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Anti-cancer Properties of Dietary Supplement CELNORM against Colon and Lung Cancer: An *in vitro* preliminary study

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Abstract: Cancer is a complex disease characterized by a cascade of events that culminate in the accumulation of several genetic alterations. Because of the high incidence and mortality rate, scientists began seeking novel medications that were harmful to cancer cells but not to healthy cells. This study aimed to investigate the effect of CELNORM and its various components individually in colon and lung cancer cells. Based on the LD50 value, colon and lung cancer cells were treated with two different dosages of curcumin, pepper, carrot and cucumber extracts, individually and CELNORM (formulation with all four ingredients). The cytotoxicity of each compound has been checked through morphological analysis and cell viability assay by using the CCK8 kit and statistically analyzed. qPCR-based Gene expression study has also been done to further validate the anti-cancerous properties of the compounds. Curcumin extract decreased cancer cell growth, as evidenced by cytotoxicity and morphological analyses, but pepper, carrot, and cucumber extracts showed a less significant reduction in cancer cell growth. CELNORM, on the other hand, had the highest significant effect in suppressing cell proliferation, indicating the novel health supplement CELNORM renders more efficacy than individual components. Gene expression analysis also verifies the anti-cancerous properties of CELNORM, though some gene expressions are not in the expected line. The morphological analysis and cell viability result has shown the efficacy of the CELNORM over the individual compounds of the CELNORM and gene expression analysis of proliferative genes such as *Cyclin D, CDK6, PCNA* and *BCL-2* further validates the anti-cancerous properties of CELNORM and all four components.

Introduction

Cancer has emerged as one of the biggest threats to humanity over the years with an estimated 9.6 million annual deaths globally, and predictions show that by 2030, the statistic will rise to 13 million deaths per year (Jemal et al., 1999; GLOBOCAN, 2018). Colon cancer is the second most common cause of cancer-related death which typically begins as a polyp on the epithelial lining of the intestinal tract and gradually progresses into cancer. It disrupts the intestinal linings and invades into nearby lymph nodes and can metastasize into different tissue locations such as the liver, pancreas and kidney etc. (Sakr et al., 2022). Lung cancer is the most common type of cancer detected world-wide. Fifteen percent of all lung cancers are Small-Cell Lung Cancer (SCLC) which happens mostly due to smoking habit. In U.S. alone, the annual death due to lung cancer is around 0.13 million (Yuan et al., 2022; Kirubhananda et al., 2023). Due to the increasing incidence and mortality rates, researchers started looking for new therapeutics that can combat cancer with the minimum level of side effects (Krasteva and Georgieva, 2022). The conventional treatment for

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cancer such as chemotherapy, radiotherapy, bone marrow transplantation etc. have shown limited control over cancer and comes with several side effects to the healthier tissues (Gunjal et al., 2015; Hussein et al., 2023; Naik et al., 2022; Yang et al., 2019). Chemotherapy, sometimes, may cause taste Alterations, oral mucositis, and fatigue along with other side effects like anaphylaxis, cytopenias, hepatotoxicity, cytotoxicity, cardiotoxicity etc. (Jacobsen and Stein, 1999; Naidu et al., 2004; Oun et al., 2018; Zabernigg et al., 2010). There are evident cases of Parkinson-like syndrome and Raynaud's Phenomenon, which occur due to extreme cytotoxicity of chemotherapeutics (Vogelzang et al., 1981). Further, some genes and microRNAs prove to be targets of gene silencing through RNA interference and might serve as effective therapeutic targets in understanding and treating colorectal cancer (Ganesan et al., 2022). Additionally, to vividly studying the cellular landscape of tumour microenvironment of colon cancer and lung cancer might aid in future perspectives for designing therapeutic regimens (Banerjee et al., 2021). The regular cytotoxic drugs are generally toxic to tumour cells as well as to the normal cells in the vicinity of the cancer tissue. To minimize the hazard associated with traditional therapeutics, natural compounds as alternative supplements can be used. More than a hundred novel pharmaceuticals are being tested in clinical trials, most of which are anti-cancerous and anti-infective drugs (Butler, 2008; Newman and Cragg, 2007). According to the World Health Organization (WHO), increasing the consumption of natural substances can impact crucial molecular signalling cascades that eventually stop cancer cells from proliferating and trigger apoptosis. These alternative therapies could prevent one-third of all cancer deaths (Bode and Zigang, 2011; Lee et al., 2011).

Curcumin's anti-cancer properties show its ability to inhibit tumour cell proliferation, down-regulate transcription factors NF-B, AP-1, and Egr-1, and the expression of *COX2, LOX, NOS, MMP-9, uPA, TNF genes*, chemokines, cell surface adhesion molecules, and *CYCLIN-D1*; growth factor receptors (such as EGFR) and it has been discovered to be a potent antioxidant and anti-inflammatory agent in a variety of systems (Aggarwal et al., 2006; Rao, 2007; Saja et al., 2007; Zhou et al., 2012). In addition, according to studies, curcumin is pharmacologically safe in reducing tumour formation, progression, and metastasis (Aggarwal et al., 2003). Curcumin has been reported to reduce the colon cancer growth by downregulating genes such as *BCL-2, Bcl-xl, COX-2 genes* (Ojo et al., 2022). Curcumin is also reported to act on lung cancer cell line A549 where it is found to be down regulator of VEGF and NF-kB pathways, important for tumour growth and viability (Li et al., 2018). Large curcumin concentration in plasma and tissues cannot be achieved or maintained even after being supplemented with plentiful amounts due to low bio-availability. These represent a substantial barrier to the drug's clinical development and demonstrate the limitation of curcumin's medical applications (López-Lázaro, 2008). As a result, it prompted a systematic search for various combinations of plant components in a large number of conceivable combinations to enhance the efficacy and bioavailability of curcumin. As a result, the combined treatment has a synergistic effect and may be more effective than a single compound intervention with low doses and minimal side effects.

The anti-cancerous properties of the compounds derived from *Piper nigrum* (black pepper), particularly Piperine were attributed to broad-spectrum bioactivities (Deng et al., 2016). The carrot (*Daucus carota*) is rich in beneficial compounds, such as bioactive substances with antioxidant properties like Carotenoid, phenolic compound, ascorbic acid etc. (Sharma & Karki, 2012). *Cucumis sativus L* (cucumber) has been used to treat headaches, burning, and insomnia. The seeds are cooling and diuretic and the fruit juice is used as a nutritious and demulcent in anti-acne creams (Saeed and Waheed, 2017).

Our previous study has shown combinational treatments of multiple extracts are beneficial when used as a treatment for breast cancer cell lines (Sathya et al., 2020), apart from this, the direct prevention or elimination of harmful factors (such as cancer cells or pathogens), as well as the synergistic stimulation of defences or healing processes of the human body, is also the focus of combinational therapies. Thus, cancer can be lessened by increasing the consumption of natural compounds that might alter important molecular signalling cascades, ultimately inhibiting cancer cells' proliferation and inducing apoptosis (Surh, 2003).

This study aimed to investigate the synergistic effect of 1) curcumin, 2) the extract of pepper, 3) Carrot, and 4) cucumber individually and in combinations that include the sets as follows- a) 'Curcumin and Pepper extract'; b) 'Pepper and Carrot extract'; c) 'Carrot and Cucumber extract'; 'd) Cucumber and Curcumin' and CELNORM– a novel formulation (combination of all the extract mentioned in a different proportions) on the colon cancer and lung cancer cell lines.

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Methods

The CELNORM was provided by RETORT Pharmaceuticals Pvt. Ltd., Chennai and the synthesis or preparation of these extracts is not related to the study and personal involved in preparation of the CELNORM is not involved in testing its efficacy as an anti-cancer drug. Researcher conducting the experiments was completely unaware of the composition of the CELNORM and the experiments were performed double-blinded.

Cell line culture expansion

HCT116 (colon cancer cells) and A549 (lung cancer cells) cell lines were purchased from the National Centre for Cell Science (NCCS), Pune, India. Both the cells are adherent cells and were cultured- as per the methods described by Ganesan et al. (2022) and Jothimani et al. (2022). The cells were expanded in standard DMEM (low glucose, Glutamax supplement) (Cat. No. 10567014, GIBCO, USA) supplemented with 10% FBS,1% Antibiotic-Antimycotic at 37ºC.

