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Extraction and Isolation of capsaicin from *Capsicum annum* L (CO-1) variety by High-performance liquid chromatography and its Bioactivities

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Abstract- Capsaicin is a nutritional factor, the active component of chilli, which is responsible for the pungent component of chilli. Chilli has diverse uses as a spice, condiment, culinary supplement, Medicine, vegetable and ornamental plant.

Aim of the study: Stimulation and enhancement of capsaicin content by polyploidy induction, quantification of enhanced capsaicin, its cytotoxicity and Bioactivity studies.

Materials and methods: Healthy 3 varieties (CO-1, K2, Jwala) of *Capsicum annum* L. (chilli) was selected for enhancement of capsaicin content by polyploidy induction. The variety showing high capsaicin content in the fifth generation was chosen for further study. The enhanced capsaicin was isolated by silica gel column chromatography and purified using HPLC. Cytotoxicity was studied in onion root tips by root tip squash method and *Vero* cell lines. Anti-inflammatory activity by inhibition of Albumin denaturation method and Anti-cancer activity by MTT assay was studied using the enhanced capsaicin.

Results: Of the 3 chilli varieties selected, CO-1 showed high and enhanced capsaicin content in the fifth generation. By HPLC, with a retention time of 1.368 min, 72.93% purity was obtained against control having a retention time of 1.337min of 41.4% purity recorded. Cytotoxicity of onion root tips showed no cell abnormalities and hence was found to be non-toxic to the cells. In the Vero cell line assay, the IC_{50} value and the viability of the cell line was 60.39% in treated and 58.79% in control of 100 µg/ml concentration. Anti-inflammatory activity of enhanced Capsaicin also showed maximum inhibition rate of 81.23% against 76.26% in control using 100 µg/ml. In Anti-cancer activity, IC_{50} dose was 6.25µg/ml in treated whereas untreated capsaicin showed 25µg/ml. Thus, enhanced Capsaicin shows a significant anti-inflammatory and anti-cancer activity.

Keywords: *chilli, capsaicin, HPLC, inhibition, anti-inflammatory, anti-cancer.*

I. INTRODUCTION

Chilli is from the genus Capsicum and belongs to the Solanaceae family. There are several domesticated species of chilli peppers, among them, *Capsicum annum*, *C. frutescence* and *C. Chinese* are the common varieties. Chilli is widely used in many parts of the world for their valued and characteristic sensory properties: colour, pungency and aroma. Pungency, a commercially important attribute of peppers, is due to the presence of phytochemicals from the characteristic capsaicinoids group [1].

The two most abundant capsaicinoids in chilli are capsaicin and dihydrocapsaicin, both constituting about 90%, with capsaicin accounting for 71% of the total Capsaicinoids in most of the pungent varieties. Capsaicin is considered as an active principle which accounts for the pharmaceutical properties of chilli. It has been used as an analgesic against arthritis pain and inflammation [2], effects on the gastrointestinal tract, the cardiovascular and respiratory systems, as well as the sensory and thermoregulation systems[3]. It has also been reported to show anticancer effect [4] and to be active against neurogenic inflammation (burning and stinging of hands, mouth and eyes) [5].

The productivity of chilli in India is 1.50 tonnes ha-1, which is far below its potential. Hence it can be increased by artificial induction of polyploidy. This method is used to enlarge variability of a species within a short time in superior variants with greater vigour, fertility, increasing fruit size, fruits characters and yield [6, 7, 8].

Thus the polyploidy induced chilli have been extracted and then isolated by TLC and column chromatography technique which is used to separate non-volatile mixtures of the capsaicinoids compounds. And the purity of selected bioactive fractions of Capsaicin, secondary metabolite were analysed by HPLC. The

purified Capsaicin is checked for cytotoxicity studies determining the potential toxicity of Capsaicin showing negligible toxicity.

Bioactivity of Capsaicin

As Phenols, flavonoids, Capsaicinoids and capsinoids present in chilli have been reported to exhibit anti-inflammatory agents [9, 10] and the response of macrophages play a central role in the inflammatory and serve as an essential interface between the innate and adaptive immunity [11]. In the inflammatory response process, macrophages release nitric oxide (NO). Once NO is formed in the cell, it can react with superoxide anions and form peroxynitrite, a potent oxidizing and nitrating molecule [12]. Excessive NO production leads to the development of many inflammatory related diseases [13, 14].

The phytochemicals present in chilli pepper trigger apoptosis of malignant cells. Capsaicin elicits the apoptosis pathway by repressing the plasma membrane NADH oxidoreductase enzyme in mitochondria [15,16]. Capsaicin also binds with the ATP generating coenzyme Q, inhibiting its activity and destabilizing the electron flow in the mitochondria, thus producing ROS and triggering apoptosis [17,18,19,20]. The carotenoid pigments present in the pepper such as capsanthin, capsorubin and cryptocapsin possess high free radical scavenging activity [21].

Anti-oxidants such as eugenol (clove), curcumin (turmeric) and capsaicin were experimentally demonstrated to manage the cellular oxidative stress due to their capability to block the reactive oxygen species production, anti-oxidant properties and interfere with signal transduction pathways and might decrease the risks of some tumours [22].

