,1322,1237,1136, 836 , and 681cm^{-1}. ^{1}H- NMR spectrum (400 MHz, in DMSO-d_6) **δ** 6.18 (d , j= 7.2 Hz, H-3), **δ** 7.90 (d, j= 9.6 Hz H-5), **δ** 7.50(1H, d, j = 8.8 Hz, H-4), **δ** 6.77(1H, d, j =, 2.4 Hz, H-6) and **δ** 6.70 (1H, d, j = 2.0 Hz, H-8) ^{13}C-NMR (DMSO-d_6, 100 MHz): **δ** 102.08 (C-8), **δ** 111.23 (C-2), **δ** 111.32 (C-10), **δ** 113.12 (C-3), **δ** 129.71 (C-5), **δ** 144.56 (C-4), **δ** 155.40 (C-9), **δ** 160.55 (C-6), **δ** 161.20 (C-7) .

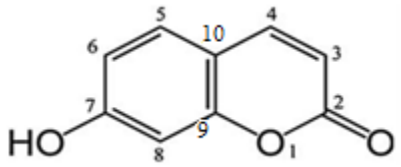

Fig. 1. Chemical structure of Umbelliferone

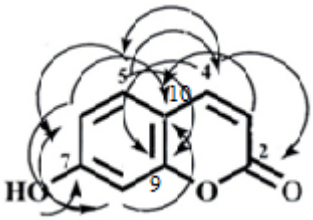

Fig. 2. HMBC Correlation of Umbelliferone

2.4.2 Structure of Compound- II

UV λmax nm (EtOH): 2224, 299. IR (TlF): √ cm^{-1}: 2930, 2965,1665 , 1604,1459,1359,1251,1191 and ^{1}H- NMR spectrum (400 MHz, in $CDCl_3$) **δ** 7.26 (1H d , j= 16.4 Hz, H-3), **δ** 6.10 (1H d, j= 16.4 Hz H-4), **δ** 2.29(3H, s, H-6), **δ** 2.08 (2H, t, j =, 6.4 Hz, H-8 **) ,** **δ** 1.62 (2H, p, j = 2.8 Hz, H-7), **δ** 1.47 (2H, t, j = 3.2 Hz, H-2), **δ** 1.23 (3H,3H, s,s, H-12,H-13), **δ** 1.08 **δ** 1.77 (3H, s, , H-11) . ^{13}C-NMR (100 MHz .in $CDCl_3$): **δ** 138.79 (C-6), **δ** 131.70 (C-5), **δ** 39.77 (C-2), **δ** 34.11 (C-1), **δ** 33.72 (C-4), **δ** 18.78 (C-3) , **δ** 28.68 (C-12), **δ** 28.84 (C-13), **δ** 21.41 (C-11), **δ** 198.51 (C-9), **δ** 142.68 (C-7), **δ** 132.19 (C-8), **δ** 27.49 (C-10). Compounds I-II were identified by UV ,IR, ^{1}H-NMR ,^{13}C-NMR and HMBC techniques. Compound I, and II were identified as Umbelliferone($C_9H_6O_3$) and Beta – Ionone($C_{13}H_{20}O$) respectively by direct comparison of the spectra with those reported in literature [16, 17].

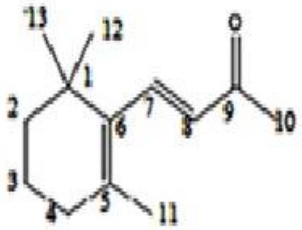

Fig. 3. Chemical structure of β-ionone

Fig. 4. HMBC Correlation of β-ionone

2.5 In Vitro Cytotoxicity Activity

2.5.1Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Minimum Essential Medium (MEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

2.5.2Cell lines and Culture medium

A-549 (Human Small Lung Cacinoma), HT-29 (Human Colon Carcinoma) ,HeLa (Human Cervical Carcinoma) RPMI (Human–Nasal Septum Cacinoma) and HEp G2 (Human Liver Carcinoma) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.5.3.Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of

1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

2.5.4Determination of cell viability by MTT Assay

The ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line [20,21].

$$\% \text{ Growth Inhibition} = 100 - \left[\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} X100 \right]$$

Table 1: Cyotoxic properties of RR 2386 (Umbelliferone) and RR2387(β-Ionone) against A-549 cell line .

Sl. No	Name of Test Compound	Test Conc. (%)	% Cyctotoxicity	CTC_{50} (µg/ml)
1	RR 2386 (Umbelliferone)	1000 500 250 125 62.5	33.33±2.2 24.51±2.9 24.95±7.3 24.37±4.3 23.25±3.9	>1000
2	RR2387 (β-Ionone)	1000 500 250 125 62.5	50.58 ±3.8 22.76±2.5 22.42±7.6 22.32±7.1 24.17±0.5	958±7.6

Table 2: Cyotoxic properties of RR 2386 (Umbelliferone) and RR2387(β-Ionone) against HT-29 cell line**.**

Sl. No	Name of Test Compound	Test Conc. (%)	% Cytotoxicity	CTC_{50} (µg/ml)
1	RR 2386 (Umbelliferone)	1000 500 250 125 62.5	32.51±5.0 12.62±2.2 8.93±2.9 8.98±4.0 4.14±2.8	>1000
2	RR2387 (β-Ionone)	1000 500 250 125 62.5	67.49±1.9 9.70±0.6 10.63±6.3 6.79±5.3 1.80±2.3	848±10.4

Table 3: Cyotoxic properties of RR 2386(Umbelliferone) and RR2387(β-Ionone) against HeLa cell line.

