Anti-T-Cell Acute Lymphoblastic Leukaemia Effect Using Methanolic Fraction of the Leaves of Tomato (*Solanum Lycopersicum L*)

Krishan Raj Singh

SOBAS, Sanskriti University, Mathura, Uttar Pradesh

Email Id- hodbio-tech@sanskriti.edu.in

ABSTRACT: "T-cell Acute Lymphoblastic Leukaemia" (T-ALL) has emerged as one the most infamous of all the cancers currently afflicting mankind. However, its treatment is marred by the high cost and many side-effects which in turn have spurred the need for alternative anti-cancer agents. Nowadays many anti-cancer drugs have been isolated from plants. In this paper, Methanolic fraction of the leaves of Tomato (Solanum lycopersicum L) has been evaluated for its anti-T-ALL activity. The methanolic fraction of the Tomato leaves was treated on a T-ALL; MOLT-3 and on a normal human embryonic kidney (nHEK) and its growth inhibitory activity was noted. The IC_{50} value after 24 h came at 40.48 µg/ml on MOLT-3 cells however; no activity was seen on nHEK cell lines. Based on these findings, it can be safely assumed that the methanolic fraction of Tomato leaves can be utilized as an anti- T-ALL agent. The discovered carotenoids and phenolic acids, as well as a number of minor chemicals exerting their synergistic impact, might be attributed to these biological characteristics.

KEYWORDS: Apoptosis, Cells, IC_{50} value, Methanolic fraction, MOLT-3, T-Acute Myelogenous Leukaemia (T-ALL), Tomato leaves.

1. INTRODUCTION

One hall mark of "T-Cell-Acute Lymphoblastic Leukaemia" (T-ALL) is its high rate of relapse and development of chemo-resistance. One of the major agents used for treating ALL is Prednisolone, a glucocorticoid. However, resistance soon develops against this agent as well, necessitating the importance of targeting of factors which prevent chemotherapeutic agent instigated cell death (apoptosis) in ALL cells[1]–[5].

One of the hallmarks of cancer is the overexpression of BCL-2 family proteins namely MCL-1, BCL-X and BCL-2, which has also been observed in ALL cells as well. Thereby BCL-2 family could be an attractive target for agents targeting ALL cells. One of the many promising agents of botanical origin having known anticancer properties is Resveratrol (3, 4, 5-trihydoxystilbene) which is phytoalexins member, found in berries, grapes, red wine etc. A promotion in BAX: BCL-2 protein ratio helps to induce apoptosis as BAX is a known pro-apoptotic or cell death protein [1].

1.1 Signaling routes active in the T-cell Acute Lymphoblastic Leukaemia:

Cross-talks between various aberrant signalling pathways have been implicated in various ALL cells and some of them have discussed in this paper. There is an (Figure 1) increase in kinase signalling via the (i) AKT/PI3K/mTOR [AKT/PI3K/ /mTOR] route, owing to *PTEN* mutations/deletions, *PTPN2* deletion, *PIK3R1* and/or *AKT1* alterations (ii) "IL-7R/ STAT/JAK pathway", via alterations that activate "IL-7Rα" gene, "Janus kinase 1" (JAK1), "Janus kinase 3" "JAK3" or "STAT5B"; (iii) "RAS/MAPK" signalling via mutations in the 'neurofibromin 1" ("NF1") and KRAS; and via (iv) chimeric ABL1 fusion genes such as nucleoporin 214kDa-Abelson murine leukaemia viral oncogene homolog 1 (NUP214)-ABL1 and ETS Variant 6-Abelson murine leukaemia viral oncogene homolog 1 (ETV6)-ABL1; (2) altered epigenetic modulation via mutations involving (i) PHF6; (ii) PRC2 components EZH2, EED and SUZ12; (iii) KMT2A [also called ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX)]; (3) functional mutations Comprising "CCR4-NOT transcription complex subunit 3" (CNOT3), "Ribosomal protein L10" (RPL10), "Ribosomal protein L5" (RPL5), "Ribosomal protein L22" (RPL22) (4) alteration within oncogenic miRNAs (onco-miRs) namely miR-20a, or miR-26a, or miR-19b, or miR-223 or miR-92, and or long noncoding RNAs (lncRNAs) namely "leukaemia-prompted non coding activator RNA 1 " ("LUNAR1") [3]–[5]. Moreover, factors like growth factors, hypoxia etc promotes cellular growth and proliferation via activation of signaling pathways like PI3K/ mTOR signalling/AKT, signalling of AMP-activated protein kinase (AMPK) etc. [2].