Preparation of CELNORM and treatment with CELNORM and its constituents

CELNORM was manufactured and supplied by RETORT Pharmaceuticals Pvt. Limited. All the individual constituents were also supplied by the same company. CELNORM is constituted with equal portions of extracts of carrot, fruit extract of cucumber, black pepper and a bioactive component Curcumin (95% purity) from the rhizome of turmeric. All the constituents were devoid of microbial contamination. At first, stock of CELNORM and its constituent have been made at a concentration of 1mg/ml in DMEM containing 300 μl of DMSO. Then two groups (A and B) of extract supplemented with DMEM were prepared at different concentrations individually with all four extracts and CELNORM according to the previously published work (Sathya et al., 2020). Two cell lines (HCT116 and A549 cells) were used separately to study the effect of CELNORM and its components in the two treatment groups individually. The first group – '**Group A**' was supplemented with lower concentrations of CELNORM (31.25 µg/ml) and all of its four constituents individually (7.81 µg/ml individually) as well as in combination. The experimental groups are: Group A-1 – cells were treated with 7.81 µg/ml of curcumin; Group A-2- cells were treated with 7.81 µg/ml pepper; Group A-3 - cells were treated with 7.81 µg/ml Carrot; Group A-4 - cells were treated with 7.81 µg/ml cucumber; and Group A-5- cells were treated with 31.25 µg/ml of CELNORM.

Additionally, for cell viability assessment the extracts were also taken in combination with two as follows-

Group A-6 - cells were treated with 15.62 µg/ml curcumin + pepper; Group A-7 - cells were treated with 15.62 µg/ml pepper + Carrot; Group A-8 - cells were treated with 15.62 µg/ml Carrot + cucumber; Group A-9 - cells were treated with 15.62 µg/ml cucumber + curcumin.

Similarly, the second group - **'Group B'** was supplemented with higher concentrations of CELNORM (62.5 µg/ml) and all of its four constituents individually (15.62 µg/ml each) as well as in combination. The treatment groups are: Group B-1- cells were treated with 15.62 µg/ml of curcumin; Group B-2 - cells were treated with 15.62 µg/ml pepper; Group B-3 - cells were treated with 15.62 µg/ml Carrot; Group B-4 - cells were treated with 15.62 µg/ml cucumber; Group B-5 - cells were treated with 62.5 µg/ml of CELNORM and for the cell viability assessment the extracts were taken in pairs as follows-

Group B-6- cells were treated with 31.25 µg/ml curcumin + pepper; Group B-7 - cells were treated with 31.25 µg/ml pepper + Carrot; Group B-8 - cells were treated with 31.25 µg/ml Carrot + cucumber; and Group B-10 - cells were treated with 31.25 µg/ml cucumber+ curcumin.

Assessment of cytotoxicity activity

Morphological assessment

Both colon and lung cancer cell lines were cultured in the 10% FBS supplemented DMEM with 1% antibiotic and antimycotic solution. Cells were seeded at a seeding density of $50x10^4$ cells/well in a 6-well plate with 10% FBS supplemented DMEM and incubated at 37^0C under normoxic conditions for 24 hours for cell attachment. After 24 hours of incubation and the drug-supplemented DMEM from both Group A and Group B as described before, were added to the cells in two different sets and incubated at 37^0C under normoxic conditions for 48 hours. The images were captured with Leica Optica inverted microscope at 10x magnification.

Cell viability assessment

Both of the cell lines (HCT116 and A549) were seeded at a seeding density of 4000 cells per well of a 96-well plate with 10% FBS-supplemented DMEM and incubated at 37^0C under normoxic conditions for 24 hours for cell attachment. After 24 hours of incubation, and the drugs were administrated according to 'Group A' and 'Group B' and incubated at 37^0C for 48 hours. After 48 hours of incubation, 10µl of CCK8 reagent was added to the cells containing medium and incubated for 4 hours. The optical density was measured at 450nm and the percentage of cell viability was calculated.

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Gene expression study

RNA was extracted from the cells treated with all four extracts individually and with CELNORM using TriZol RNA isolation reagent and subjected to quantification of total RNA spectrophotometrically at 260 nm. cDNA was synthesized from the RNA with a reverse transcription kit (Eurogentec, Belgium) and qPCR was performed using SYBR-qPCR master mix (Takyon, Eurogentec, Belgium) followed by the gene expression analysis of the four genes – *Cyclin-D, PCNA, CDK-6,* and *BCL-2*- expressed in HCT116 and A549 cells in normal conditions. CT values were obtained and normalization of CT values of targeted genes was done using the CT values of housekeeping gene GAPDH to calculate ΔCT, followed by the calculation of ΔΔ CT and finally the relative quantification (RQ) values.

Statistical analysis

The optical density was measured at 450nm and the percentage of cell viability was calculated. As the study has comprises with more than two treatment groups, One-Way-Anova test was performed to determine the p-value of data. *P-values of <0.05, <0.01 and <0.001* were considered statistically significant, and are indicated by asterisks (*, ** and *** respectively).

Results

Assessment of cytotoxicity activity on colon cancer cell line HCT116

Morphological assessment after treatment

In Group-A:

After 48 hours of incubation, cells treated with Group – A supplemented medium have attained the spherical morphology in both Curcumin (b) and CELNORM (e) as shown in Fig.1 and have started to detach from the culture plate which is evident from the Fig.1. Comparatively a large number of cells have detached in CELNORM rather than the Curcumin. Whereas, *Piper nigram* (c), Carrot (d), and Cucumber (e) don't show any significant morphological difference compared with solvent control (a) which remained in the epithelial morphology.

In Group – B:

In the Group - B with the higher concentration of treatments, depicted in ***Fig.2***, the cells appeared with a spherical morphology in both Curcumin (b) and CELNORM(e) and all the cells detached from the plate. After the treatment with CELNORM for 48 hours, the cells were reduced in size and completely dissociated from the plate. Similar to low dose, *Piper nigram* (c), Carrot(d), and cucumber (e) don't show significant morphological difference compared with solvent control (a).

Cell viability assessment

The CCK-8 results showed that low doses of curcumin (Group A-1) and CELNORM (Group A-5) inhibited colon cancer cell proliferation significantly (*p<0.001 and p <0.0001* respectively). The CELNORM has greatly reduced the cell viability more than curcumin which is evident from Fig. 3a**.** Whereas, the other components like *Piper nigram*, Carrot, and cucumber did not show any significant reduction in the cell viability which is similar to our previous findings in morphological assessment (Sathya C et al., 2020). However, the synergistic effect of all the components together in the formulation of CELNORM inhibited the colon cancer cell proliferation more significantly than the individual components. Curcumin supplemented Group' B-1 has significantly reduced the cell proliferation *(p <0.001).* Whereas CELNORM supplemented (Group B-5) has turned down the proliferation significantly (p<0.0001). The combinational treatment, Group B-6 and Group B-9 has significant changes whereas Group B-7, and Group B-8 do not show any significant reduction in the cell viability (shown in Fig. 3b). This suggests that CELNORM is a better anti-cancer agent when compared with curcumin + pepper, cucumber + curcumin and curcumin alone. All the natural compounds in a synergistic manner reduce the cell proliferation of colon cancer cells significantly.

Assessment of cytotoxicity activity on lung cancer cell line A549

Morphological assessment

In Group A:

After 48 hours of incubation, with various concentrations as mentioned previously, of each component of CELNORM individually and the whole formulation, the cells have reduced their proliferation in both Curcumin (b) and CELNORM (e) with lesser colonies which is evident from Fig. 4. Comparatively fewer colonies of cells were observed in CELNORM (f) rather than the Curcumin (b). Whereas, *Piper nigram* (c), Carrot (d), and cucumber (e) did not display any significant difference compared with solvent control (a) which remained in the epithelial morphology.

