Antioxidant activity and induction of cell cycle arrest by *Curcuma amada* roxb extracts in acute T lymphoblastic leukemia cell lines

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Abstract

This study was undertaken to evaluate the phytochemical, antioxidant and anti-cancer efficacy of Curcuma amada rhizome extracts against acute T lymphoblastic leukemia cell lines. The antioxidant activity of the extracts was studied by DPPH assay, TAC and by estimating the phyto-constituents present. The reducing property of the extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution. The cell viability, proliferation and resistance towards cell toxicity were evaluated by MTT assay and the effect of Curcuma amada test extracts on the cell cycle of acute T lymphoblastic leukemia cell lines (JURKAT) was analyzed by flow cytometry. The Curcuma amada extract treatment of 100 and 200 µg/ml was found to be arrested to 13.37% and 25.96 % at G_2M phase of cell cycle respectively when compared to untreated Jurkat cells (8.32 %). Asparaginase showed 49.92 % of cell cycle arrest at G_2M phase.

When the concentration of Curcuma amada roxb extracts increased from 100 µg/ml to 200 µg/ml, a greater number of cells were seen in G2/M phase and lesser number were there in the S phase indicating that there was a decline in the formation of new daughter cells i.e. it was not allowing the cells to multiply faster. The number of doublets seen was very few when compared to the cells seen while using the standard drug Asparaginase. The cytopathology observed after Curcuma amada extract treatment was rounding and clumping of cells, detachment of cells, cell flagging and apoptosis. The results demonstrate the strong antioxidant and protecting potential of Curcuma amada rhizome extract towards DNA damage and mark it as a potential source of natural antioxidant and anti-cancerous activity at various acute phases of T lymphoblastic leukemia.

Keywords: *Curcuma amada*, MTT assay, Flow cytometry, Jurkat cells, T lymphoblast, Acute lymphoblastic leukemia.

Introduction

Curcuma amada Roxb is annual herb commonly called Mango-ginger which belongs to the family Zingiberaceae. The genus Curcuma of family Zingiberaceae comprises of more than 80 species of rhizomatous herbs[15]. It is a unique spice having morphological resemblance with ginger but imparts a raw mango flavour. The genus originated in the Indo-Malayan region and is widely distributed in the tropics of Asia to Africa and Australia as shown in fig. 1.

The rhizomes and leaves of most of the Curcuma species are aromatic, indicating the presence of volatiles/essential oils possessing a wide range of pharmacological properties which are commercially important plant volatiles employed extensively in pharmaceutical, natural colorant, flavouring and perfumery industries. The essential oil of *Curcuma longa* has been well studied and reported to contain ar-turmerone, turmerone, turmerol and zingiberene as the major constituents[1,6]. Major chemical components include curcuminoids, volatile oil, phenolic acid, starch and terpenoids like difurocumenonol, amadannulen and amadaldehyde. Bioactive components are deposited in *Curcuma amada*, hence it can serve as important raw material for pharmaceutical manufactures. The mango ginger rhizome is found to be a rich source of fibres and starch. Mango ginger is used medicinally as a coolant, aromatic, astringent, to promote digestion and to heal wounds, cuts and itching.

Figure 1: Mango ginger rhizomes: raw and powdered form

The external use of the rhizome paste for sprains and skin diseases is also an old practice. The rhizome (Fig. 1) has carminative properties, as well as being useful as a stomachic. Ayurveda and Unani medicinal systems have given much importance to mango ginger as an appetizer, alexteric, antipyretic, aphrodisiac, diuretic, emollient, expectorant and laxative and to cure biliousness, itching, skin diseases, bronchitis, asthma, hiccough and inflammation due to injuries. The biological activities of mango ginger include antioxidant activity, antibacterial activity, antifungal activity, anti-inflammatory activity, platelet aggregation inhibitory activity, cytotoxicity, antiallergic activity, hypotriglyceridemic activity, brine-shrimp lethal activity, enterokinase inhibitory activity, central nervous system (CNS) depressant and analgesic activity[3,15,18].