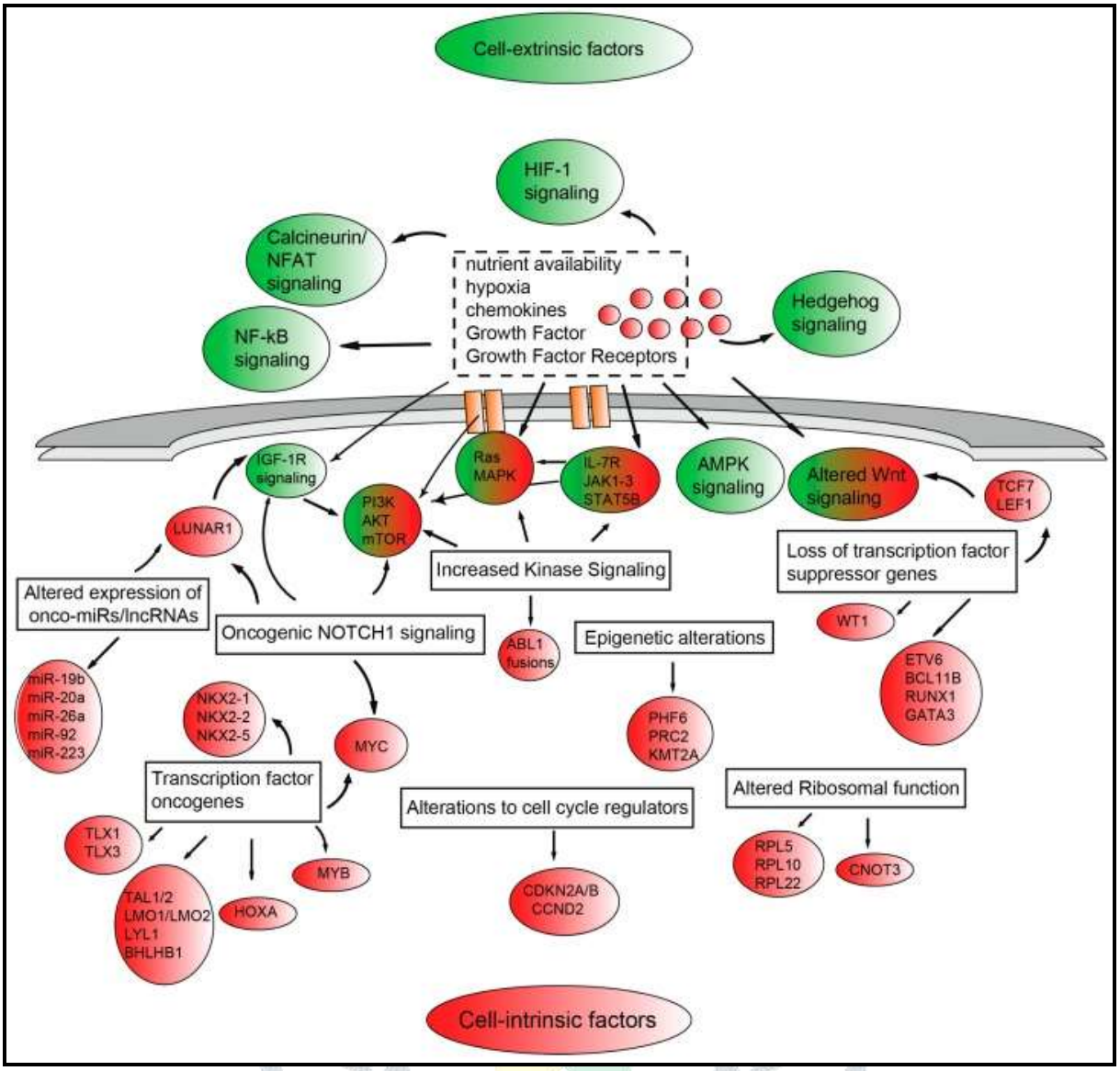

Figure 1. Flowchart based representation of signalling routes active in "T-cell Acute Lymphoblastic Leukaemia" ("T-ALL"), divided on basis of green shaded coloured cell-extrinsic and shaded red coloured cell-intrinsic aspects or dappled green and red coloured combined. (Courtesy [1]).