Sl. No	Name of Test Compound	Test Conc. (%)	% Cyctotoxicity	CTC_{50} (µg/ml)
1	RR 2386 (Umbelliferone)	1000 500 250 125 62.5	44.34±5.2 7.94±4.0 5.65±3.8 6.68±2.8 1.51±5.0	>1000
2	RR2387 (β-Ionone)	1000 500 250 125 62.5	71.88±3.4 15.74±5.5 10.06±6.0 10.89±6.4 9.50±4.9	821±10.4

Table 4 : Cyotoxic properties of RR 2386(Umbelliferone) and RR2387(β-Ionone) against RPMI cell line.

Sl. No	Name of Test Compound	Test Conc. (%)	% Cyctotoxicity	CTC_{50} (µg/ml)
1	RR 2386 (Umbelliferone)	1000 500 250 125 62.5	10.25±4.4 5.15±3.6 5.90±3.5 4.18±3.3 1.60±0.9	>1000
2	RR2387 (β-Ionone)	1000 500 250 125 62.5	31.84±2.1 16.49±8.2 3.38±4.4 7.04±3.0 9.16±1.9	>1000

Table 5: Cyotoxic properties of RR 2386(Umbelliferone) and RR2387(β-Ionone) against HEpG2 cell line.

Sl. No	Name of Test Compound	Test Conc. (%)	% Cyctotoxicity	CTC_{50} (µg/ml)
1	RR 2386 (Umbelliferone)	1000 500 250 125 62.5	35.49±4.6 10.52±3.4 6.52±1.3 1.85±1.7 1.45±1.8	>1000
2	RR2387 (β-Ionone)	1000 500 250 125 62.5	73.50±3.5 56.92±2.9 26.37±9.8 6.26±1.9 4.67±0.8	458.33±7.6

2.6 Graphical Representation

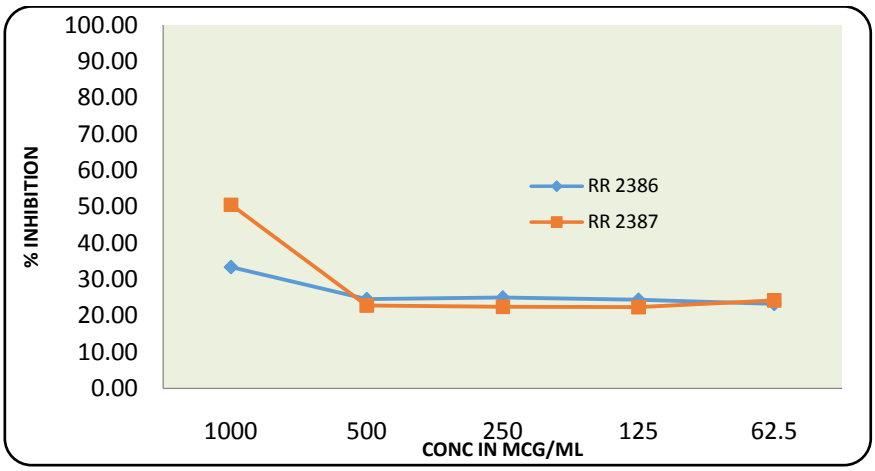

Fig. 5: Cyotoxic effect of the sample RR RR 2386 (Umbelliferone) and RR2387 (β-Ionone) against A-549 cell line .

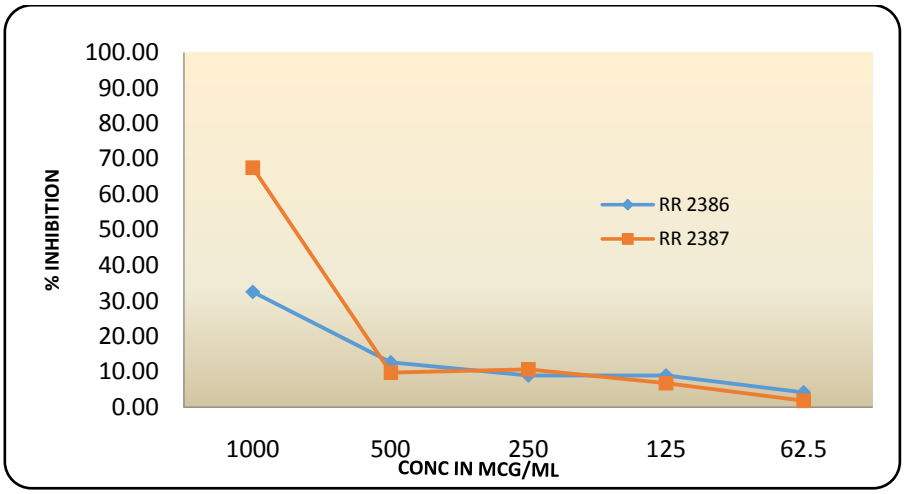

Fig. 6: Cycotoxic effect of the sample 2386 (Umbelliferone) and RR2387 (β-Ionone)against HT-29 cell line**.**