In Group B:

In the Group B supplemented medium the cells have greatly reduced their proliferation and started to appear with a spherical morphology in both Curcumin (b) and CELNORM (e). After incubation for 48 hours, the cells have reduced in size and no colonies were formed which is evident from Fig. 5. In Group B, *Piper nigram* (c) and

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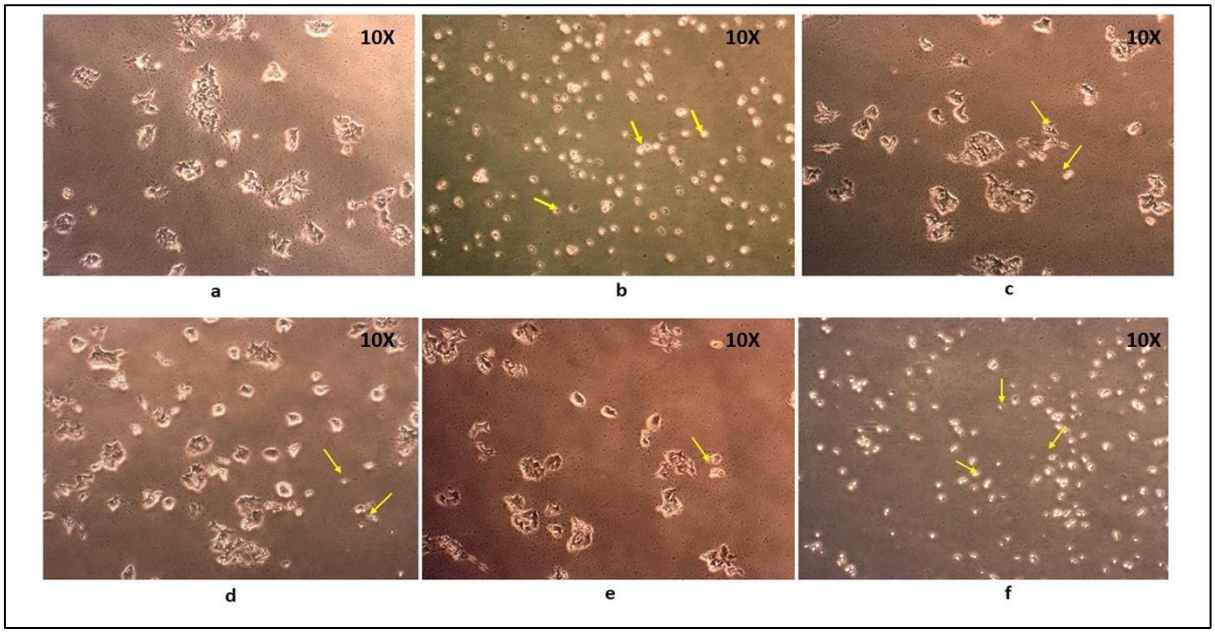

Figure 1. Morphological assessment of HCT116 cells after treatment with Solvent control and Group -A subgroups (a) solvent control (DMSO); (b) Curcumin; (c) *Piper nigram*; (d) Carrot; (e) Cucumber; (f) CELNORM. Images were captured at 10x magnification

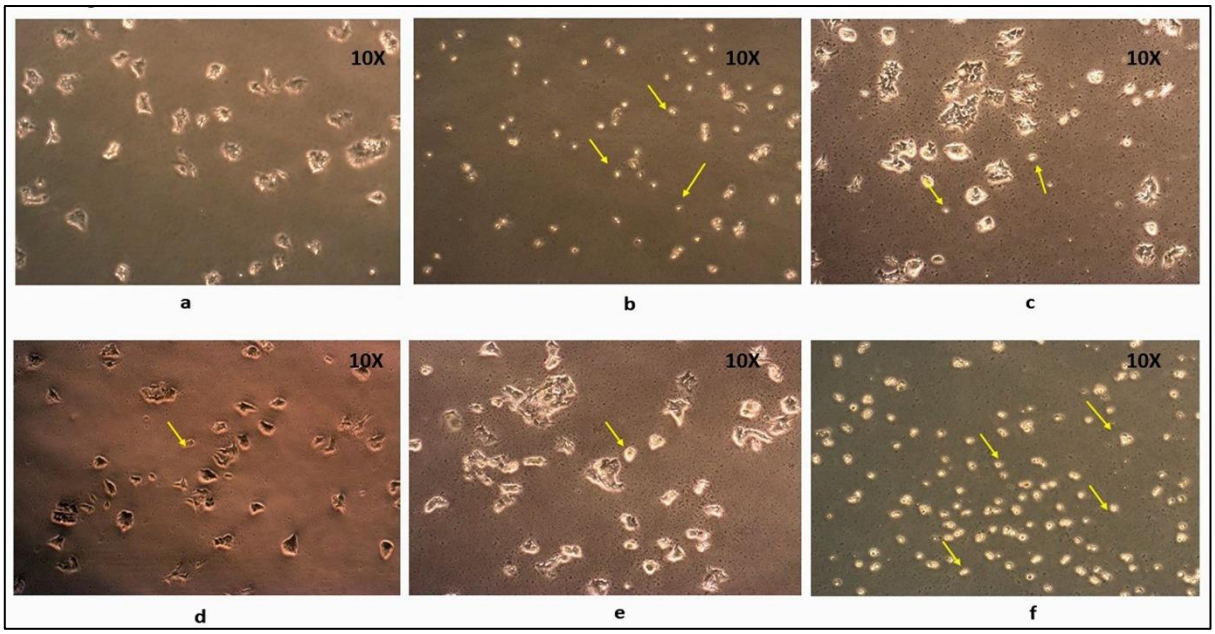

Figure 2. Morphological assessment of HCT116 cells after treatment with Solvent control and Group- B subgroups (a) solvent control (DMSO); (b) Curcumin; (c) *Piper nigram*; (d) Carrot; (e) Cucumber; (f) CELNORM. Images were captured at 10x magnification

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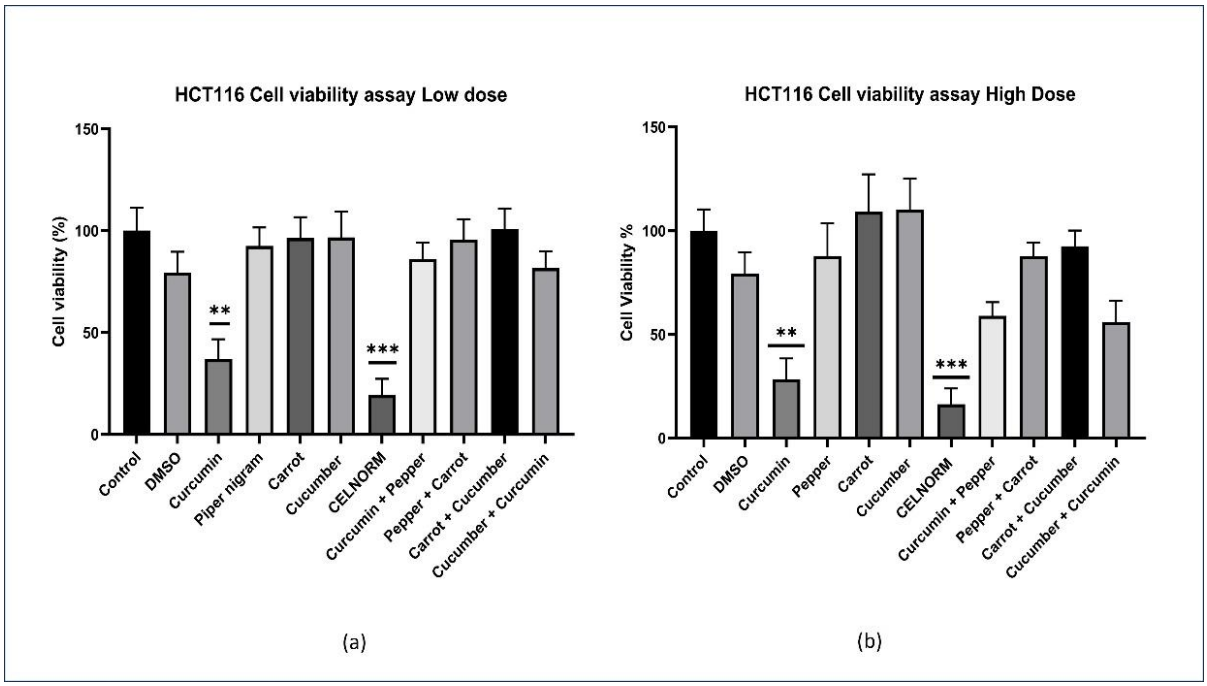