In a study by Sivaprabha et al[19], the DNA damage inducing ability of the methanolic extract of leaves and rhizomes of *Curcuma amada* was also studied against MCF-7 and MDA MB 231 breast cancer cell lines. A non-cancerous breast cell line HBL-100 and eukaryotic model organism yeast was also used for comparison. In another study by Jambunathan et al[7], various staining techniques using acridine orange/ethidium bromide, Giemsa, ethidium bromide, propidium iodide and Hoechst 33342 were employed to study the mechanism of cell death induced by the extract. The cytotoxic effect of different concentrations such as 18.75 µg, 37.5 µg, 75 µg, 150 µg and 300 µg of *Curcuma amada* extract was studied by MTT assay on human cervical cancer cell lines called HeLa and the percentage of cell inhibition was found to be 7.51, 24.49, 79.13, 99.86 and 100% respectively[16].

The different concentrations of *Curcuma amada* ethanolic extracts such as 20, 40, 60, 80, 100, 120 and 140 µg/ml showed different levels of radical scavenging activity like 0.4, 1.46, 6.4, 10.8, 22, 37 and 54 % of inhibition respectively with the IC50 value of 475 µg/ml[12]. Glioblastoma multiforme (GBM) is one the most aggressive and lethal human neoplasms with poor prognosis. The antitumor effect of supercritical CO_2 extract of mango ginger (*Curcuma amada* roxb) (CA) with and without irinotecan (IR) was analyzed in U-87MG human glioblastoma multiforme (GBM) cells *in vitro* and in nude mice xenografts. CA inhibits tumor growth rate in GBM xenografts, the inhibition rate being higher than in IR treated group. GBM xenograft mice treated with IR + CA combination showed almost complete inhibition of tumor growth rate.

Gene expression analysis of xenograft tumors indicated that IR + CA treatment significantly downregulated anti-apoptotic (Bcl-2 and mutant p53). The natural products made inside the plants may be non-essential for the existence of plants and their usual functioning, but these secondary metabolites could act as stimulants or hallucinogens for humans due to their medicinal values. On the other hand, free radicals are by-products of natural metabolism, but react with the proteins, enzymes and nucleic acids present in the body and cause their mutation and loss of function, leading to tissue damage. But antioxidants act as protective agents by reducing the oxidative impairment caused in the body, constraining the peroxidation of lipids[10]. This study brings to light the major active components present in *Curcuma amada* along with their biological activities such as antioxidant and anti-cancer efficacy which is important from the pharmacological point of view**.**

Material and Methods

Rhizome extracts: Fresh *Curcuma amada* rhizome (4 kg) was collected from Mysore (Southern region of India) in the month of February and March 2015. The handpicked *Curcuma amada* rhizomes were washed well using tap water and twice using distilled water. Then the rhizomes were chopped into small pieces or slices and half of it was dried in shade for a period of 5-6 days, at an ambient temperature of 22˚C. The dried samples were grinded properly using a mortar and pestle and later using a grinder to obtain the powdered form. The other half was immersed into ethyl alcohol and subsequent extracts were withdrawn at regular intervals of 20 hours till it became colourless. After which under reduced pressure, the crude extract of 28.2 gm was obtained which was viscous, dark yellowish liquid with an aromatic smell.

The aqueous extract and ethanolic extract were prepared by treating the suitable solvents in the Soxhlet extractor along with the rhizome samples. Extraction was done at 70˚C for 30 minutes, followed by filtering of the extracts using Whatmann filter paper no.1[9].

Subject and study design: This was a 52 days research work for which acute T lymphoblastic leukemia cell lines were procured from Skanda Lifescience (Bangalore, India) to study the effect of *Curcuma amada* rhizome extracts in different concentration. The investigational set ups and the experiments were carried out in a good manufacturing practices certified facility.

Phytochemical analysis: Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties. They are not required by the human body for life sustenance, but they offer protection against pathogens. There are different ways in which a phytochemical can work. It can act as an antioxidant and protect cells against free radical damage e.g. polyphenols, carotenoids etc. or it can stimulate certain enzymes; thereby reduce risk for breast cancer. It may act as an anti-bacterial and hormonal-stimulant component. It may even act as binders which may prevent the adhesion of pathogens to the human cell walls[9]. In the case of *Curcuma amada* rhizome, a wide spectrum of chemical constituents was identified by several tests which were done in triplicate.