Hence the present study was conducted to meet the high-value demand and to optimize techniques for enhancement and purification of capsaicin to find out the effect of acetone crude extract of *Capsicum annum* and its Bioactivities.

Organization of the Paper

Section I contains the introduction of chilli and Bioactivity of the capsaicin compound, Section II contains the Materials and Methodology of the chilli extraction, purification and chromatographic and bioactive studies, Section III contains the results and discussion of the isolation, purification and bioactive study and Section IV contains the conclusion of all the studies.

II. MATERIALS AND METHODS

2.1 Collection of Seed varieties

Seeds of 3 Chilli varieties CO-1, K2 and Jwala were collected from Coimbatore Agricultural University. The seeds were treated with Panchakavya 3%, Vermicompost 3%, its consortia and Sodium azide 0.2% separately. 0.02% of Colchicine was sprayed to the 25day old treated seedlings to induce polyploidy and the First generation plants (M1) were raised. The untreated plants were taken as control. The seeds were harvested and grown-up to M5 generation. The variety showing highest capsaicin content was analysed by spectrophotometrically and later proceeded for further study.

2.2 Extraction of Capsaicinoids

The Panchakavya treated CO-1 variety chilli pods of the M5 generation was shade dried, powdered and Capsaicinoids was extracted using the method of [23] with slight modifications. 5 g of sample was dipped in 5ml acetone in a 120 mL glass bottle equipped with a Teflon lined lid and placed in a water bath at 80 °C for 4 hours, then swirled manually every hour. The sample was removed from the water bath, cooled to room temperature and left undisturbed. The supernatant layer of each sample (5 mL) was filtered through 0.45 µm filter paper into an HPLC sample vial using a 5 mL disposable syringe (Millipore, Bedford, MA, USA). The vial was capped and stored at 5 °C in a refrigerator until analysis.

2.3 Purification of Capsaicin

2.3.1 Thin layer Chromatography (TLC)

TLC is a chromatography technique used to separate non-volatile mixtures [24]. The CO-1 fractions were spotted in TLC 5x5 cm glass slide coated with silica gel 60 F254 to detect the presence of capsaicin. The dried glass TLC plates were activated by hot air oven at 100^{o}. Next TLC plates were immersed in the mobile phase (Hexane: ethyl acetate in the ratio 4:1 for capsaicin isolation) for 2min and removed from the mobile phase, dried and sprayed with iodine vapour for detection of Capsaicin, the spot was compared with standard Capsaicin spot and the Rf value were noted.

Calculation of Rf value:

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

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2.3.2 Silica gel Column Chromatography

The acetone Chilli extracts of Panchakavya treated CO-1 variety were subjected to Silica gel column chromatography for the isolation of the bioactive compound. An appropriate column sized 2.5cm diameter and 40cm length was used. It was washed with water and rinsed with solvent and then dried completely. Silica gel mesh size 70-150 was packed about 1/3rd of the column. TLC solvent system was chosen for the chilli extract was a Hexane and Ethyl acetate (4:1) was poured into the column up to ¾th level. Little cotton was placed on top of a silica gel- extract mixture pack to get a neat column

pack. Chilli extract (1g) was mixed with 1ml of running solvent separately and poured evenly in the rim of the silica gel using a micropipette. Each 2ml of the elution was considered as one fraction. The fractions were eluted at the rate of 1ml/min. The collected fractions were evaporated and the Rf value of the spot was identified again by TLC pre-coated plates. The fractions of same Rf value were pooled, weighed and stored at 4°C for further use.

2.3.3 High-Performance Liquid Chromatographic Analysis

The purity of the chosen bioactive fractions of capsaicin of 1mg/ml concentration was analysed by HPLC and the chromatographic conditions were as follows: Betasil C18 column (particle size 3 µm, dimension 150 × 4.6 mm) from Thermo Electron (USA), column temperature at 60 °C, sample temperature at 20°C, sample volume was taken as 5 µL, the wavelength at 222 nm for UV detection, mobile phase: binary mixture water-acetonitrile (CH3CN) at a 50:50 ratio, flow rate: 1.5 mL/min and the obtained standard curve plots of peak area were plotted against concentration. During HPLC sample analyses, the standard solution was injected every 10 samples to evaluate the retention time reproducibility and instrument calibration.

2.4 Cytotoxicity Activity of purified enhanced Capsaicin.

2.4.1 Cytological studies using onion root tips:

A stock solution of Capsaicin was taken for the different concentrations 0.2, 0.4, 0.6, 0.8, 1.0 ml to check the cytotoxicity of the cells. 1mm sized onion root tips were taken and soaked in different concentrations of Capsaicin. The root tips with a few ml of the Aceto-carmine stain was heated using a spirit lamp. The onion root tips were squashed and observed under a microscope. The cells were checked for abbreations and tabulated.