Figure 3. Cell viability analysis of HCT116 cells cultured in both (a) Group A and (b) Group B treatment groups

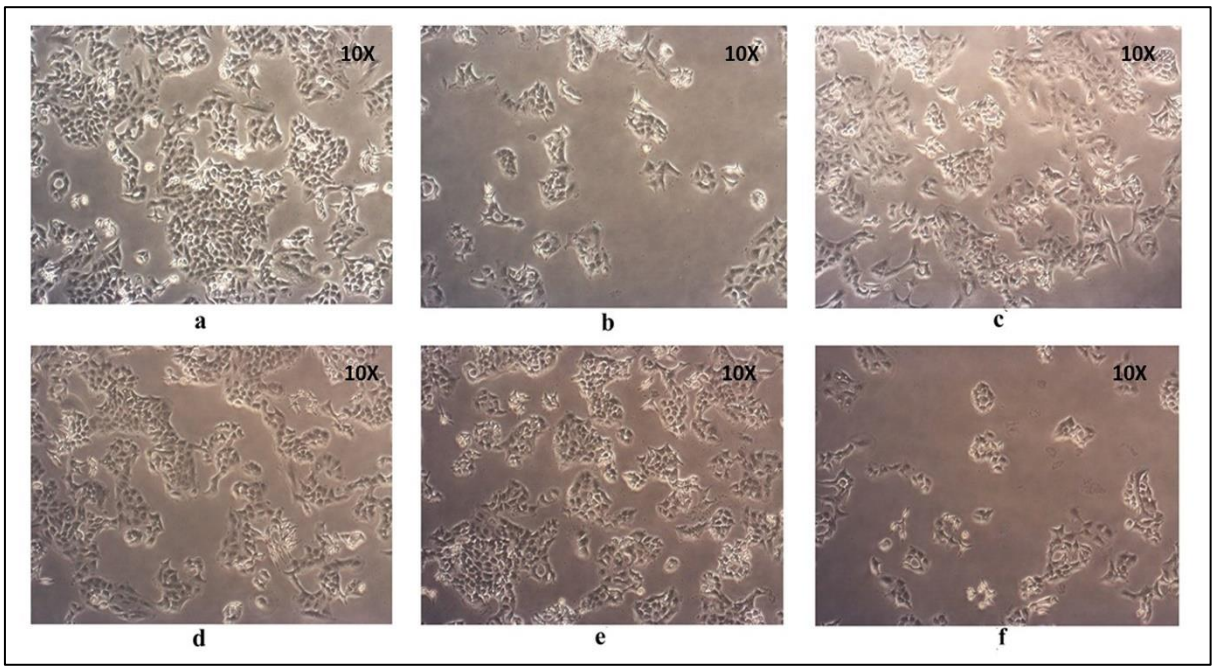

Figure 4. Morphological assessment of A549 cells after treatment with solvent control and Group -A subgroups (a) solvent control (DMSO); (b) Curcumin; (c) *Piper nigram*; (d) Carrot; (e) Cucumber; (f) CELNORM. Images were captured at 10x magnification

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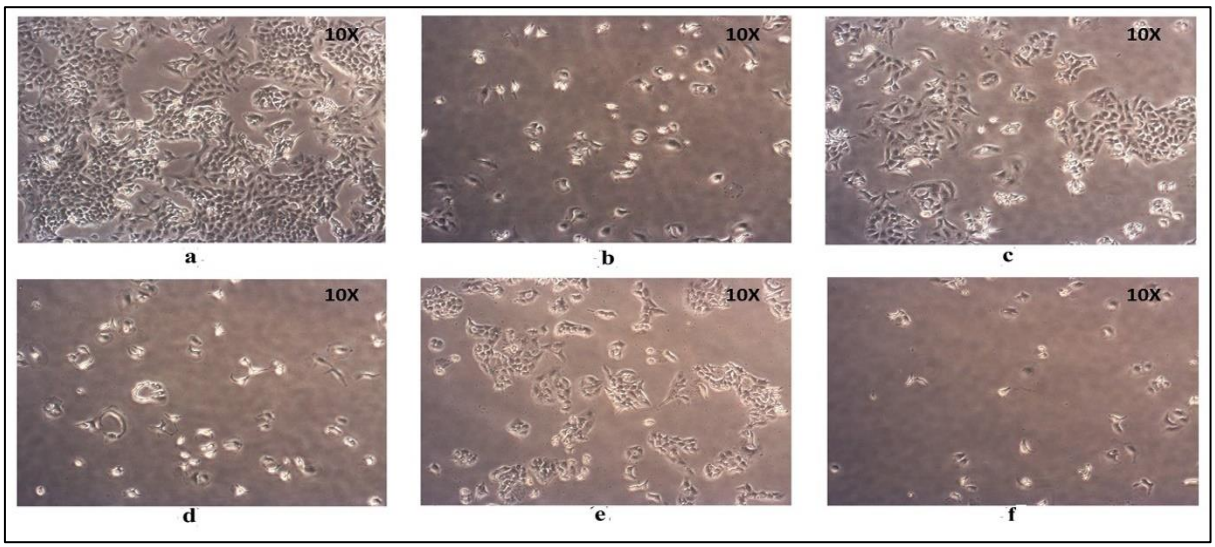

Figure 5. Morphological assessment of A549 cells after treatment with Solvent control and Group -B subgroups (a) solvent control (DMSO); (b) Curcumin; (c) *Piper nigram*; (d) Carrot; (e) Cucumber; (f) CELNORM. Images were captured at 10x magnification

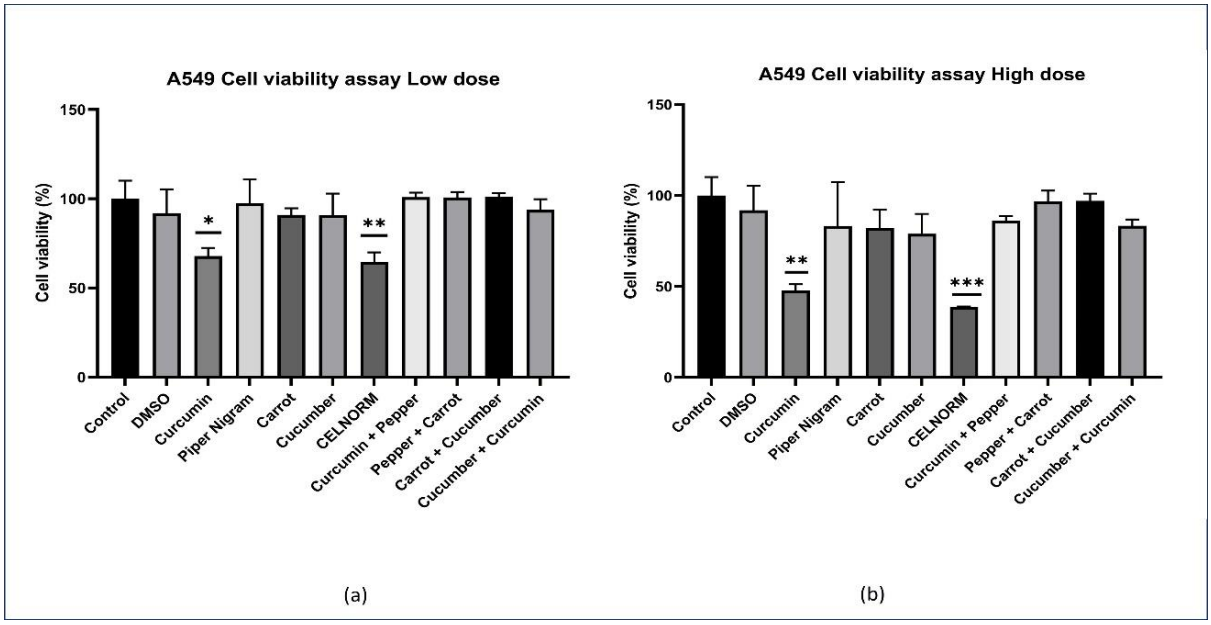

Figure 6. Cell viability analysis of A549 cells cultured in both (a) Group A and (b) Group B treatment groups

cucumber (e) don't show any significant morphological difference compared with solvent control (a). However, the cells treated with Carrot (d) also had a reduced proliferation rate.