Test for carbohydrates: Molisch's reagent was added to 2 ml of aqueous extract. A little amount of concentrated

sulphuric acid was added to it and allowed to form a layer. The mixture was shaken well and allowed to stand for few more minutes which was then diluted by adding 5 ml of distilled water. Purple precipitate ring showed the presence of carbohydrates[20].

Test for saponins: 0.5 gm of ethanolic extract was boiled and the mixture was filtered. To 2.5 ml of the filtrate, 10 ml of distilled water was added in a test tube. It was shaken well for few minutes and was allowed to stand for some time. Frothing along with the formation of honey comb indicated the presence of saponins.

Test for tannins: Few drops of 10% lead acetate solution were added to 5 ml of aqueous extract. Formation of white precipitate indicated the presence of tannins.

Test for fixed oils and lipids: Small quantity of extracts was separately pressed between two filter papers and allowed to dry. Appearance of an oil stain or a grease spot on the filter paper when observed under direct sunlight indicated the presence of fixed oils.

Test for proteins: 0.5 ml of aqueous extract was treated with equal volume of 1% sodium hydroxide, to which few drops of copper sulphate solution were gently added. The solution turning to purple colour, indicated the presence of proteins.

Test for steroids: 0.5 ml of the extract was dissolved in 3 ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube which formed a lower layer. A reddish-brown colour ring with a slight greenish fluorescence indicated the presence of steroid[11].

Test for proteins and amino acids: A few drops of Millon's reagents were added to the sample extract, which was then heated gently. A reddish-brown coloration or precipitate indicated the presence of tyrosine residue, which occurs in most of the proteins[23].

Antioxidant Activity analysis: The metabolism that takes place within a human body is aerobic in nature that is, it depends on oxygen. One of the crucial problems is the tendency of the oxygen molecule to create free radicals. These radicals are by-products of the natural metabolism, but react with the proteins, enzymes and nucleic acids present in the body and cause their mutation.

When these free radicals attack a specific region in the body, it leads to oxidative stress, which causes cells to lose their function and they also have an essential role in the development of a variety of physiological conditions including mutagenesis, cellular ageing, coronary heart disease, diabetes etc. This eventually results in the destruction of those affected cells or tissues. On the other hand, antioxidants act as protective agents, by reducing the oxidative damage caused in the body, inhibiting the peroxidation of lipids and by retarding the progress of many diseases[10].

The antioxidant activity of the rhizome was evaluated in this study[10]. A small quantity of sample was taken and infused into 200 ml of double distilled water at 100°C for 2 min. Then the sample was centrifuged at 12,000 rpm for 5 min. The total antioxidant capacity (TAC) was determined by phosphomolybdenum method using different concentrations of ethanolic extracts by phosphomolybdenum method. The reducing property of the extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution[13,24]. The test for phenols and flavonoids was also done by using Folin-Ciocalteu reagent and $AlCl_3$ solution respectively[10,25]. Another assay method done here was by using a commercially available free radical (DPPH•+, 2,2 diphenyl- 1-picrylhydrazyl) which is soluble in methanol and the antioxidant activity was measured by decrease in absorbance at 515 nm[5,12].

Test for phenols: An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent and 4 ml of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40˚C for colour development. An appearance of blue colour indicates the presence of phenols[24].

Test for flavonoids: 0.5 ml of sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture. Water was added immediately to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 min. An appearance of pink colour indicates the presence of flavonoids[25].

DPPH free-radical scavenging assay: 1,1-diphenyl-2-picrylhydrazyl assay stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at −20°C until needed. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.10 ± 0.02 units at 515 nm using spectrophotometer. 100 µl of rhizome extract solution was allowed to react with 1,900 µl of the DPPH solution for 2 hours in the dark. Then the absorbance was measured at 515 nm. This study was done in triplicate and Butylated hydroxytoluene (BHT) was used as standard for the preparation of calibration curve[2].