There are many genetic faults in the T-cell acute lymphoblastic leukaemia (T-ALL) such as 9p deletions which results in the deactivation of CDKN2B (p15) and CDKN2A (p16) and the T-cell receptor (TCR) genes that's gets affected by chromosomal translocations which all leads to faulty expression of 1 particular transcription factor namely TLX1, TLX3, TAL1 etc. Nearly 100 gene shave been identified that have the potential to be mutated in T-ALL namely by the usage of transcriptome and exome sequencing or complete-genome sequencing. It was observed that the genes; CDKN2A/2B and NOTCH1 were mutated in 50% of "T-ALL" cases whereas a great number of genes were altered at smaller occurrence. Centered over such information, there has been a report of nearly 10 genetic wounds which converts the normal T cells into leukaemia cells having deviant differentiation, enhanced proliferation and survival features with homing properties, altered metabolism and cell cycle. All such modifications aids "T-ALL" cells to survive, proliferate via altering their nature which leads to the growth of stem cell like properties of these transformed cells.

1.2 Transcription factors:

Various T-ALL groups, identified through certain transcriptional identity and deviant countenance of one of transcription factors, due to chromosomal fault have been reported via gene expression pattern of T-ALL cases. Leading subclass is addressed via deviant TAL1 countenance (along with LMO1/LMO2), whereas other groups have shown reciprocal complete expression of NKX2-1, TLX3, TLX1, LMO2, HOXA9/10.. Moreover, the T-cell antecedent group of ALL ("ETP-ALL") ties up with to undeveloped T-ALLs that expresses ETP/stem cell genes which is identified by ectopic expression of LYL12; and also, such as ETV6

and RUNX1 which is hematopoietic transcription factors are often mutated in this genetically non-uniform group.

Four major mechanisms that causes deviant expression of transcription factors in T-ALL have been indicated below: (1) chromosomal translocations that involve one of TCR genes, (2) regulatory sequences having chromosomal rearrangements (3) transcription factors having amplification/duplication (4) enhancers which are small insertions or mutations having a role in generating novel regulatory sequences. The latter mechanism was revealed due to alteration within chromatin constituent nearby "TAL1 gene", suggestive for location of novel surger area.

1.3 T-ALL action:

Currently, T-ALL's curation regime comprises of great power grouping chemo. which though ends within survival for infant patients have very high long-term and short-term side effects. Moreover, there is a small chance of a relapse occurring mainly due to the presence of a minor genetic subclone or by the clonal expansion of an ancestral cell or maybe due to secondary malignancies. One of the major causes of relapse is due to triggering mutations in the gene encoding for cytosolic -nucleotidase II (NT5C2) by utilizing whole-exome sequencing of the T-ALL cells from relapsed patients. Incidentally, NT5C2 is an enzyme which inactivates nucleoside-analog chemotherapy drugs especially 6-thioguanine (6-TG) & 6-mercaptopurine (6-MP). As both ABL1 and JAK kinases are many a times triggered within "T-ALL", obtainable "JAK restrictors" & "ABL1 restrictors" (imatinib, nilotinib, dasatinib,) may further be reused for curation of "T-ALL" circumstances along with alterations causing ABL1 or JAK/STAT actuation. Many preclinical studies have shown the role of tofacitinib or ruxolitinib for repression of "T-ALL cells" with JAK1/ JAK3, IL7R alterations, while some action of dasatinib or imatinib for the therapy of NUP214-ABL11 T-ALL has been reported.

2. LITERATURE REVIEW

In this modern era, green vegies have really hailed as panacea for good health. Out of these, tomatoes have caught the attention of researchers world-wide for its rich source of phyto-chemicals. Tomato (*Solanum lycopersicum*) of the nightshade family has generally a fruit of red colour and fruit is itself rich in Lycopene and other carotenoids such as Phytofluene, α-arotene, β-carotene, Phytoene, Neurosporene and Gammacarotene. The various phyto-chemicals present in the tomato fruit has been tabulated in Table 1, whereas the various chemical structures of the carotenoids present have been illustrated in Figure 2 while the chemical structures of various carotenoids found in the tomato fruit has been illustrated in Figure 3. Moreover, an anti-cancer property of the methanolic extract of tomato leaves has already been reported which has formed the basis of further investigation in this paper [6], [7].

Table 1 Composition of phyto-chemicals in a tomato fruit. The tomato fruit is known for its rich source of phyto-molecules, some of which has been tabulated in this table [7].