Cell viability assessment

The CCK-8 results showed that curcumin supplemented Group A medium inhibited Lung cancer cell proliferation and only 68% of cells were viable after 48 hours. CELNORM reduce the cell proliferation even more and the cell viability was observed near 64.5 % with a significant *p-value* of *0.01* and *0.004* respectively. The CELNORM has greatly reduced the cell viability more than curcumin which is evident from Fig. 6 a. Whereas, the other components like *Piper nigram*, Carrot, and cucumber and its combinations treatment did not show any significant reduction in the cell viability which correlates to our previous findings in morphological assessment. Although some visible morphological differences were evident in the components when compared to the solvent-treated cells. However, the synergistic effect of all the components together (CELNORM) inhibited lung cancer cell proliferation more significantly than the individual components.

In case of Group B, Curcumin has significantly reduced the cell proliferation to cell viability to 47.7% *(p <0.001)*. Whereas CELNORM decrease the proliferation to cell viability of 38.7% *(p <0.0001)*. The combinational treatment of unlike curcumin alone and combinational

CELNORM; *Piper nigram*, Carrot, and cucumber and their combination do not show any significant reduction in the cell viability (shown in Fig. 6b) which correlates to our previous findings in morphological assessment.

Gene expression analysis:

Four genes - *Cyclin-D, PCNA, CDK-6,* and *BCL-2* have been used as the targets as they are normally expressive in both HCT116 and A549 cell lines. Therefore, a reduction in the level of their expression was expected in the treated group than in the control group

CELNORM treated A549 cell line has shown a great reduction in the expression of the *Cyclin D* genes whereas the *PCNA* expression has elevated in CELNORM treated cell line than control. Though CELNORM has reduced the expression of *CDK 6*, and *BCL-2*, the individual compounds were found to be more effective than CELNORM as shown in (Fig. 8).

Discussion

Cancer is a heterogeneous disease that develops

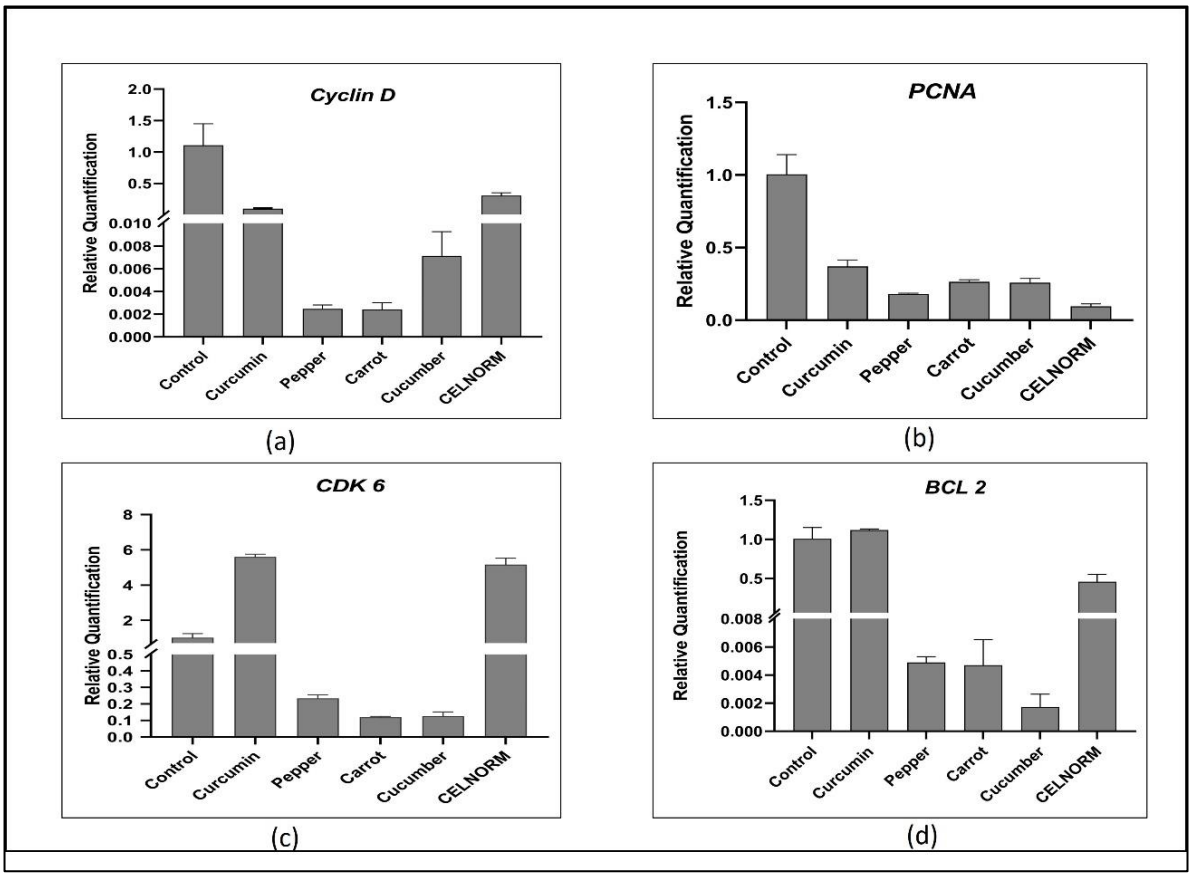

Figure 7. Gene expression results from qPCR of HCT116 cell line without treatment (control) and with treatment (curcumin, pepper, Carrot, cucumber and CELNORM) (a) relative expression of *Cyclin- D* (b) relative expression of *CDK6* (c) relative expression of *PCNA* d) relative expression of *BCL2*

due to cell death. *GAPDH* has been used as the housekeeping gene and the CT value of the targeted gene has been normalized according to the CT value of the housekeeping gene. The RQ value has been calculated for each gene and has been plotted in the graph. The HCT116 cell line treated with CELNORM has shown a reduction in the expression of *Cyclin-D, PCNA* and *BCL-2* genes but overexpression of the *CDK-6* gene has been observed in curcumin and CELNORM treated cell line. The individual components are found to be more effective to reduce the gene expression of *Cyclin-D, CDK-6,* and *BCL-2* than their synergistic effects in CELNORM (Fig.7).

through a multistep process that culminates in the accumulation of multiple genetic alterations (Dagogo-Jack and Shaw, 2018; Guo et al., 2019; Hesketh, 1997; Sriramulu et al., 2021). It is distinguished by uncontrolled proliferation, invasion, and metastasis. It encompasses a series of disrupted molecular pathways that result in abnormal protein expression as a result of genetic mutations (Boland & Goel, 2010; Pino & Chung, 2010; Sherr & McCormick, 2002). Cancer cells can acquire drug resistance in a variety of ways. To find an effective cancer treatment, a variety of components, including inflammatory cytokines, growth factors, proteases, adhesion molecules, coagulation factors, hormones, and apoptotic agents, have been studied. Extensive research

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has revealed that phytochemicals such as polyphenols, flavones, and flavonoids have powerful anti-cancer properties against a variety of cancers (Thomasset et al., 2007; Ventura et al., 2007; Paramita et al., 2022).