Cell viability and proliferation test: The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. It is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, which is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. 100 µl of dimethyl sulfoxide (DMSO)

was added to solubilize the formazan rapidly and the absorbance was measured at 590 nm. The percentage (%) of cell inhibition was determined using the following formula:

% Cell inhibition = 100 - Abs (sample) / Abs (control) x100

Flow Cytometry: The analysis of cell cycle was done by flow cytometry. This analysis is based on the ability to stain the cellular DNA in a stoichiometric manner. A variety of dyes are having high binding affinities for DNA which was considered as the location to which these dyes bind on the DNA molecule. Blue-excited dye Propidium Iodide (PI) was selected[2] as it is an intercalating dye which binds to DNA and double stranded RNA[8,21]. Later, cells were incubated for 15 min at room temperature in 500 µl of PI solution containing 0.05 mg/ml PI and 0.05 mg/ml RNase A in PBS.

The percentage of cells in various stages of cell cycle in compounds treated and un-treated populations was determined using FACS Calibur (BD Biosciences, San Jose, CA). The cells used to study the anticancer activity were Jurkat cells, an immortalized line of human T lymphocyte cells that are used to study acute T cell leukemia, T cell signaling and the expression of various chemokine receptors susceptible to viral entry, particularly HIV. Jurkat cells are also useful because of their ability to produce interleukin 2. It can also determine the mechanism of differential susceptibility of cancers to drugs and radiation.

Statistical analysis: Data was determined from the triplicate analysis. The concentration which caused 50% of inhibition of the system assessed (IC50) by *Curcuma amada* extracts was determined using GraphPad Prism software. Data were statistically analyzed by one-way Anova followed by t-test for evaluation of the formulation influence in the antioxidant activity assays. Results were presented as SEM (standard error mean) and were measured to be significantly different when $P < 0.001$ was obtained.

Results

Phytochemical analysis: The general phytochemical analysis of *Curcuma amada* rhizomes has interpreted more than 130 chemical constituents of which 121 have been identified. In our study, it has shown the presence of proteins, oils and fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrates as the major phytochemical groups listed in table 1.

Antioxidant Activity analysis: The Total Antioxidant capacity (TAC) was found to increase from lower to higher concentration. The TAC of different concentrations like 200, 400, 600, 800 and 1000 µg/ml was found to be 123.62, 217.17, 315.34, 395.96 and 491.10 µg/g of ascorbic acid equivalents respectively, with $r^2 = 0.963$ as seen in table 2. Data is presented as the means ± SEM of triplicate.

DPPH assay*:* The free radical scavenging capacity of ethanolic extracts was found by DPPH method and the percentage of free radical scavenging capacity is represented in fig. 2.

Table 1
Preliminary phytochemical screening

Phytochemicals	Aqueous ex.	Ethanolic ex.
Phenolic compounds	+	++
Flavanoids	+++	+++
Sterols	-	-
Steroids	-	-
Tannins	-	-
Reducing sugar	+++	+++
Alkaloids	+	++
Terpenoids	++	+++
Volatile oil	++	+++
Fixed Oils and lipids	+	+
Carbohydrates	+	+
Proteins and amino acids	+	+

- Absent, + Present (+ trace quantity, ++ medium quantity, +++ high quantity)

Table 2
Total antioxidant capacity (TAC)

Concentration of ethanolic extract in µg/ml	TAC µg/g (ascorbic acid equivalents)
200	123.62
400	217.17
600	315.34
800	395.96
1000	491.10