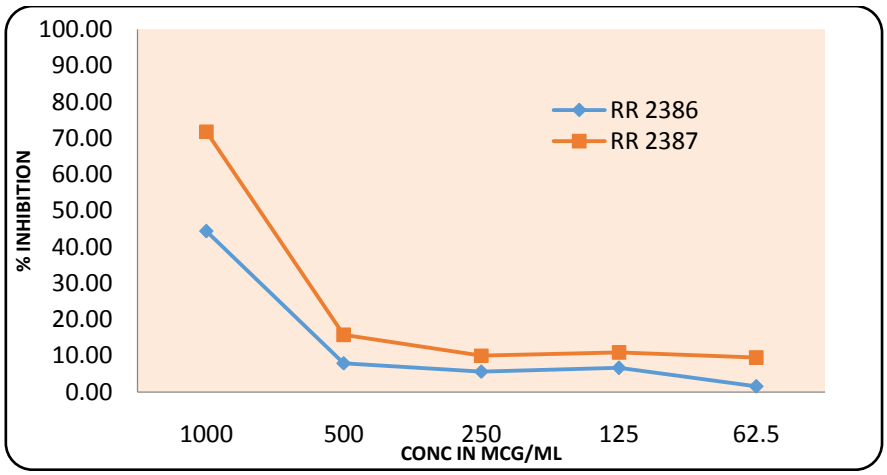

Fig. 7: Cyotoxic effect of the sample RR 2386(Umbelliferone) and RR2387(β-Ionone) against HeLa cell line.

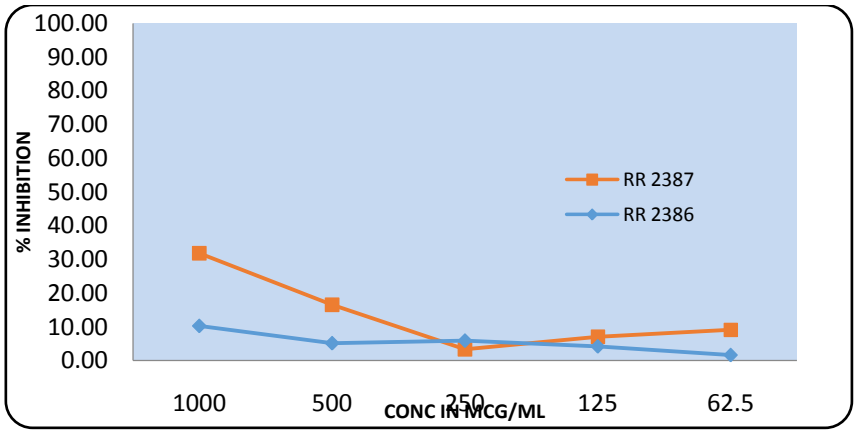

Fig. 8: Cyotoxic effect of the sample RR 2386 (Umbelliferone) and 2387 (β-Ionone) on HeLa Cell line . (β-Ionone) on RPMI Cell line .

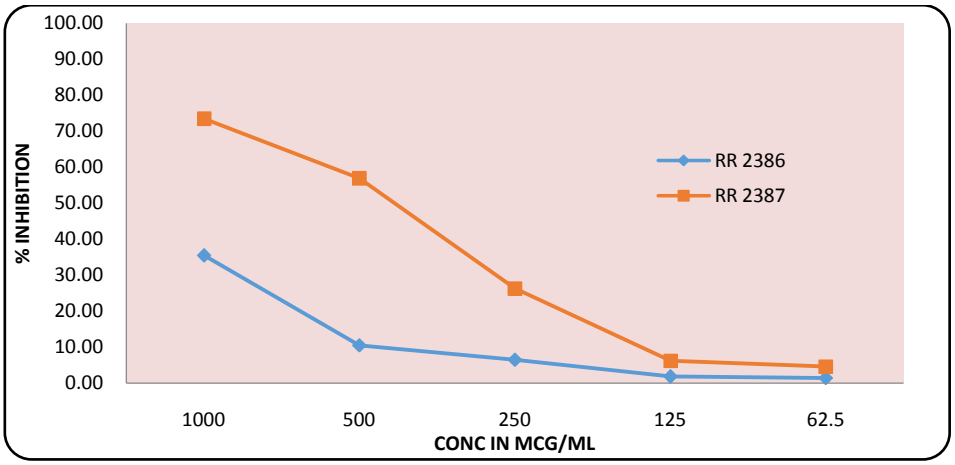

Fig. 9: Cytotoxic effect of the sample RR 2386(Umbelliferone) RR2387(β-Ionone) against HEpG2 cell line