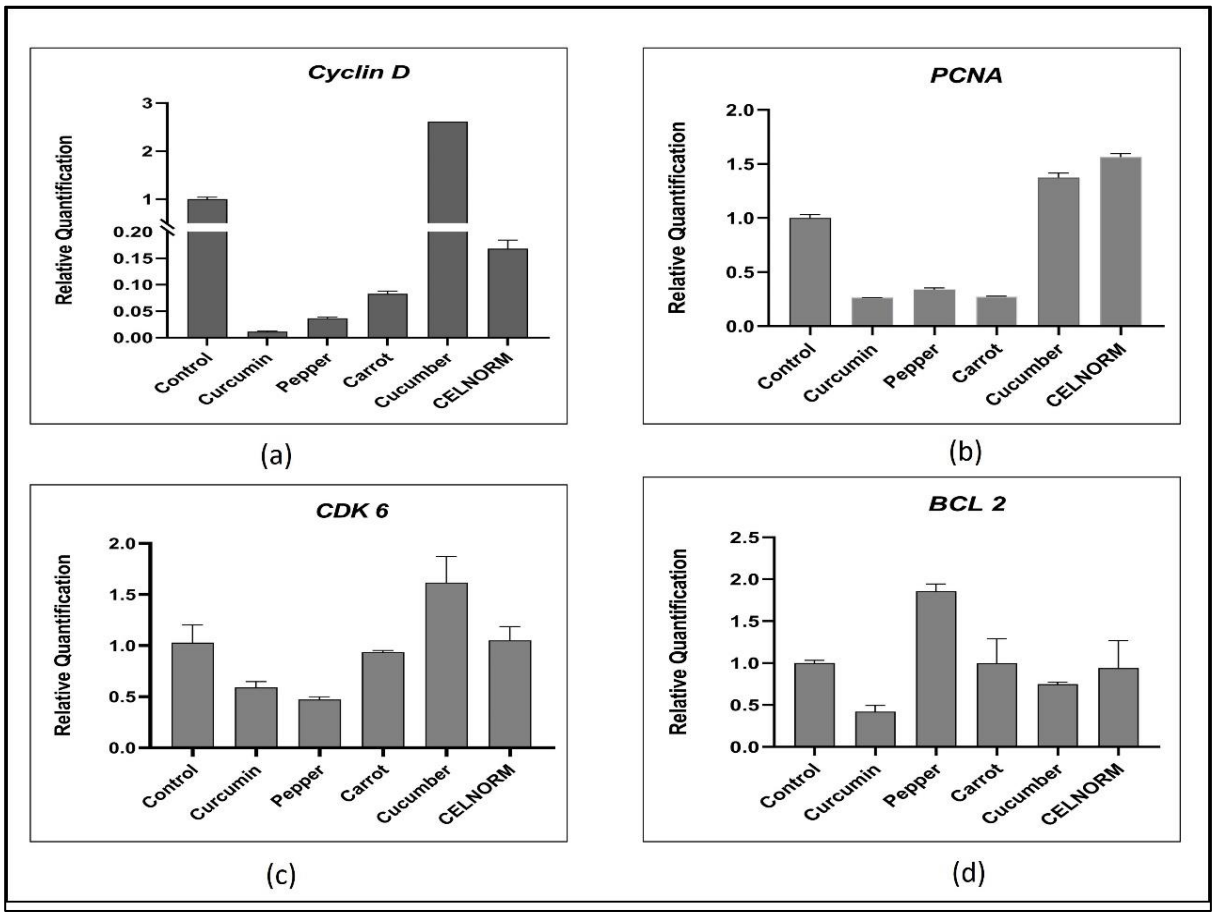

Figure 8. Gene expression results from qPCR of A549 cell line without treatment (control) and with treatment (curcumin, pepper, Carrot, cucumber and CELNORM) (a) relative expression of *Cyclin D* (b) relative expression of *CDK-6* (c) relative expression of PCNA d) relative expression of *BCL-2*

Cancer, as previously said, is a complex disease that may demand the use of medicines that target different intracellular components. The popular medicines in the market today are designed to target a single intracellular target. Unfortunately, the physiological and molecular deregulations that lead to cancer initiation and development might involve hundreds of genes or signaling pathways, necessitating multi-target therapy to treat complex human diseases such as cancer (Benjamin et al., 2021). Along with mainstream therapeutics, alternative supplementation of natural compounds elevating anti-cancer properties can be helpful to cure cancer as well as minimize the recurrence of tumors. In the present study, the effect of curcumin, pepper, Carrot, cucumber, and CELNORM (combination of all four extracts) on the control of cell proliferation and apoptosis leading to malignant cell death, leveraging the multiple therapeutic benefits of natural substances in cancer prevention. Curcumin individually in both low dose (Group A) and high dose (Group B) shows a significant reduction in cell viability in HCT116 and A549 cell lines. In case of Group A, the cell viability comes down to 36 % for HCT116 cells and 67% for the A549 cell line. Whereas in Group B, the cell viability was reduced to 28.37% for HCT116 cells and 47% for A549 cells. However, the treatment with other components (pepper, Carrot and cucumber) do not show any significant change in cell viability of both HCT116 and A549 cell lines for both concentrations. But the combination of all four compounds in CELNORM in a 1:1:1:1 ratio shows a synergistic effect and a significant reduction of cell viability to 19% in case of Group A and 16% in Group B for HCT116 cell line. Similarly, for A549 cell line cell, viability reduced down to 64% in Group A and 38% in Group B. These data also show the efficacy of the CELNORM against cancer cell line is more than the anti-cancerous efficacy of curcumin alone. Overall, the synergistic effect of curcumin, pepper, Carrot and cucumber in CELNORM makes it more efficient as a potential therapeutic for cancer. In case of gene expression studies, conflicting result has been observed as the individual compound of CELNORM reduces the gene expression of four targeted genes (*Cyclin-D, PCNA, CDK-6,* and *BCL-2*) more effectively than CELNORM

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itself. CELNORM was found to reduce the expression of targeted genes in both cell lines in most of the cases except *CDK-6* in HCT116 and *PCNA* in the A549 cell line, which shows the anti-cancerous properties of CELNORM.

Curcumin is a polyphenol natural chemical that has been utilized in traditional medicine for millennia. Experiments were carried out to understand more about the intracellular pathways targeted by curcumin, which have been connected to its promising therapeutic potential. Curcumin has been reported to re-sensitize the resistant cancer cell to 5-flurouracil. But the bioavailability of curcumin at cancer site after the oral administration is yet to be established (Jin et al., 2021). The limitation associated with curcumin is its poor bioavailability of it beyond the gastrointestinal tract as it is not absorbed easily. Therefore, to increase the bioavailability of curcumin in plasma, several combinational therapies have been introduced and trialled (Prasad et al., 2014). In the present study, the synergistic effect of curcumin along with pepper, Carrot, and cucumber extract has been studied. Carrot is a great source of dietary fibres and antioxidants. A regular intake of dietary fibre has manifested a reduction in incidence and mortality rates of cancer (Masrul and Nindrea, 2019; S Shankar, 1991), as well as cardio- and cerebrovascular disorders, most likely due to the antioxidants found in vegetables (Clarke and Armitage, 2003; Hertog et al., 1993; John et al., 2002). Antioxidants can scavenge reactive oxygen species (ROS) created by the human body lethal to healthy tissues and cells (da Silva Dias, 2014; Poljsak et al., 2013; Yngve, 2013). Cucumber also contains antioxidants that block oxidation by interacting with free radicals, chelating free catalytic metals, and functioning as oxygen scavengers, among other ways (Kumar et al., 2010; Shahidi et al., 1992). Curcumin, when combined with other natural substances, significantly increased clonogenic inhibition and induced cell apoptosis. One previous study that showed the efficacy of CELNORM on breast cancer has detailed its action against halting the proliferation of breast cancer cells when tested in MCF7 cell lines (Sathya et al., 2020). In terms of morphological features and cell proliferation inhibition, this combination of therapies generated a consistent response in both colon and lung cancer cell lines. Because of its synergistic action, CELNORM may deliver increased bioavailability of curcumin outside of the gastrointestinal system in higher organisms if taken as a dietary supplement.

Conclusion

In summary, curcumin inhibited cancer cell growth more clearly than other natural compounds, whilst the other natural compounds had no significant influence on cell proliferation or morphological changes of cancer cells. However, treatment with all the extracts together (CELNORM) have a significant impact on cell growth, implying that the synergistic effect of pepper, Carrot and cucumber enhanced cancer cell apoptosis and reduce proliferation. When compared to curcumin alone, CELNORM had a higher apoptotic activity on both colon and lung cancer cells. However, subsequent investigations concerning the detailed mechanism of action and more specific role in proliferation and apoptosis and its mechanism of action is required for its use in animal models on cancer and subsequent clinical trials.

Ethics approval and consent to participate

Not applicable.

Human and animal rights

No animals/humans were used for studies that are the basis of this research.

Consent for publication

Not applicable.

Availability of data and materials

All data are provided along with manuscript.

Conflict of interest

There is no conflict of interest.