2.4.2 Cytological studies using Cell line and culture:

The *Vero* cell line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

In Vitro assay for Cytotoxicity activity (MTT assay) [25]

Cells (1 × 105/well) were plated in 24-well plates and incubated in 370C with 5% CO2 optimum condition. After the cell reaches the confluence, the capsaicin with concentrations 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µg/ml was added and incubated for 24hrs, 48 hrs and 72 hrs. After incubation, the capsaicin was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells of the plate. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were carried out and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability } = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}}\ x\ 100$$

Graphs were plotted using the Cell Viability % at Y-axis and sample concentration at X-axis. Control of the cell and control of the sample is included in each assay to compare the full cell viability assessments.

2.5 Bioactivity of Capsaicin

2.5.1 Assessment of in vitro Anti-inflammatory activity

2.5.1.1 Inhibition of albumin denaturation

The anti-inflammatory activity of Capsaicin compound was studied by using inhibition of albumin denaturation technique according to [26, 27] followed with minor modifications. The reaction mixture was consists of the test sample(capsaicin 1mg/1ml concentration) and 1% aqueous solution of bovine albumin fraction, the pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample was incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm. (UV-Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was carried out in triplicate.

The inhibition percentage of protein denaturation was calculated as follows:

$$\text{Inhibition percentage} = \frac{(\text{Abs Control} - \text{Abs Sample})\ \text{X}\ 100}{\text{Abs control}}$$

2.6 ANTICANCER ACTIVITY

2.6.1 Cell line and culture:

Hela cell line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

In Vitro assay for Anticancer activity (MTT assay) [25]

Cells (1 × 105/well) were plated in 24-well plates and incubated in 370C with 5% CO2 optimum condition. After the cell reaches the confluence, the Capsaicin with concentrations 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µg/ml was added and incubated for 24hrs, 48 hrs and 72 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells

of the plate. The absorbance was measured at 570nm with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was graphically determined. The % cell viability was calculated using the following formula:

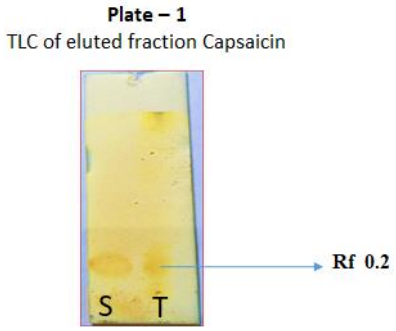
$$\text{Cell viability \%} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} x\ 100$$

Graphs were plotted using the Cell Viability % at Y-axis and the sample concentration at X-axis. Control of the cell and control of the sample is included in each assay to compare the full cell viability assessments.

III. RESULTS AND DISCUSSION

Quantification of capsaicin in chilli varieties CO-1, K2, Jwala was estimated by spectrophotometric method. CO-1 variety of Panchakavya treatment was found to have high capsaicin content and thereafter used for further isolation and purification studies.

3.1 Isolation by Column chromatography and identification by TLC method.



S – Standard Capsaicin
T – CO-1 Capsaicin

Isolation of the bioactive compound (capsaicin) was done by column chromatography. The fractions of Panchakavya treated CO-1 variety from 1 to 150 were eluted at the rate of 1ml/min. Fractions of different elutions were tested by TLC analysis. Fractions 115 to 135 showed the presence of capsaicin compound and these fractions were pooled together for purification studies. Pooled fractions were spotted in TLC plate. After 2mins TLC plate was dried and capsaicin band was marked by placing the TLC plate in iodine vapour and Rf value was noted as 0.2 compared with standard capsaicin.

3.2 Purification by HPLC Analysis

The chromatogram of untreated control of CO-1 variety indicated a peak area 41.48% observed at the retention time of 1.337 min (Fig 1). The Panchakavya treated CO-1 variety showed Capsaicin peak of about 72.93% purity in 1.368min (retention time) as revealed in HPLC chromatogram in Fig 2. A sharp increase of capsaicin content was observed in treated CO-1 variety of Panchakavya treatment. There is enhanced production of Capsaicin when compared with control and constant increase in purity and yield absorbed throughout the generation.

In [28]reported the ethanolic extracts of Capsicum fruits (Hot chilli) showing capsaicin peak eluted at 4.69 mn. The maximum absorbance corresponds to max = 228 nm and this study shows the quantitative contents of capsaicin as (4,249.0 µg/g).

In [29] analysed 16 types of capsicum fruits in Methanol extract grown at different regions in Nepal and estimated their capsaicin content by high-performance liquid chromatography was found to range from 2.19 to 19.73 mg/g of dry weight at the wavelength of 281nm.

In [30] analysed capsicum annum by accelerated solvent(methanol) extraction method giving rapid separation of capsaicin at retention time (3.98 min) and the contents of capsaicinoids were found in the range of 2,307.0-9047.3 mg/g recording emission wavelength at 310 nm.