Concentration	Carotenoid	Concentration	Polyphenol
7.8-18.1	Lycopene	0.9-18.2	Naringenin chalcone
1.0-2.9	Phytoene	0.5-4.5	Rutin
0.2-1.6	Phytofluene	0.7-4.4	Quercetin
0.1-1.2	ß-Carotene	1.4-3.3	Chlorogenic acid
0.05-0.3	ɣ-Carotene	0.1-1.3	Hydroxycinnamic acid
0-0.2	Δ-Carotene	0-1.3	Salipurol
0.09	Luteine	0-0.8	Nicotiflorin
0-0.03	Neurosporene	0-0.6	Coumaric acid

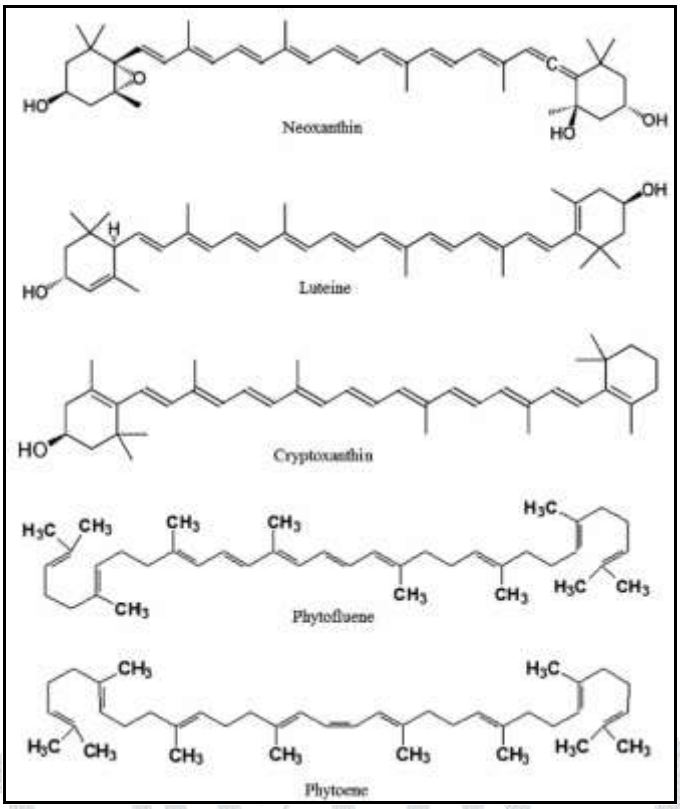

Figure 2 Chemical structures of various phytochemicals present in the tomato fruit (Courtesy [8]**). All these phyto-molecules play an important role in human health.**

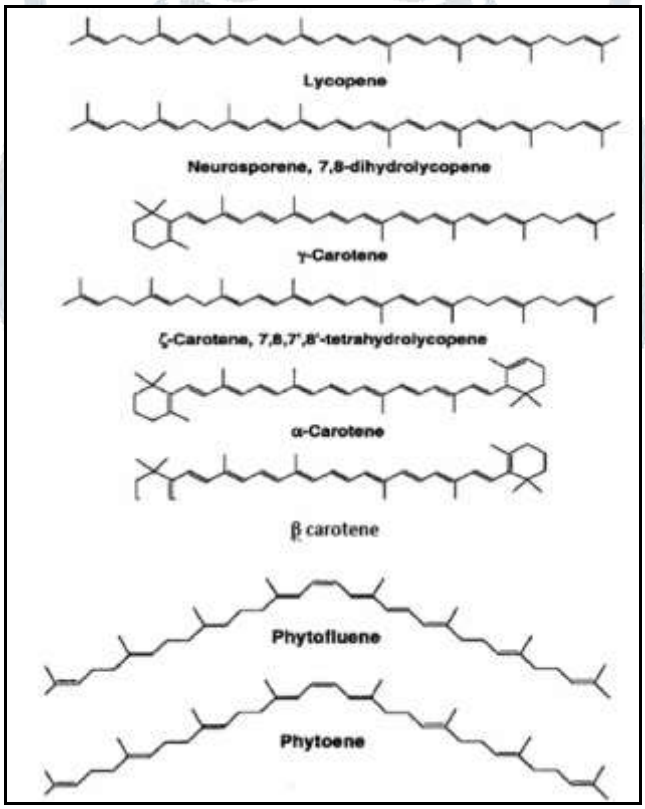

Figure 3 Chemical structures of the myriad of carotenoids found in the tomato fruit. All these phyto-molecules play an important role in human health (Courtesy [8]).