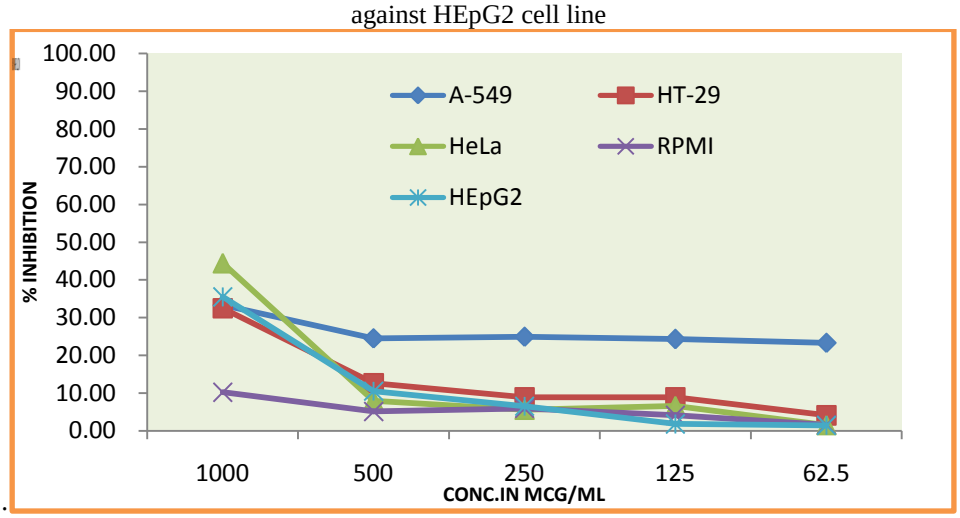

Fig. 10: Shows A-549, HT-29, HeLa, RPMI and HEpG2cell lines after 24 h of incubation ,RR 2386(Umbelliferone)1000,500 ,250,125,62.5 µg/ml .

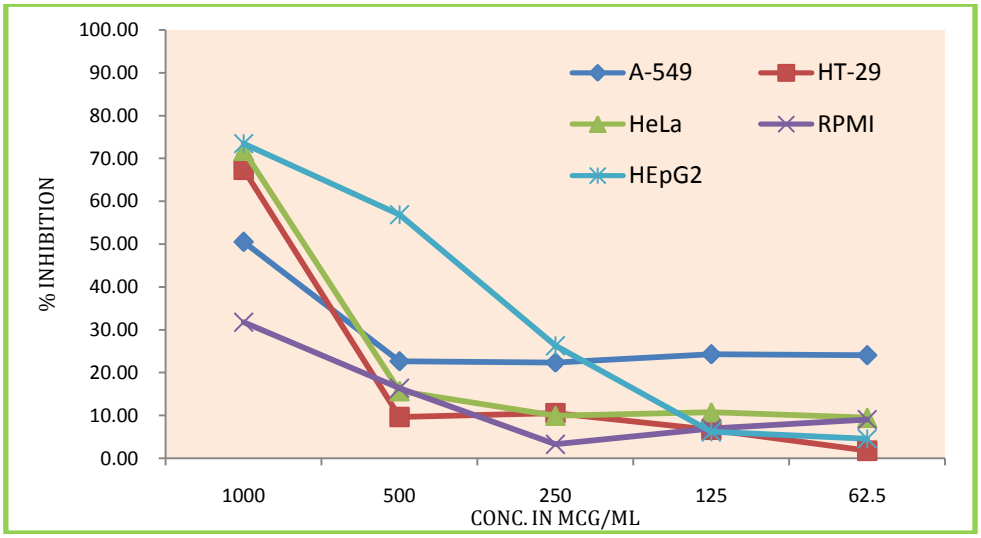

Fig. 11: Shows A-549, HT-29, HeLa , RPMI and HEpG2 cell lines after 24 h of incubation2387 (β-Ionone) 1000,500 ,250,125,62.5 µg/ml .

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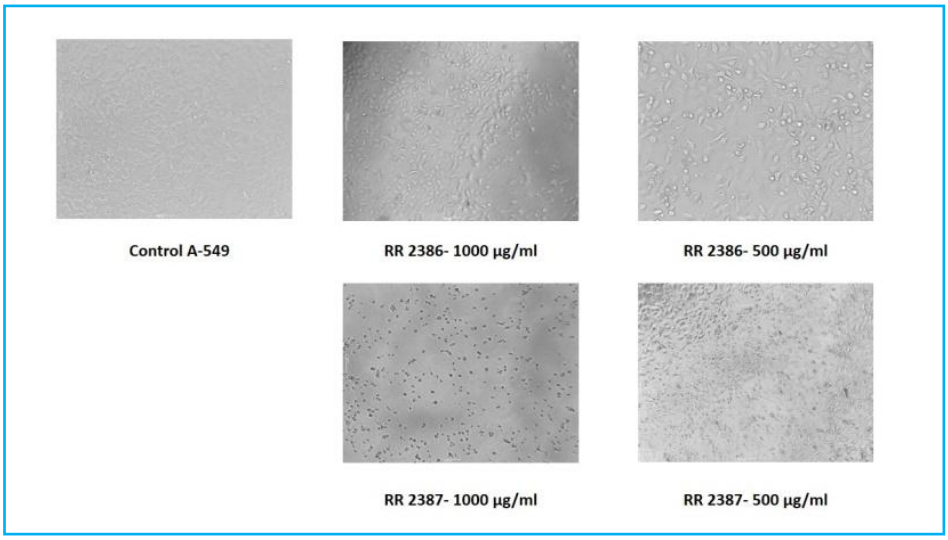