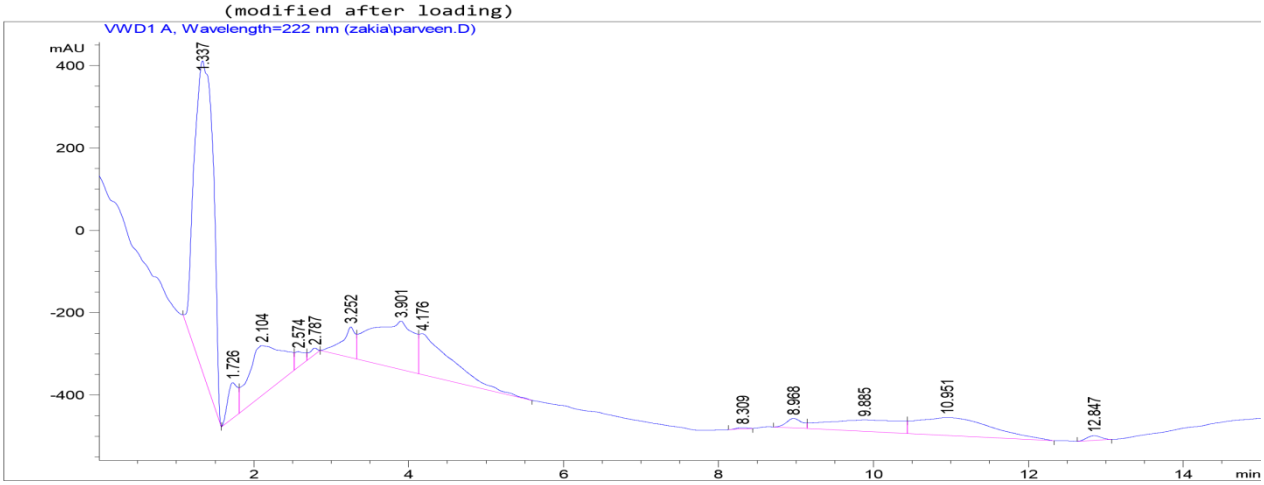


Figure 1: Chromatogram of the Capsaicin (control) solution

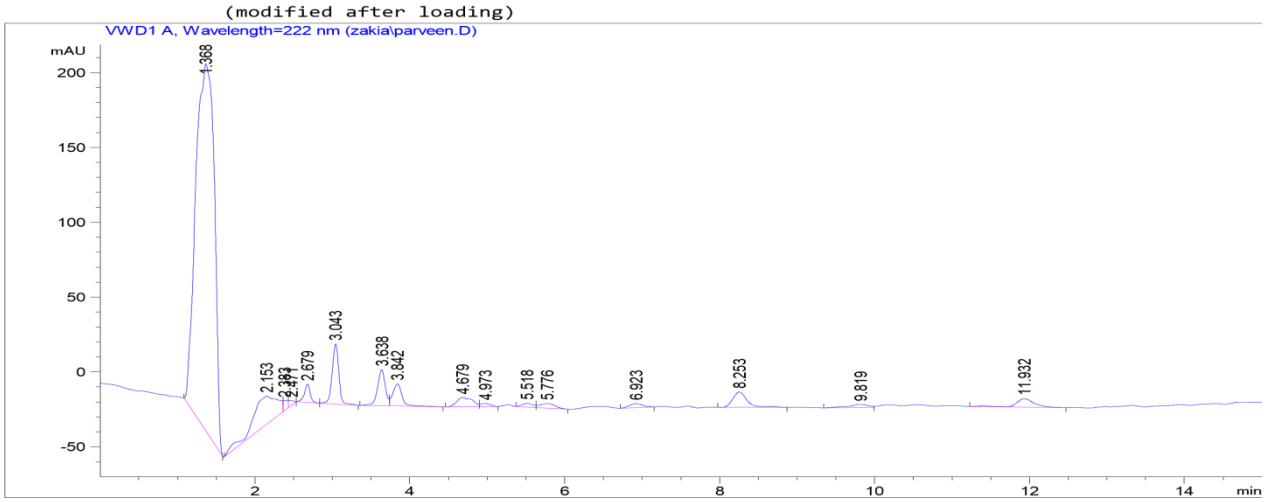


Figure 2: Chromatogram of the induced Capsaicin (treated -5th generation)

3.3 Cytotoxicity of purified capsaicin

3.3.1 Cytotoxicity in onion root tip cells by root tip squash method.

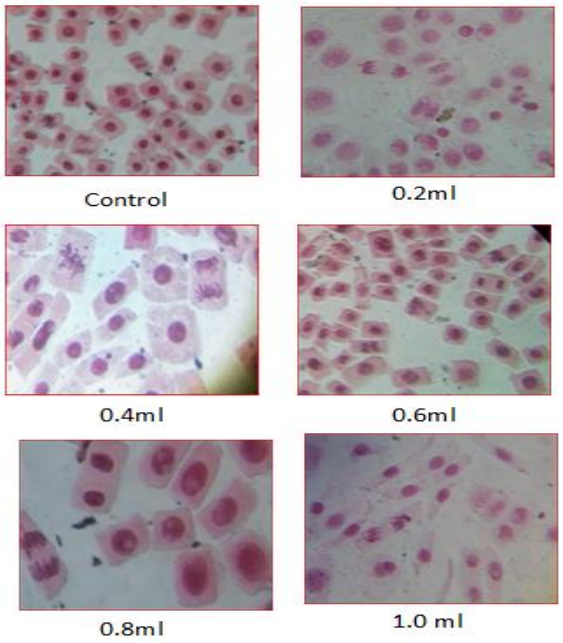
The cytotoxicity was studied in **purified capsaicin** of Panchakavya treated CO-1variety using onion root tips. Different concentrations of Capsaicin compound were used such as 0.2, 0.4, 0.6, 0.8, 1.0 ml respectively were used to study the chromosomal aberrations.