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the composition was given to him with only mentioning the group name as described in the text. One of the funders (Retort Pharmaceuticals) is only involved in formulating the composition of the supplement and is not involved in executing the experiment, analysis and interpretation of the data.

References

Aggarwal, B.B., Kumar, A., & Bharti, A.C. (2003). Anticancer potential of curcumin: Preclinical and clinical studies. *Anticancer Research*, *23*(1 A), 363–398.

Aggarwal, S., Ichikawa, H., Takada, Y., Sandur, S. K., Shishodia, S., & Aggarwal, B. B. (2006). Curcumin (diferuloylmethane) down-regulates expression of cell proliferation and antiapoptotic and metastatic gene products through suppression of IκBα kinase and Akt activation. *Molecular Pharmacology*, *69*(1), 195–206. https://doi.org/10.1124/mol.105.017400

Banerjee, A., Chabria, Y., Kanna N. R, R., Gopi, J., Rowlo, P., Sun, X. F., & Pathak, S. (2021). Role of Tumor Specific niche in Colon Cancer Progression and Emerging Therapies by Targeting Tumor Microenvironment. *Advances in Experimental Medicine and Biology*, *1341*, 177–192. https://doi.org/10.1007/5584_2019_355

Benjamin D. Granta, Chelsey A. Smithb, Philip E. Castlec, d, Michael E. Scheurere, and R. R.-K. (2017). HHS Public Access. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1002/anie.201209804.A

Bode, A., & Zigang, D. (2011). Cancer Prevention Research–Then and Now Ann. *Bone*, *23*(1), 1–7. https://doi.org/10.1038/nrc2646.Cancer

Boland, C.R., & Goel, A. (2010). Microsatellite Instability in Colorectal Cancer. *Gastroenterology*, *138*(6), 2073-2087.e3. https://doi.org/10.1053/j.gastro.2009.12.064

Butler, M. S. (2008). Natural products to drugs: Natural product-derived compounds in clinical trials. *Natural Product Reports*, *25*(3), 475–516. https://doi.org/10.1039/b514294f

Clarke, R., & Armitage, J. (2003). SYMPOSIUM : VITAMIN THERAPY AND ISCHEMIC HEART DISEASE Antioxidant Vitamins and Risk of Cardiovascular Disease . Review of Large-Scale Randomised Trials. *Cardiovascular Drugs and Therapy*, pp. 411–415.

da Silva Dias, J. C. (2014). Nutritional and Health Benefits of Carrots and Their Seed Extracts. *Food and Nutrition Sciences*, *05*(22), 2147–2156. https://doi.org/10.4236/fns.2014.522227

Dagogo-Jack, I., & Shaw, A. T. (2018). Tumour heterogeneity and resistance to cancer therapies. *Nature Reviews Clinical Oncology*, *15*(2), 81–94. https://doi.org/10.1038/nrclinonc.2017.166

Deng, Y., Sriwiriyajan, S., Tedasen, A., Hiransai, P., & Graidist, P. (2016). Anti-cancer effects of Piper nigrum via inducing multiple molecular signaling in vivo and in vitro. *Journal of Ethnopharmacology*, *188*, 87–95. https://doi.org/10.1016/j.jep.2016.04.047

Ganesan, H., Nandy, S. K., Banerjee, A., Pathak, S., Zhang, H., & Sun, X. F. (2022). RNA-Interference-Mediated miR-122-Based Gene Regulation in Colon Cancer, a Structural In Silico Analysis. *International Journal of Molecular Sciences*, *23*(23), 15257. https://doi.org/10.3390/IJMS232315257/S1

GLOBOCAN (2018). counting the toll of cancer. In *The Lancet*, *392*(10152), 985. https://doi.org/10.1016/S0140-6736(18)32252-9

Gunjal, P. M., Schneider, G., Ismail, A. A., Kakar, S. S., Kucia, M., & Ratajczak, M. Z. (2015). Evidence for induction of a tumor metastasis-receptive microenvironment for ovarian cancer cells in bone marrow and other organs as an unwanted and underestimated side effect of chemotherapy/radiotherapy. *Journal of Ovarian Research*, *8*(1), 1–11. https://doi.org/10.1186/S13048-015-0141-7/FIGURES/5

Guo, M., Peng, Y., Gao, A., Du, C., & Herman, J. G. (2019). Epigenetic heterogeneity in cancer. *Biomarker Research*, *7*(1), 1–19. https://doi.org/10.1186/s40364-019-0174-y

Hertog, M. G. L., Feskens, E. J. M., Kromhout, D., Hertog, M. G. L., Hollman, P. C. H., Hertog, M. G. L., & Katan, M. B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, *342*(8878), 1007–1011. https://doi.org/10.1016/0140-6736(93)92876-U

Hesketh, R. (1997). The Multistep Nature of Cancer. *The Oncogene & Tumour Suppressor Gene Factsbook*, *9*(4), 54–60. https://doi.org/10.1016/b978-012344548-3/50010-8

Hussein, R. M., Mohammed, A. T., Hussein, D. M., Fadhil, A. A., Al-Ani, M. S., Bayati, S., &

Sahib, A. A. (2023). The Influence of Fish Oil Supplementation on the Adverse Effects of Chemotherapy in Patients with Hepatocellular Carcinoma. *Journal of Chemical Health Risks*, *13*(1), 125–133. https://doi.org/10.22034/JCHR.2022.697645

Jacobsen, P. B., & Stein, K. (1999). Is fatigue a long-term side effect of breast cancer treatment? *Cancer Control*, *6*(3), 256–263. https://doi.org/10.1177/107327489900600304

Jemal, A., Bray, F., & Ferlay, J. (1999). Global Cancer Statistics: 2011. *CA Cancer J Clin*, *49*(2), 1,33-64. https://doi.org/10.3322/caac.20107.Available

Jin, M., Kong, L., Han, Y., & Zhang, S. (2021). Gut microbiota enhances the chemosensitivity of hepatocellular carcinoma to 5-fluorouracil in vivo by increasing curcumin bioavailability. *Phytotherapy Research*, *35*(10), 5823–5837.https://doi.org/10.1002/PTR.7240

John, J. H., Ziebland, S., Yudkin, P., Roe, L. S., & Neil, H. A. W. (2002). Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: A randomised controlled trial. *Lancet*, *359*(9322), 1969–1974. https://doi.org/10.1016/S0140-6736(02)98858-6

Jothimani, G., Ganesan, H., Pathak, S., & Banerjee, A. (2022). Molecular characterization of primary and metastatic colon cancer cells to identify therapeutic targets with natural compounds. *Current Topics in Medicinal Chemistry*, *22*. https://doi.org/10.2174/1568026622666220401161511

Kirubhanand, C., Merciline, L J.,, Anitha, S., Sangeetha, R., Nachammai, K.T., Langeswaran, K., Gowtham, K. S. (2023) Targeting potential receptor molecules in non-small cell lung cancer (NSCLC) using in silico approaches. *Front Mol Biosci*. 10:1124563. https://doi.org/10.3389/fmolb.2023.1124563.

Krasteva, N., & Georgieva, M. (2022). Promising Therapeutic Strategies for Colorectal Cancer Treatment Based on Nanomaterials. *Pharmaceutics*, *14*(6). https://doi.org/10.3390/PHARMACEUTICS14061213

Kumar, D., Kumar, S., Singh, J., Narender, Rashmi, Vashistha, B. D., & Singh, N. (2010). Free radical scavenging and analgesic activities of Cucumis sativus L. fruit extract. *Journal of Young Pharmacists*, *2*(4), 365–368. https://doi.org/10.4103/0975-1483.71627

Lee, K. W., Bode, A. M., & Dong, Z. (2011). Molecular targets of phytochemicals for cancer prevention. *Nature Reviews Cancer*, *11*(3), 211–218. https://doi.org/10.1038/nrc3017

Li, X., Ma, S., Yang, P., Sun, B., Zhang, Y., Sun, Y., Hao, M., Mou, R., & Jia, Y. (2018). Anticancer effects of curcumin on nude mice bearing lung cancer A549 cell subsets SP and NSP cells. *Oncology Letters*, *16*(5), 6756–6762. https://doi.org/10.3892/OL.2018.9488/HTML