Fig. 12: Shows A-549 cell lines after 24 h of incubation Control, RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) 1000, 1000, 500 µg/ml 500 µg/ml.

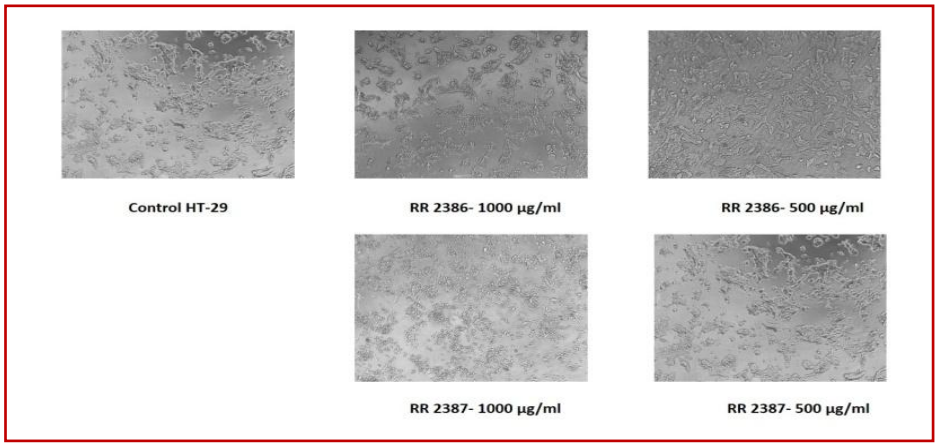

Fig. 13: Shows HT-29 cell lines after 24 h of incubation Control, HT-29, ,RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) 1000, 1000, 500 µg/ml 500 µg/ml

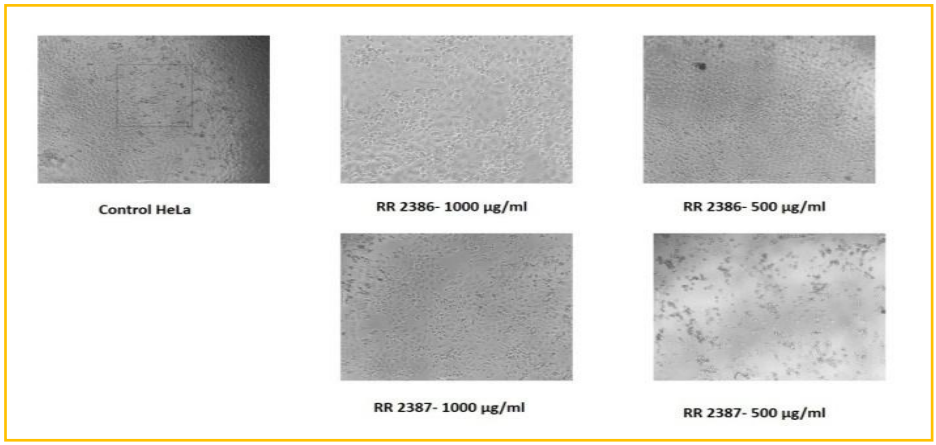

Fig. 14: Shows HeLa cell lines after 24 h of incubation Control, HeLa , ,RR 2386

(Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) 1000, 1000, 500 µg/ml 500 µg/ml.

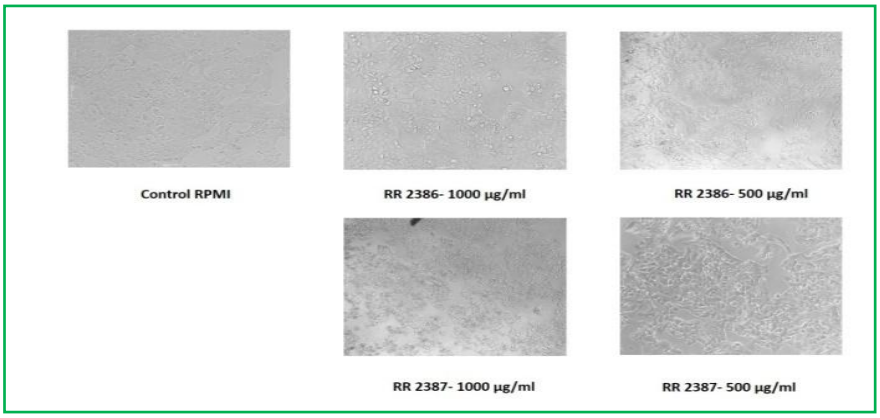

Fig. 15: Shows RPMI Cell line after 24 h of incubation Control, RPMI, ,RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) 1000, 1000, 500 µg/ml 500 µg/ml .