Table 1: Cytotoxicity effect of capsaicin in onion root tips

S.No	Concentrations in ml/ 1ml of water	Observations
1	0.2	No abbreations
2	0.4	No abbreations
3	0.6	No abbreations
4	0.8	No abbreations
5	1	No abbreations

Plate- 2

Cytotoxicity testing in onion root tips



3.3.1 Cytotoxicity in onion root tips

The onion root tips did not show have any abnormalities in any of the cell stages and hence proved to be non-toxic to the cells.

[31] reported the Mitotic Index (MI) of root meristem cells of *Allium cepa* treated with the ethanolic extracts of *Capsicum frutescens* in concentration (13.6, 13.4 and 10.50) does not show variation among the analyzed species. MI of *Capsicum frutescens* did not differ significantly from mineral water as a negative control (13.2) even concerning the extract. From this study, it is concluded that the Capsaicin of different concentrations does not show any abnormalities and hence the treated capsaicin is non-toxic to the cells.

3.3.2 In vitro assay for cytotoxicity

The cytotoxicity activity is carried out by using the MTT assay. Cell lines derived from NCCS, Pune. Percentage cell viability of cell line was carried out by using the tetrazolium dye exclusion technique. (Table 3) it showed that IC50 value and the viability of the Vero cell line is 58.79% in control and 60.39% in treated in 100 µg/ml concentration, which is most suitable to perform cytotoxicity study. The percentage viability was found to be increasing with decreasing concentration of capsaicin. From the results, the efficiency of treated capsaicin was more efficient when comparing the non-treated capsaicin.

[32 studied ethanolic extracts of *Capsicum annum* and isolated 4 different genotypes of hot species of *Capsicum annuum* L. and capsaicin solutions in different concentrations and time of exposures were investigated. Capsaicin (500nM, 1 µM, 10µM) did not influence significantly viability or cell death of B104 cells when it was applied for 1 or 24 hours incubation.

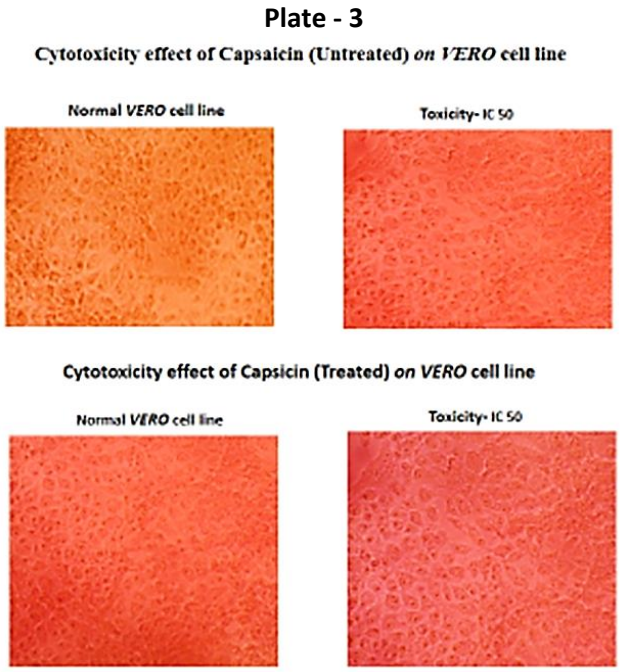
[33]studied cytotoxicity activities of Flavonoid extract of *Capsicum annum L.* Seeds and results showed that the flavonoid compound had no cytotoxicity against the human red blood cells in all the concentration tested

(ranging between 0.5-200 ppm), by using DMSO solution as a control.

From the above results, it denotes that the percentage viability of cells was found to be increasing with decreasing concentration of capsaicin and efficiency of treated capsaicin was more efficient when compared with non-treated capsaicin.

Table 2: Cytotoxicity effect of Capsaicin (Untreated and treated) *on VERO* cell line

S.No	Concentration (µg/ml)	Untreated		Treated	
		Absorbance (O.D)	Cell viability (%)	Absorbance (O.D)	Cell viability (%)
1	100	0.478	58.79	0.491	60.39
2	50	0.511	62.85	0.533	65.55
3	25	0.562	69.12	0.578	71.09
4	12.5	0.614	75.52	0.621	76.38
5	6.25	0.657	80.81	0.677	83.27
6	3.12	0.691	84.99	0.723	88.92
7	1.56	0.742	91.26	0.787	96.8
8	0.78	0.787	96.8	0.809	99.5
9	Cell control	0.813	100	0.813	100