López-Lázaro, M. (2008). Anticancer and carcinogenic properties of curcumin: Considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. *Molecular Nutrition and Food Research*, *52*(SUPPL. 1), 103–127. https://doi.org/10.1002/mnfr.200700238

Masrul, M., & Nindrea, R. D. (2019). Dietary fibre protective against colorectal cancer patients in Asia: A meta-analysis. *Open Access Macedonian Journal of Medical Sciences*, *7*(10), 1723–1727. https://doi.org/10.3889/oamjms.2019.265

Naidu, M. U. R., Ramana, G. V., Rani, P. U., Mohan, I. K., Suman, A., & Roy, P. (2004). Chemotherapy-induced and/or radiation therapy-induced oral mucositis - Complicating the treatment of cancer. *Neoplasia*, *6*(5), 423–431. https://doi.org/10.1593/neo.04169

Naik, K., Chaudhary, S., Ye, L., & Parmar, A. S. (2022). A Strategic Review on Carbon Quantum Dots for Cancer-Diagnostics and Treatment. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/FBIOE.2022.882100

Nasab, S. H., Amani, A., Ebrahimi, H. A., & Hamidi, A. A. (2021). Design and preparation of a new multi-targeted drug delivery system using multifunctional nanoparticles for co-delivery of siRNA and paclitaxel. *Journal of Pharmaceutical Analysis*, *11*(2), 163–173. https://doi.org/10.1016/j.jpha.2020.04.005

Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, *70*(3), 461–477. https://doi.org/10.1021/NP068054V/SUPPL_FILE/NP068054V_SA.PDF

Ojo, O. A., Adeyemo, T. R., Rotimi, D., Batiha, G. E. S., Mostafa-Hedeab, G., Iyobhebhe, M. E., Elebiyo, T. C., Atunwa, B., Ojo, A. B., Lima, C. M. G., & Conte-Junior, C. A. (2022).

Anticancer Properties of Curcumin Against Colorectal Cancer: A Review. *Frontiers in Oncology*, *12*. https://doi.org/10.3389/FONC.2022.881641

Oun, R., Moussa, Y. E., & Wheate, N. J. (2018). The side effects of platinum-based chemotherapy drugs: A review for chemists. *Dalton Transactions*, *47*(19), 6645–6653. https://doi.org/10.1039/c8dt00838h

Paramita, P., Sethu, S.N., Subhapradha, N., Ragavan, V., Ilangovan, R., Balakrishnan, A., Srinivasan, N., Murugesan, R., Moorthi, (2022) A. Neuro-protective effects of nano-formulated hesperetin in a traumatic brain injury model of *Danio rerio*. *Drug Chem Toxicol*. 45(2):507-514. doi: 10.1080/01480545.2020.1722690.

Pino, M. S., & Chung, D. C. (2010). The Chromosomal Instability Pathway in Colon Cancer. *Gastroenterology*, *138*(6), 2059–2072. https://doi.org/10.1053/j.gastro.2009.12.065

Poljsak, B., Šuput, D., & Milisav, I. (2013). Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. *Oxidative Medicine and Cellular Longevity*, *2013*. https://doi.org/10.1155/2013/956792

Prasad, S., Tyagi, A. K., & Aggarwal, B. B. (2014). Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. *Cancer Research and Treatment*, *46*(1), 2–18. https://doi.org/10.4143/crt.2014.46.1.2

Rao, C. V. (2007). Regulation of COX and LOX by curcumin. *Advances in Experimental Medicine and Biology*, *595*, 213–226. https://doi.org/10.1007/978-0-387-46401-5_9

S Shankar 1, E. L. (1991). Dietary fiber and cancer prevention - PubMed. *Hematology/Oncology Clinical North America*, *5*(1), 25–41. https://pubmed.ncbi.nlm.nih.gov/1851150/

Saeed, H., & Waheed, A. (2017). A REVIEW ON CUCUMBER (Cucumis sativus). *International Journal of Technical Research & Science A*, *2*(6), 365–374.

Saja, K., Babu, M. S., Karunagaran, D., & Sudhakaran, P. R. (2007). Anti-inflammatory effect of curcumin involves downregulation of MMP-9 in blood mononuclear cells. *International Immunopharmacology*, *7*(13), 1659–1667. https://doi.org/10.1016/j.intimp.2007.08.018

Sakr, A. S., Soliman, N. F., Al-Gaashani, M. S., Pławiak, P., Ateya, A. A., & Hammad, M. (2022). An Efficient Deep Learning Approach for Colon Cancer Detection. *Applied Sciences 2022, Vol. 12, Page 8450*, *12*(17), 8450. https://doi.org/10.3390/APP12178450

Sathya, T. N. Mehta, V. A., Senthil, D., Navaneethakrishnan, K. R., Murugan, S. S., Kumaravel, T.S. (2020). Cytotoxicity evaluation of CELNORM, a nutritional health supplement, on MCF7 breast cancer cells. *Indian Journal of Science and Technology*, *13*(30), 2170–2175. https://doi.org/10.17485/ijst/v13i30.1150

Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic Antioxidants. *Critical Reviews in Food Science and Nutrition*, *32*(1), 67–103. https://doi.org/10.1080/10408399209527581

Sharma, K. D., & Karki, S. (2012). *Chemical composition , functional properties and processing of carrot — a review*. *49*, 22–32. https://doi.org/10.1007/s13197-011-0310-7

Sherr, C. J., & McCormick, F. (2002). The RB and p53 pathways in cancer. *Cancer Cell*, *2*(2), 103–112. https://doi.org/10.1016/S1535-6108(02)00102-2

Sriramulu, S., Sun, X. F., Malayaperumal, S., Ganesan, H., Zhang, H., Ramachandran, M., Banerjee, A., & Pathak, S. (2021). Emerging role and clinicopathological significance of aeg-1 in different cancer types: A concise review. *Cells*, *10*(6), 1–18. https://doi.org/10.3390/cells10061497

Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer*, *3*(10), 768–780. https://doi.org/10.1038/nrc1189

Thomasset, S. C., Berry, D. P., Garcea, G., Marczylo, T., Steward, W. P., & Gescher, A. J. (2007). Dietary polyphenolic phytochemicals - Promising cancer chemopreventive agents in humans? A review of their clinical properties. *International Journal of Cancer*, *120*(3), 451–458. https://doi.org/10.1002/ijc.22419

Ventura, A., Kirsch, D. G., McLaughlin, M. E., Tuveson, D. A., Grimm, J., Lintault, L., Newman, J., Reczek, E. E., Weissleder, R., & Jacks, T. (2007). Restoration of p53 function leads to tumour regression in vivo. *Nature*, *445*(7128), 661–665. https://doi.org/10.1038/nature05541

Vogelzang, N. J., Bosl, G. J., Johnson, K., & Kennedy, B. J. (1981). Raynaud's phenomenon: a common toxicity after combination chemotherapy for testicular cancer. *Annals of Internal Medicine*,

95(3), 288–292. https://doi.org/10.7326/0003-4819-95-3-288

Yang, S., Zhang, Z., & Wang, Q. (2019). Emerging therapies for small cell lung cancer. *Journal of Hematology and Oncology*, *12*(1), 1–11. https://doi.org/10.1186/S13045-019-0736-3/FIGURES/2

Yngve, A. (2013). Fruit and vegetable consumption revisited. *Public Health Nutrition*, *16*(11), 1911. https://doi.org/10.1017/S136898001300270X

Yuan, M., Zhao, Y., Arkenau, H. T., Lao, T., Chu, L., & Xu, Q. (2022). Signal pathways and precision therapy of small-cell lung cancer. *Signal Transduction and Targeted Therapy*, *7*(1), 1–18. https://doi.org/10.1038/s41392-022-01013-y

Zabernigg, A., Gamper, E.-M., Giesinger, J. M., Rumpold, G., Kemmler, G., Gattringer, K., Sperner-Unterweger, B., & Holzner, B. (2010). Taste Alterations in Cancer Patients Receiving Chemotherapy: A Neglected Side Effect? *The Oncologist*, *15*(8), 913–920. https://doi.org/10.1634/theoncologist.2009-0333

Zhou, H., S. Beevers, C., & Huang, S. (2012). The Targets of Curcumin. *Current Drug Targets*, *12*(3), 332–347. https://doi.org/10.2174/138945011794815356

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