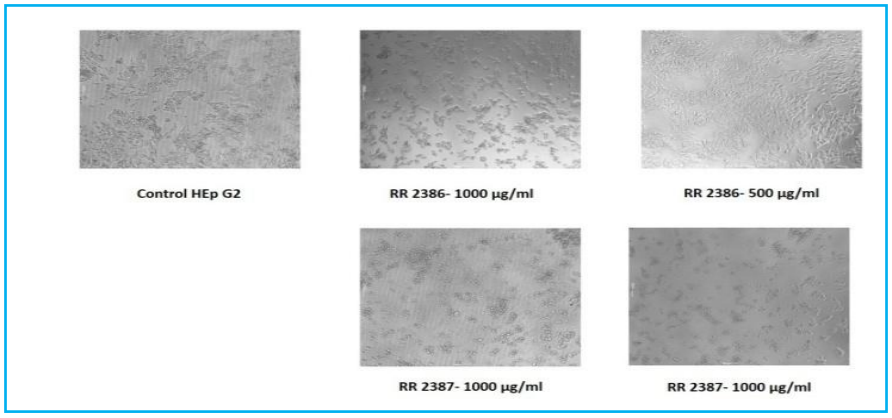

Fig. 16: Shows HEpG2 cell lines after 24 h of incubation Control, HEpG2 ,RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) RR 2386 (Umbelliferone) 1000,500 µg/ml , RR2387(β-Ionone) 1000, 1000, 500 µg/ml 500 µg/ml.

III. Result and Discussion

3.1 Structure of Umbelliferone

The isolation of organic compound from Coriandrum sativum Linn was subjected to column chromatographic separation analysis. Its UV spectrum in neutral (EtOH) displayed an absorption bands at 222 (-C=O) nm and 326nm (-OH). Compound exhibited UV absorption bands at 330 nm indicating Coumarin derivative. Thr IR absorption showed the absorption bands of hydroxyl (-OH) at 3175 cm^{-1},carbonyl (-C=O) stretching at 1625cm^{-1}.The position of the absorption bands and the shape of the spectra were in complete agreement with the reference Umbelliferone. The compound exhibited the expected molecular ion peak (M^+ = 162) which is also the base peak of the spectrum. The main fragmentation process is the loss of one molecule of carbon monoxide leading to the peak m / e = 134 followed by a loss of a second molecule of CO or a formyl radical (CHO) giving the peaks at m / e = 106 and m / e = 105 respectively[17].The ^{1}H-NMR spectrum showed five signals in the aromatic region. AB type doublet of α, β- olefinic protons at **δ** 6.18 (H-3) and **δ** 7.50(H-4). The tri substituted benzene ring was proposed from an ABX signal of aromatic protons H-5,H-6 and H-8 at **δ** 7.90 (H-5), **δ** 6.77(H-6) and **δ** 6.70 (H-8) respectively. The decoupled ^{13}C-NMR spectrum revealed nine signals indicating presence of nine types of carbons. ^{13}C-NMR (DMSO-d_6, 100 MHz): spectrum shows five signals at **δ** 102.08 (C-8),111.23 (C-6), 113.12 (C-3), 129.71 (C-5) 144.56 (C-4) and shows the presence of four quaternary carbons. One signal at **δ** 161.20 (C-2) was described to carbonyl function (C-2) of Coumarin derivative. Another downfield intense signal at **δ** 160.55(C-7) was indicative of hydroxyl substitution at C-7 position and two others were **δ** 111.32 (C-10) and **δ** 155.40 (C-9). The HMBC correlation of H-4 (**δ** 7.90) to C-5 (**δ** 129.71) and H-5(**δ** 7.50) to C-4(**δ** 144.56) confirmed the aromatic proton H-5 peri to olefinic proton H-4. A hydroxyl was assigned

for 7-OH due to the HMBC correlations of H-5 and 7-OH to C-2(**δ** 160.55)[18]. Coumarins are classified as a member of the benzopyrone family, all of which consist of a benzene ring joined to a pyrone ring , of which the flavonoids are principal members the isolated component was confirmed as Umbelliferone.