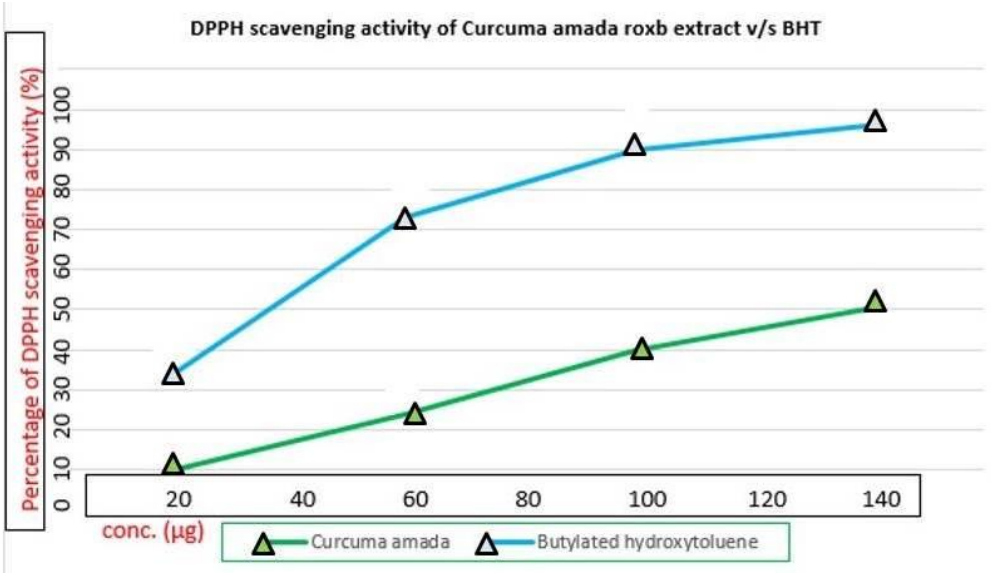

Figure 2: Free radical scavenging capacity of ethanolic extracts by DPPH

Table 3
Percentage inhibition and IC_{50} value of *Curcuma amada* rhizome extract

Samples	Conc. µg/ml	OD 590 nm	% Inhibition	IC_{50}µg/ml
	1% DMSO	0.5296	0.00	
Curcuma amada extract	5	0.4764	11.74	43µg/ml
	10	0.3991	24.64	
	20	0.3422	35.39	
	40	0.3014	43.09	
	80	0.2034	61.59	
	160	0.1279	75.85	
	320	0.1018	80.78	

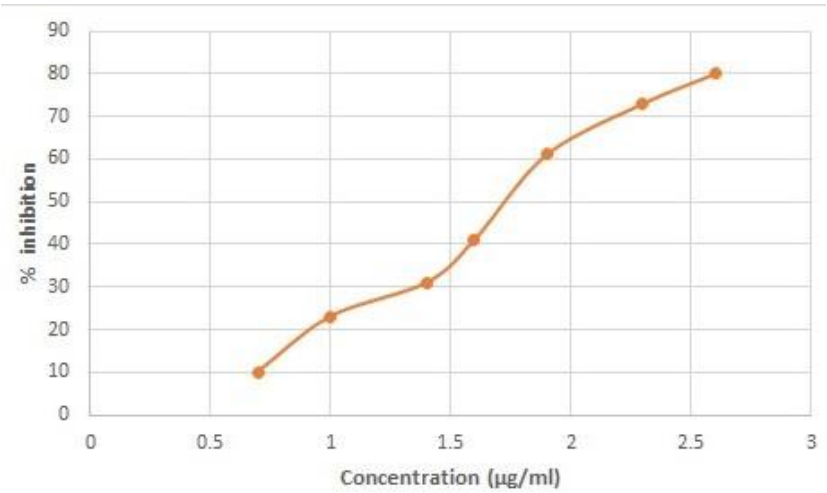

Figure 3: Plot for percentage inhibition of *Curcuma amada* rhizome extract

The radical scavenging activity of the extract was the highest at the maximum dose of 140 µg/mL with an IC50 value of 14 ± 0.05 µg/mL (r^2 = 0.976). Ascorbic acid showed an IC50 value of 16 ± 0.06 µg/mL (r^2 = 0.987). The effect was found to be concentration-dependent. DPPH is a stable, nitrogen-centred free radical that accepts hydrogen from the antioxidants existing in the polyphenolic extracts and converts it into a stable diamagnetic molecule called as diphenyl-picryl hydrazine[22]. The observed reduction of DPPH by the extract was either due to the transfer of a hydrogen atom or the transfer of an electron.