3. METHODOLOGY

3.1 Experimental design:

The experiment design is inspired from earlier reported publications [6]. Firstly, the methanolic extracts of the tomato leaves were suspended in DMSO and its growth inhibitory potential was observed on "T-ALL" cell lines; MOLT-3 and normal human embryonic kidney; (nHEK) cells for 24 h in order to obtain an IC_{50} value. The results would be analysed for statistical significance.

3.2 Chemicals and reagents:

Dimethyl sulfoxide (DMSO), MTT, Cell culture media, Foetal Bovine Serum (FBS), Antibiotics were availed commercially.

3.3 S. lycopersicum leaves methanol extract preparation

Leaves from *S. lycopersicum* plants were procured, oven dried, pulverized and then suspended in methanol in a 1:20 ratio (w/v) ratio for 48 h. The mixture was then filtered and the filtrate was re-suspended in cell culture grade DMSO for future use in a process as reported earlier [6].

3.4 Cell culture:

Human T-ALL cell line MOLT-3 and the "normal human embryonic cell lines" ("NHEK") procured through "National Facility of Animal Tissue and Cell Culture", "Pune", "India" followed by culturing over RPMI 1640 ("Roswell Park Memorial Institute") media along with 105% FBS and antibiotics under 5% CO2 conditions at 37°C as reported earlier [9], [10].

3.5 *Cell viability assay*:

Both the MOLT-3 and NHEK-293 cells (1×10^4) were administered with varying concentrations of methanolic extract of tomato leaves (0, 10, 20, 30, 40 and 50 µg/ml) for 1day and following which cells viability was noted by conducting MTT as reported earlier [9], [10].

3.6 Statistical test:

Experiments were conducted for thrice and value of statistical significance was considered over $p < 0.05$ [9], [10].

4. RESULTS AND DISCUSSION

4.1 Growth Inhibitory role of methanolic extract of tomato leaves on MOLT-3 cells:

To understand the role of the tomato leaves' methanolic extract against "T-ALL", compounds were observed for their growth inhibitory effect on MOLT-3 cell line by undergoing MTT assay. Further, after 24 h curation, observation concluded, methanolic extract showed a concentration dependent growth inhibition in MOLT-3 cell lines, with an IC_{50} values of 40.48 µg/ml, respectively (Figure 4) without any growth inhibitory activity on the Normal anthropoid embryonic kidney 293 ("HEK-293") cell lines (data not shown). This implies that methanolic extract of tomato leaves unlike on T-ALL cells has no growth inhibitory activity on normal cells.

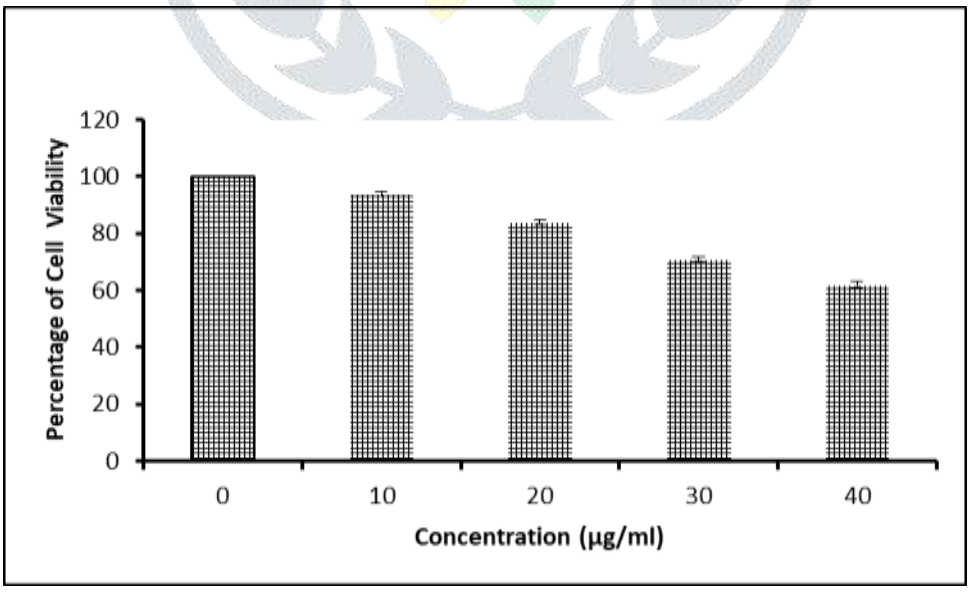

Figure 4 Graph showing growth inhibitory efficacy of methanolic extract of tomato leaves. The T-ALL cells; MOLT-3 showed a dose dependent loss in the total percentage of viable cells after 24 h. The values were statistically significant with respect to the control or un-treated cells with P<0.05.