3.2 Structure of β-Ionone

The UV spectrum showed absorption band at λmax 224 nm (-C=O) and 299 nm (-CH=CH-). IR spectrum of the compound showed characteristic bands at 1604(olefinic -CH=CH-) and a strong absorption band at 1665cm^{-1} for a α, β- unsaturated carbonyl group. The presence of β-carotene skeleton was also supported by the mass spectrum of the compound, which showed prominent fragments ion peak characteristic of molecular ion peak (M^+ = 177) which is also the base peak of the spectrum. The main fragmentation process is the loss of one molecule of 2-propene(m / e = 56) leading to the peak m / e = 92 , in agreement with the molecular formula $C_{13}H_{20}O$ .The ^{1}H-NMR spectrum showed the presence of an olefinic protons resonating at δ 6.10(1H) which was assigned to H-7 and H-8 respectively. The same spectrum also showed singlet for four methyls in the region δ 1.23-2.29 and two triplet signals in the region **δ** 1.47-2.08 were assigned to H-2 and H-4. The ^{13}C-NMR spectrum with the signals in the region δ 21.41- 28.68 ppm for four methyl groups to C-1, C-11, C-12 and,C-13 and four quaternary carbons in the region of δ 131.70-198.50. One signal at **δ** 198.51 (C-9) was described to α, β- unsaturated carbonyl group of cyclohexyl derivative[18]. Three signals at **δ** 18.78-39.77 were showed presence of secondary carbons and the olefinic carbons were in the region at **δ** 132.19-142.68.The spectrum of β-ionone shows that H-4 is coupled to H-8. There are two long range (4 bonds) couplings between H-4 and 3-CH_2 and 2-CH_3 protons. The cross peaks are small since the couplings are weak. The coupling of 3-CH_2 to 4-CH_2, and that of 3-CH_2 to 5-CH_2 are clearly observed. This was confirmed by the HMBC experiment which showed correlations between the oxygenated methine signal H-2 (2H, t, j = 3.2 Hz) and C-1, C-4, C-11 and C-12. The relative configuration of compound –II was deduced on the basis of the J values in the ^{1}H NMR spectrum .The coupling constants of H-3 (1H d , j= 16.4 Hz, H-3 and H-2 (2H, t, j = 3.2 Hz) and H-4 (1H d, j= 16.4 Hz) in the ^{1}H NMR spectrum were almost same as those of H-3/H-4 and H-3/H-2 in Indaquassin B. Moreover, NOESY correlations between H-3 (**δ** 7.26) and H-4 (**δ** 6.10) and no correlations between H-2 and H-3 and H-4 were observed .In the spectrum on the following slide, the olefinic protons H-7 and H-8 show NOE (cross peaks) with all -CH_3 groups because the side chain of β-Ionone is flexible and does not have a fixed conformation[19].

3.3 Cell Growth Profile in MTT Assay

The MTT assay results for 24 hrs and 72 hrs incubation of RR 2386(Umbelliferone) and RR2387(β-Ionone) samples at the concentrations 1000,500,250,125 and 62.5% showed increased A-549 (Human Small Lung Cacinoma), HT-29 (Human Colon Carcinoma) ,HeLa (Human Cervical Carcinoma) RPMI (Human-Nasal Septum Cacinoma) and HEpG2 (Human Liver Carcinoma) cell viability as the concentration got diluted. In the present study, the treatment with the R2386 (Umbelliferone) and RR2387(β-Ionone) suppressed the cell viability up to 62.5% at 500 µg/ml concentrations when compared to the untreated cells. MTT assay is a rapid and high accuracy colorimetric approach that widely used to determine cell growth and cell cytotoxicity, particularly in the development of new drug. It measures cell membrane integrity by determining mitochondrial activity through enzymatic reaction on the reduction of MTT to formazan. The profile of cell growth after treated with Umbelliferone and β-Ionone are presented in Fig. 15 and 16 respectively. From these figures, it was found that both samples only showed a significant reduction in the number of viable cells at the concentration higher than 1000 treatment with β-Ionone(73.50±3.5 **)** was more than Umbelliferone (44.34±5.2)against HEpG2 cell lines. Therefore, the CTC_{50} value were 458.33 and >1000 µ g /ml.Isolated compounds were exhibited dose- and time-dependent killing capabilities in various human derived tumour cell lines and primary cultures established from patients' biopsies. The killing activity was specific toward tumour cells, as the compounds had no effect on primary cultures of healthy human cells. Cell death caused by the isolated compounds was via apoptosis. In addition, the pattern of tumour promotion was slower in mice treated with β-Ionone compared with the Umbelliferone. Therefore, β-Ionone might be valuable as a cancer chemo preventive agent than Umbelliferone. Cancer has developed multiple mechanisms to escape regulated growth and avoid apoptosis Cancer chemoprevention is defined as inhibiting, delaying or reversing the carcinogenic process using non-toxic chemicals, and is considered to be a promising strategy for controlling cancer progression. A variety of chemical compounds have been reported to protect against chemical carcinogenesis and thus, are considered to be cancer chemo preventive agents. Among these, RR 2386 (Umbelliferone) and RR2387 (β-Ionone) are promising phytochemical agents that have attracted interest due to its cancer chemo preventive activity in multistage carcinogenesis.