3.3 Anti-inflammatory studies

3.3.1 Inhibition of albumin denaturation

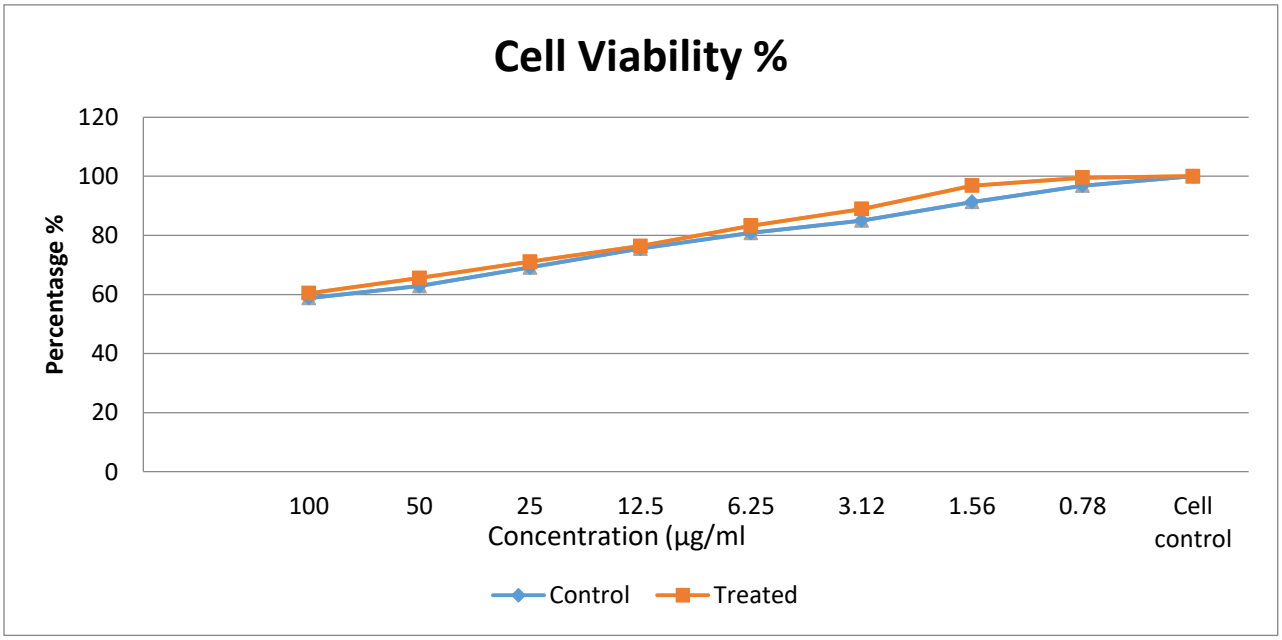


Figure 3: Cytotoxicity effect of Capsaicin (Untreated and treated) on Vero cell line

3.4 Bioactivity Results

3.4.1 Anti-inflammatory activity

The anti-inflammatory activity of Capsaicin compound was studied by using inhibition of albumin denaturation technique and it was efficient in inhibiting heat-induced albumin denaturation. Maximum inhibition of control and treated was observed as 76.26% and 81.23% in 100 µg/ml. Respectively Aspirin was used as a standard anti-inflammatory drug which shows 68.4% as maximum inhibition in 100 µg/ml. Results are shown in Table – 1.

According to [34], the Red pepper stalk extract at a concentration of 20 µg/mL, exhibited the most significant NO inhibitory effect (53.5%) in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.). Capsaicin, a major ingredient of hot pepper, is known to exhibit an anti-inflammatory property.

[35], reported anti-inflammatory activity of curcumin, capsaicin and their combination in agar suspension induced leukocyte mobilization assay, the combined curcumin and capsaicin had shown 39.5±1.58 % of inhibition compared to individual curcumin and capsaicin, which showed moderate inhibition of 16.0 and 21.6 % respectively.

[36], studied anti-inflammatory properties of Capsaicin and Ethyl Acetate extract of *Capsicum frutescens* and showed capsaicin (2.5 mg/kg, i.p.) significantly inhibited paw swelling at (95%) compared to distilled water-treated control.

[37], observed Anti-inflammatory activities of Capsaicin in the methanolic extracts of Bhut Jolokia variety and determined the bioactivities of Capsaicin using in vitro Cyclooxygenase enzyme inhibitory assays and the results showed Cyclooxygenase -1 enzyme by 50% inhibition at 42 µg/mL. Similarly, the Cyclooxygenase -2 enzyme inhibitory concentration at 50% for Capsaicin was at 75 µg/mL, respectively.

From the above results of anti-inflammatory activity, it is concluded that the treated capsaicin has more efficient in inhibiting the protein denaturation.

Table 3: Effect of Capsaicin on albumin denaturation

Concentration (µg/ml)	Positive control % of inhibition	Untreated % of inhibition	Treated % of inhibition
20	47.3	57.84	68.77
40	50.4	61.34	70.64
60	59.3	65.51	76.87
80	62.7	70.57	79.58
100	68.4	76.26	81.23

Table 4: Anti-cancer activity of Capsicin (Untreated) *on HeLa* cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	100	Neat	0.268	34.89
2	50	1:1	0.312	40.62
3	25	1:2	0.371	48.30
4	12.5	1:4	0.433	56.38
5	6.25	1:8	0.496	64.58
6	3.12	1:16	0.550	71.61
7	1.56	1:32	0.602	78.38
8	0.78	1:64	0.659	85.80
9	Cell control	-	0.768	100