MTT assay: Sample *Curcuma amada* rhizome extracts showed dose dependent inhibitory cytotoxic effect on Jurkat cells and its IC_{50} value are revealed in table 3. The cytotoxicity inhibition percentage of *Curcuma amada*

rhizome extracts according to the specific concentration is shown in fig. 3. This study was performed in triplicate.

Flow cytometry: The effect of *Curcuma amada* test extract on cell cycle in JURKAT cells as analyzed by flow cytometry with 1% DMSO and 20 µM Asparaginase is depicted in fig. 4a and fig. 4b respectively. On the other hand, fig. 5a and fig. 5b shows 100 µg/ml and 200 µg/ml of *Curcuma amada roxb* treated Jurkat cells respectively.

Discussion

The major chemical constituents of *Curcuma amada* based on percent yield are myrcene, ocimene, ar-turmerone, (Z)β -farnasene, guaia-6,9-diene, cisβ -ocimene, cis-hydroocimene, transhydroocimene, α -longipinene, α -guaiene, linalool, β -curcumene and turmerone. *Curcuma amada* rhizome is considered to be rich in phytochemicals. The aqueous and ethanolic extracts of the rhizome revealed the presence of carbohydrates, alkaloids, tannins, fixed oils, reducing sugars, proteins, steroids, phytosterols, phenols and flavonoids. This study provides an explicit perception about the *Curcuma amada* rhizome as to which phytochemicals are more in number[15]. The free radical scavenging capacity of ethanolic extracts was found by DPPH method.

The molecule of 1,1-diphenyl-2-picrylhydrazyl i.e. DPPH is regarded as a stable free radical due to the delocalisation of the extra electron over the molecule, so that the molecules do not dimerise, as is the case with maximum other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 520 nm. When a solution of DPPH is mixed with a substance that can contribute a hydrogen atom, then there would be a change from the residual pale-yellow colour due to the picryl group present giving rise to the reduced form resulting in the loss of violet colour.

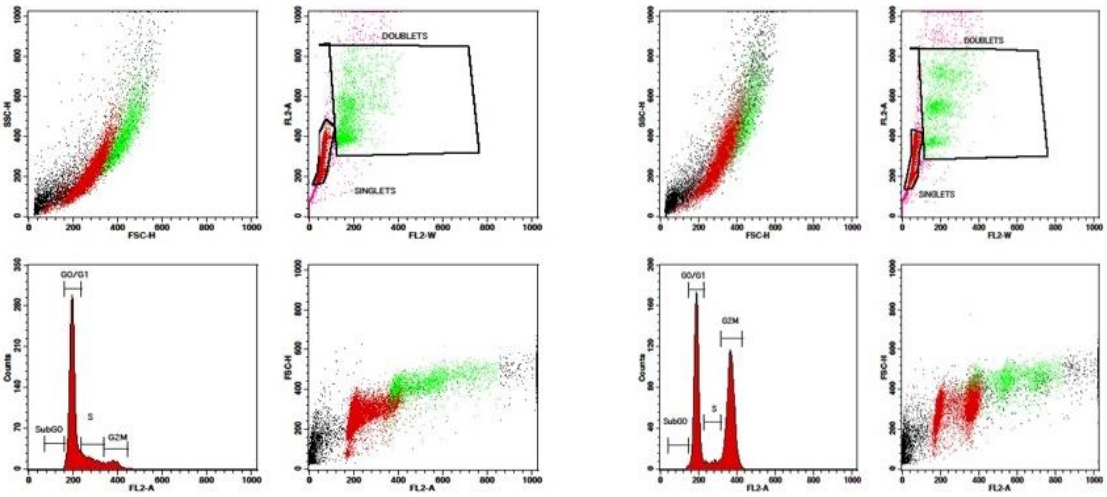

Figure 4: Flow Cytometry plots of a) JURKAT cells; b) JURKAT cells treated with 20 µM of Asparaginase (Elspar)