A quick literature search has shown that methanolic extract of tomato leaves have shown growth inhibition against breast cancer cells; MCF-7 after 48 h whereas in this paper, methanolic extract of tomato leaves has shown growth inhibition against the T-ALL cells; MOLT-3 after 24 h. The inference here is that the tomato

leaves' methanolic extract showed anti-T-ALL activity as well which can be used to develop it as a lead agent in the future.

5. CONCLUSION

T-ALL has emerged as one of the major causes of lethality in Leukaemia patients. Most of the agents used to treat cases of T-ALL have high toxicity thereby there is an urgent need for development of safer alternatives. Of late, medicinal properties of plants are being investigated. Tomato (*Solanum lycopersicum*) is a common vegetable plant. There are already previous reports of the anti-cancer property of the tomato which owes its basis in the plethora of phytochemicals in the tomato fruit. In this paper, the anti-cancer role of its methanolic portion has been evaluated. Firstly, the tomato leaves were collected and dried in an oven following which they were grinded and suspended in methanol. The suspension was then filtered and the filtrate was suspended in DMSO till further use.

The methanolic extract was then evaluated for its growth inhibitory property on a T-ALL cell line; MOLT-3 and on a normal human embryonic kidney cell line (nHEK). The methanolic fraction induced growth inhibition in MOLT-3 in amount reliant way after 24 with IC_{50} value at 40.48 µg/ml. However, no such activity was noticed on the nHEK implying that the methanolic extract has anti-T-ALL activity and doesnot affect the normal cells. As tomato is a very commonly used vegetable, thereby the potential lead agents against T-ALL can be produced commercially.

REFERENCES

[1] T. Khanzadeh *et al.*, "Investigation of BAX and BCL2 expression and apoptosis in a resveratrol- and prednisolone-treated human T-ALL cell line, CCRF-CEM," *Blood Res.*, 2018, doi: 10.5045/br.2018.53.1.53.

[2] T. Girardi, C. Vicente, J. Cools, and K. De Keersmaecker, "The genetics and molecular biology of T-ALL," *Blood*. 2017, doi: 10.1182/blood-2016-10-706465.

[3] J. Zhang *et al.*, "The genetic basis of early T-cell precursor acute lymphoblastic leukaemia," *Nature*, 2012, doi: 10.1038/nature10725.

[4] B. Gerby *et al.*, "SCL, LMO1 and Notch1 Reprogram Thymocytes into Self-Renewing Cells," *PLoS Genet.*, 2014, doi: 10.1371/journal.pgen.1004768.

[5] J. M. Navarro *et al.*, "Site- and allele-specific polycomb dysregulation in T-cell leukaemia," *Nat. Commun.*, 2015, doi: 10.1038/ncomms7094.

[6] W. D. Wan Chik, A. Amid, and P. Jamal, "Purification and cytotoxicity assay of tomato (Lycopersicon esculen turn) leaves methanol extract as potential anticancer agent," *J. Appl. Sci.*, 2010, doi: 10.3923/jas.2010.3283.3288.

[7] R. Martí, S. Roselló, and J. Cebolla-Cornejo, "Tomato as a source of carotenoids and polyphenols targeted to cancer prevention," *Cancers*. 2016, doi: 10.3390/cancers8060058.

[8] R. Perveen, H. A. R. Suleria, F. M. Anjum, M. S. Butt, I. Pasha, and S. Ahmad, "Tomato (Solanum lycopersicum) Carotenoids and Lycopenes Chemistry; Metabolism, Absorption, Nutrition, and Allied Health Claims—A Comprehensive Review," *Crit. Rev. Food Sci. Nutr.*, 2015, doi: 10.1080/10408398.2012.657809.

[9] S. Mallick, B. C. Pal, J. R. Vedasiromoni, D. Kumar, and K. D. Saha, "Corchorusin-D directed apoptosis of K562 cells occurs through activation of mitochondrial and death receptor pathways and suppression of AKT/PKB pathway," *Cell. Physiol. Biochem.*, vol. 30, no. 4, pp. 915–926, 2012, doi: 10.1159/000341469.

[10] S. Chatterjee *et al.*, "New 13-pyridinealkyl berberine analogues intercalate to DNA and induce apoptosis in HepG2 and MCF-7 cells through ROS mediated p53 dependent pathway: Biophysical, biochemical and molecular modeling studies," *RSC Adv.*, 2015, doi: 10.1039/c5ra17214d.