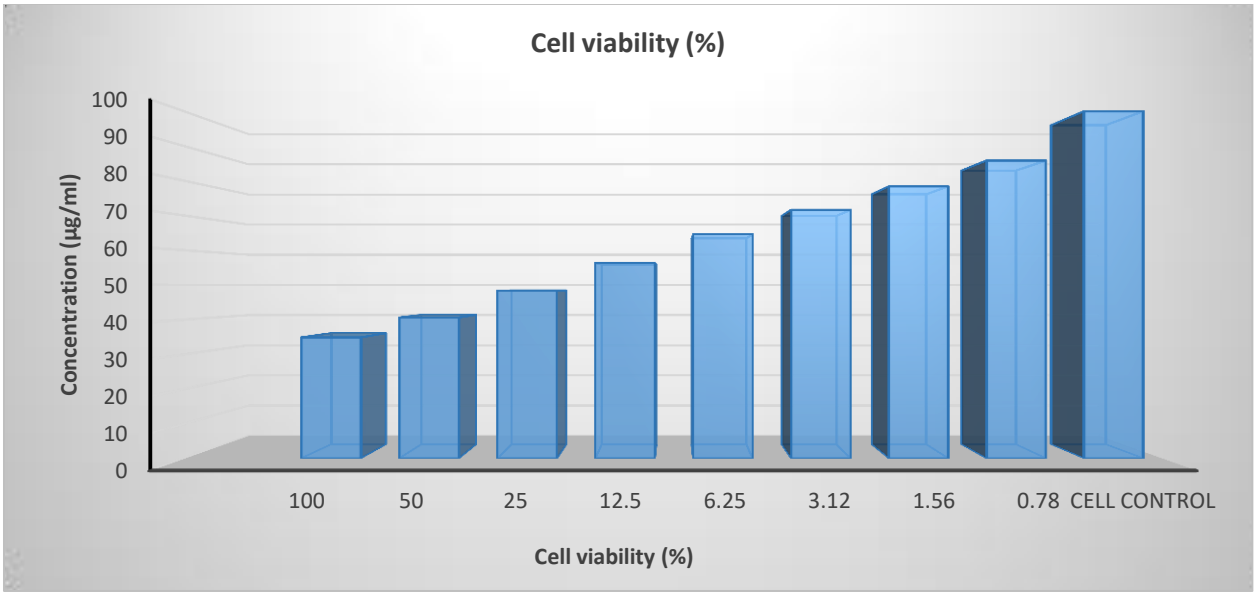


Fig 4: Anti-Cancer activity of Capsaicin (Untreated) *on HELA* cell line

Plate – 4

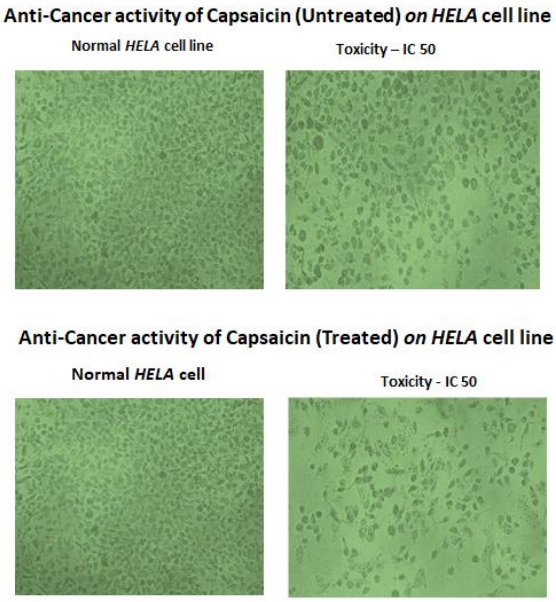


Table 5: Anti -Cancer activity of Capsaicin (Treated) *on HELA* cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	100	Neat	0.161	20.96
2	50	1:1	0.214	27.86
3	25	1:2	0.277	36.06
4	12.5	1:4	0.329	42.83
5	6.25	1:8	0.383	49.86
6	3.12	1:16	0.436	56.77
7	1.56	1:32	0.491	63.93
8	0.78	1:64	0.543	70.70
9	Cell control	-	0.768	100