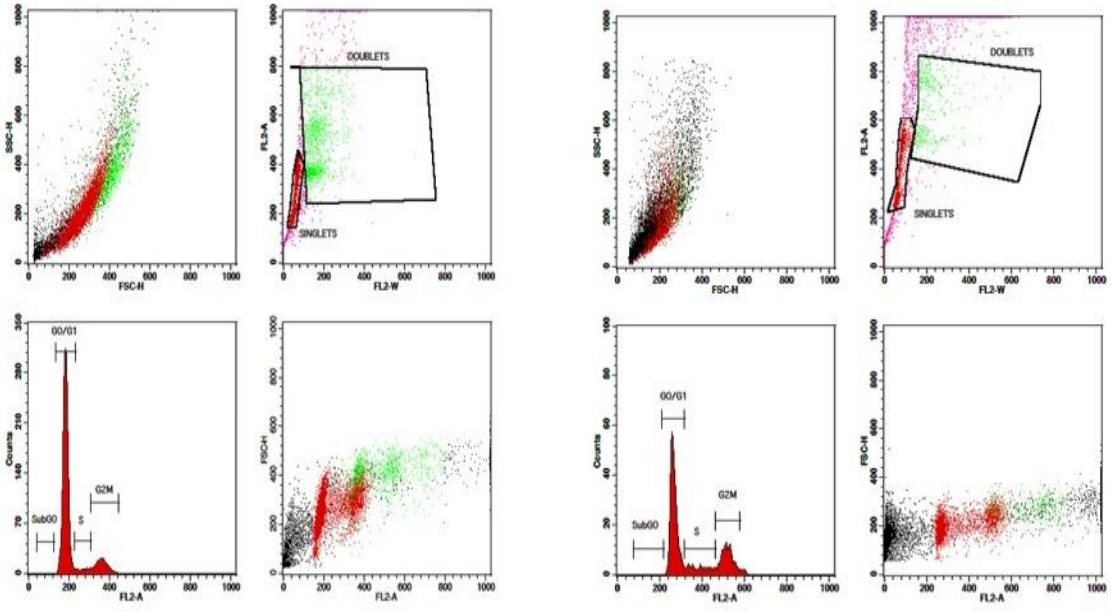

Figure 5: Flow Cytometry plots of a) JURKAT cells treated with 100 µg/ml of *Curcuma amada* roxb extract; b) JURKAT cells treated with 200 µg/ml of *Curcuma amada* roxb extract

The primary reaction where the DPPH radical by is represented Z• and the donor molecule by AH, can be given as Z• + AH = ZH + A• where ZH is the reduced form and A• is free radical produced in this first step. This later radical will then go through further reactions which control the overall stoichiometry, such as the number of molecules of DPPH condensed or decolorised by one molecule of the reductant[12].

In the MTT assay, the dissolved MTT was converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of the viable cells. This water insoluble formazan could be solubilized using DMSO, acidified isopropanol or other solvents such as pure propanol or ethanol.

The resulting purple solution was spectrophotometrically measured. An increase or decrease in cell number resulted in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material[4]. The extracts showed dose dependent inhibitory cytotoxic effect on Jurkat cells and as its concentration increased, the percentage inhibition towards leukemia cells also increased. Reproduction of cells requires cell division, with production of two daughter cells. The most obvious cellular structure that requires duplication and division into daughter cells is the cell nucleus- the repository of the cell's genetic material DNA. The two most obvious features of the cell cycle are the synthesis and duplication of nuclear DNA and the cellular division is known as the “S phase” and “M phase”. A temporal delay or gap between mitosis and the onset of DNA synthesis is termed as G1 whereas another gap between the completion of DNA synthesis and the onset of mitosis is termed as G2[17].

The cycle of G1 → S → G2 → M → G1 etc. is shown schematically in fig. 6 as done by flow cytometry. It helps to analyse the doublets or cellular aggregates along with studying a specific stage of cell cycle to determine how a cell is responding to a sample extract. Whenever two cells possessing similar G1-phase DNA content to that of to a G2/M-phase are recorded as a single event, then it results in a doublet. But if these doublets are more in any sample, it could result in a higher number of cells in the G2/M-phase.