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4. Cytotoxicity activity:

In-Vitro confirmation of their toxicity on A-549, HT-29, HeLa, RPMI and HEpG2 cell lines. Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique. The cytotoxicity activity is carried out by using MTT assay. Percentage cell viability of cell lines was carried out by using trypan blue dye exclusion technique. Results were tabulated in Table 1-5. The percentage growth inhibition was found to be increasing with increasing concentration of test compounds (Fig. 10 and Fig. 11). It was found that the % growth inhibition increasing with increasing concentration steadily up to 62.5 mg/ml on each cell lines. Now overall study evaluate that Umbelliferone has potential activity on HeLa cell (44.34±5.2) and less effect on HEpG2 cell (1.45±1.8). So these drugs have considerable anticancer activity on cervical cancer. MTT assay also shows significant effect of β-Ionone on HT-29(73.50±+3.5) cell and had significant value on HEp G2 activity on colon cancer [22].

This paper describes a comprehensive In Vitro cytotoxicity assessment of Umbelliferone and β-Ionone on A-549 cells, a human lung carcinoma epithelial cell line. In this study we found that in general, greater Umbelliferone toxicity was observed in the absence of serum, although this trend was not as marked as that observed β-ionone exposures [23]. The results show that as concentration increased, the proliferation of the HT-29 cells was markedly inhibited in a dose-and time-dependent manner. The present results demonstrated that Umbelliferone and β-Ionone were able to suppress HT-29 cell proliferation induce apoptosis and adjuvant therapeutic application in the treatment of human colon cancer [24]. Morphological analysis of the mode of HeLa cell death, together with the cell cycle analysis, showed that the treatment of HeLa cells with higher concentrations of the examined extracts induced apoptotic cell death .The RPMI 2650 cells form a polarized epithelium resembling nasal mucosa. However, different culture conditions have a significant effect on cell ultrastructure, barrier integrity, and gene expression, and should be considered when using this cell line as an in vitro model for drug permeability studies and screening of nasal drug candidates. Hepatoblastoma (HB) is an embryonal malignancy of hepatocellular origin and the most common primary liver tumor of childhood, often presenting in the first years of life . Pediatric hepatocellular carcinoma is a rare tumor associated with much worse prognosis and aggressive behavior than HB and significantly less responsive to chemotherapy.

Umbelliferone and ß- Ionone were found that high anticancer activity on HeLa cell line has shown in Fig.-7. The experiments demonstrate that these compounds did not inhibit the growth of RPMI cell line up to 250mcg/ml (Fig.10). A number of flavonoids and poly phenolic were shown to inhibit invasion In Vitro and artificial metastasis In Vivo. Some anti invasive flavonoids appear to act via targets in normal tissues confronted by tumer cells. These compounds take anti invasive congeners were found that were selectively cyctotoxic for the A-549, HT-29, HeLa, RPMI and HEpG2cell lines (Fig.10 & 11). In Fig.9 explained ß- Ionone is more inhibited while increase the concentration than Umbelliferone and fig. 13 showed both Umbelliferone and ß-Ionone having more inhibition rate with concentration. To establish the inhibition of Umbelliferone and ß-

Ionone treated with various cell lines (A-549, HT-29, HeLa, RPMI and HEpG2cell lines) after 24 h of incubation that for the different concentration that it explains Umbelliferone with RPMI have more inhibition than other cell lines . Similarly ß -Ionone with HEpG2 had more inhibition than others treated cells where as RPMI cell line has very less inhibition rate. On comparing the activity of Umbelliferone & ß- Ionone with various cell lines ß- Ionone showed more active than Umbelliferone.

IV. Conclusion

The compounds Umbelliferone and β-Ionone were isolated from Coriandrum sativum Linn and were characterized by UV, IR, ^{1}H NMR ,^{13}C NMR and HMBC. Human have always relied on the nature to survive,which has been the main source of food, clothing and remedies. Natural products have been regarded as important source that could produce potential chemotherapeutic agents . In particular anti cancer therapy have special place and recently some newer chemotherapeutic agents available for uses in a clinical setting include paclitaxel, vincristine, podophyllotoxin and camptothecin. Obviously natural products are extremely important as a source of medicinal agents. In conclusion, the results of the present study indicate that both of RR 2386(Umbelliferone) and RR2387(β-Ionone) treatment induces dose-dependent responses in A-549 human non-small cell lung carcinoma cells that involve the up-regulation of a large group of genes associated with cell growth-related signaling pathways and the down regulation of genes associated with metabolic function. This reciprocal regulatory mechanism may provide clues to further our understanding of the mechanism driving growth inhibition in human cancer cells treated with the RR 2386(Umbelliferone) and RR2387(β-ionone), especially in A-549 human lung cancer cells. examination of In Vitro cytotoxicity revealed that compounds- 1 and 2 might be a significant source of novel promising anticancer compounds in view of their pronounced cytotoxic activities against A-549 (Human Small Lung Cacinoma), HT-29 (Human Colon Carcinoma) , HeLa (Human Cervical Carcinoma) RPMI (Human-Nasal Septum Cacinoma) and HEpG2 (Human Liver Carcinoma). In conclusion this work provides a gene signature of human hepatoma cells showing genes that change their expression as a consequence of liver cancer in the absence of any genetic mutations or viral infection, evidences new differently expressed genes found in our signature compared to previous published studies and suggests some genes on which to focus future studies to understand if they can be used to improve the HCC prognosis/diagnosis.

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