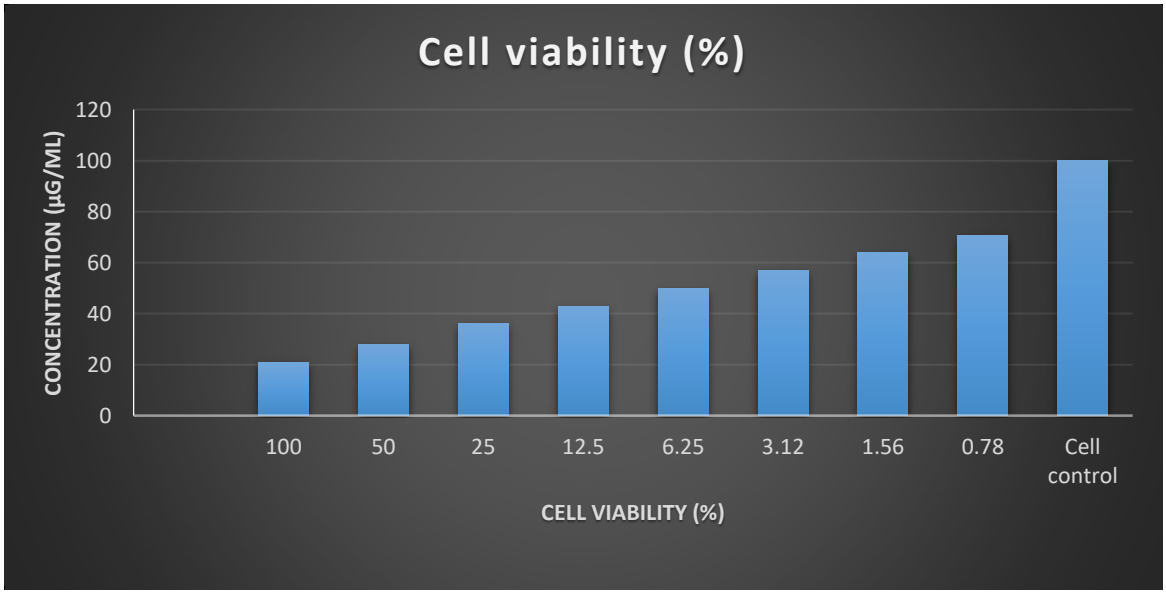


Fig 5: Anti-Cancer activity of Capsaicin (Treated) *on HeLa* cell line

3.4.2 Anticancer activity

The effect of Capsaicin compound on the viability of cancer cells was determined by MTT assay (3-[4,5 – dimethylthiazol – 2]-2,5- diphenyl tetrazolium bromide) assay on *Hela* cells. The IC_{50} doses were determined by exposing cells to various concentrations of the compound from 100µg, 50µg, 25µg, 12.5µg, 6.25ug, 3.12µg, 1.56µg, 0.78µg for 24hrs. The IC_{50} dose was defined at the concentration of the compound at which there was 50% less growth determined. In untreated capsaicin shows IC_{50} dose in 25µg/ml whereas in treated capsaicin showed IC_{50} dose in 6.25µg/ml. Which we compared with cisplatin with an IC_{50} of 10.01µg/ml.

[38] Showed reduction in the cell viability through MMT assay in *Capsicum chinese*. In his study Control, HepG2 cells showed high proliferation that has been taken as 100% and treatment with the Acetonitrile extracts suppressed the cell viability up to 50% at 50 µg/ml concentrations when compared to the untreated cells and it also significantly suppressed the release of LDH, LPO and NO production in a dose-dependent manner this result indicating that the plant extracts play a protective role on the liver diseases.

[39]reported the results of the cancer activity of capsaicin of Acetonitrile extract was confirmed by MTT assay and HepG2 cells showed high proliferation that has been treated with the Acetonitrile suppressed the cell viability up to 50% at 50 µg/ml ions when compared to the

untreated cells. The treatment with Acetonitrile extract also suppressed the release of Lactate dehydrogenase at 57.95 ± 5.1% for HepG2 cell lines and lightened the anticancer property

[40] observed Anti-oxidant activity of the *Capsicum annum* of Soxhlet dry pulp methanol extract which showed a significant in-vitro effect on *HeLa* cells (1.9µg/mL) and PC3 (<1 µg/mL) cells with a moderated impact on fibroblasts (26.1µg/mL); whereas, Soxhlet fresh red sweet pepper had a significant effect on MCF-7 cell line (2.1µg/mL) with a moderated impact on fibroblasts (25.9µg/mL). The higher antioxidant activity was found for MFP- Maceration fresh red sweet pepper (80.3%) and SFP- Soxhlet fresh red sweet pepper extracts (75.5%).

[41] reported the effect of capsaicin on transcription factors in the 3T3-L1 cell line were treated with various concentrations of capsaicin (50, 100, 150, 200 and 250 µM) during each stage. Capsaicin treatment on 3T3-L1 preadipocytes during proliferation decreased the cell viability in all treated groups ($p<0.05$). This reduction started at 50 µM capsaicin applied to group and then, cell viability continued to reduce on dose-dependent manner but this decline was not statistically significant compared with 50 µM capsaicin applied group ($p>0.05$). The percentages of cell viability comparing with the control group were 49.22 ± 2.04%, 46.34 ± 2.07%, 45.54 ± 2.29%, 43.18 ± 2.36% and 41.94 ± 1.37% for the 50, 100, 150, 200 and 250 µM capsaicin treatments respectively

IV. CONCLUSION

In this study of polyploidy induction, Panchakavya treated *Capsicum annum* (Co-1) variety shows the prominent results of increased biochemical contents compared to all the variety and treatments and it was also chosen due to their bioactive components which can reduce the risk of cancer through their antioxidant and anti-inflammation through their anti-tumorigenic activity. However, Panchakavya treated *Capsicum annum* (Co-1) variety show promising results as an anticancer agent due to its relatively high toxicity on the *HeLa* cell line (Cancer cells) and at this same concentration capsaicin compound also shows low toxicity on the Vero (normal cells) cell line.

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