Fluorescent DNA dye (FL2) generates a signal by emitting fluorescence which is recorded as flow cytometer column height (FL2H) for high stain whereas flow cytometer pulse-area (FL2A) and flow cytometer pulse-width (FL2W) measure the pulse-area and pulse-width respectively which can be plotted using a dot plot to differentiate between doublet and singlet. FL2W increases with the doublet diameter whereas G1 doublet and the G2/M single produce the same FL2A signal. G0/G1 is on lower left side, S phase is on lower middle side and G2/M in on the lower right side[14]. The black dots represent debris, red dots represent singlets whereas the green dots represent doublets. The nuclear fluorescence of each cell was examined where two cells in G0/1 which are stuck together, will have as much nuclear fluorescence as one G2/M cell.

When treated with 1% DMSO, the total percentage of cells in the G0/G1, S and G2/M phase after gating was 75.21%, 15.92% and 8.32% respectively whereas when treated with 20µM Asparaginase, the total percentage of cells in the G0/G1, S and G2/M phase after gating was 44.30%, 5.55% and 49.92% respectively as the synthesis of daughter cells in S phase came down drastically. But when treated with 100 µg/ml of *Curcuma amada* extract, the total percentage of cells in the G0/G1, S and G2/M phase after gating was 82.83%, 5.24% and 13.37% respectively. When treated with 200 µg/ml of *Curcuma amada* extract, the total percentage of cells in the G0/G1, S and G2/M phase after gating was 60.79%, 3.41% and 25.96% respectively.

The results strongly suggest that when the concentration of *Curcuma amada roxb* extracts increased from 100 µg/ml to 200 µg/ml, a greater number of cells were seen in G2/M phase and lesser number were there in the S phase indicating that there was a decline in the formation of new daughter cells i.e. it was not allowing the cells to multiply faster. The amounts of doublets seen were very few when compared to the cells seen while using the standard drug Asparaginase.

Thus, the *Curcuma amada roxb* extracts have potential anti-cancerous activity at various acute phases of T lymphoblastic leukemia. A significant decrease in the cell viability, proliferation and increase in cytotoxicity was observed along with few morphological alterations after the treatment with *Curcuma amada* extracts. The data demonstrate that this rhizome exhibits an inhibitory effect indicating apoptotic cell death. The outcome of this study recommends that the compounds of this extract may have promising use as a cancer chemotherapeutic agent. In general, the highest (75%) dose of extract was cytotoxic for cells in most assays.

It is recommended that further work should be done using the lowest concentration of the extract on other types of cancer cell lines and for longer incubation periods. This might help reduce or eliminate the toxic and damaging effects of the high concentrations of the extract on the healthy cells while, at the same time, possibly getting better effects on the cancer cells due to the extended period of incubation. Another area of research is to work on the active ingredients of the extract with different types of cancer. This research work also brings in the scope for experimentation of new anticancer drugs such as cyclophosphamide or cytoxan and Etoposide or VePesid against traditional medication such as using plant or rhizome extracts.

Conclusion

This study revealed the antioxidant activity and phytochemical analysis of *Curcuma amada roxb.* indicating the presence of phenols, carbohydrates, saponins, tannins, fixed oils and lipids, proteins, steroids and amino acids. The

in vitro cytotoxic studies conducted by MTT Assay method showed an inhibition of 43µg/ml for the extract. The studies on acute T- Lymphocytes lymphoblastic leukemia cell lines (Jurkat) confirmed an inhibitory effect which showed effective cell cycle arrest at G_2M phase. The effect of *Curcuma amada* test extract on cell cycle in Jurkat cells was analyzed by flow cytometry by considering cell lines which were untreated (control), Asparaginase 20 µM treated, *Curcuma amada* 100 µg/ml treated and *Curcuma amada* 200 µg/ml treated.

The treatment of 100 and 200 µg/ml arrested 13.37% and 25.96% of growth at G_2M phase of cell cycle respectively, compared to untreated Jurkat cells (8.32 %). Asparaginase has showed 49.92 % of cell cycle arrest at G_2M phase. This study demonstrates that these extracts induced the apoptosis proving to be a considerable plant source against cell induced damage due to acute lymphoblastic leukemia and for further drug development.

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