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A STUDY ON PHYTOCHEMICAL ANALYSIS AND CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACT OF *ANNONA MURICATA*

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Abstract:

Phytocompounds from the medicinal plants are extensively studied for their mode of action and therapeutic efficacy. It is estimated that plant derived compounds constitute more than 50% of anticancer agents in one way or the other. The modern trend of anticancer drug development research is largely based on the exploration of potential phytochemicals, with this background, the hydro alcoholic extract of *Annona muricata* leaf was subjected to the phytochemical screening and its anticancer effect was also studied using Molt-3 T cells of Acute Lymphoblastic Leukemic origin. The qualitative and quantitative phytochemical analysis showed the presence of various phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, tannins, saponins and steroids in the *Annona muricata* leaf extract which was confirmed by HPLC analysis. The leaf extract was able to scavenge free radicals effectively as determined by DPPH and H_2O_2 scavenging assay and also exhibited good cytotoxicity towards Molt-3 cell line while rendering protection against the normal/untransformed S. cerevisiae cells from oxidative damage using MTT and SRB assay. The results of annexin V/FITC staining clearly indicate that *A.muricata* leaf extract were able to induce apoptotic cell death in Molt-3 leukemic cell line. To conclude, the ethanolic extract of *A.muricata* leaf exhibit strong antioxidant and anticancer activity which might be due to the presence of various phytoconstituents.

Key Words: *Annona muricata*, Antioxidants, Anticancer & Secondary Metabolites

Introduction:

Leukemia is a chronic disease characterized by the increase in the number of abnormal blood cells, and decreases the number of normal blood cells. Leukemia is one of the hematological malignancies, with varied number of clinical and pathological types. According to the lineage cells involved, leukemia can be categorized into Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML), Chronic Lymphoblastic Leukemia (CLL) and Acute Lymphoblastic Leukemia (ALL) (Miladinia *et al.*, 2016). T cell acute lymphoblastic leukemia (T-ALL), an aggressive hematological cancer, is mainly diagnosed in children. It arises from the malignant transformation of T cell progenitors. Current treatment protocols includes high-dose, multi-agent combination chemotherapy which cures greater than 85% in children. However, the toxic nature of these aggressive treatment methods causes severe side effects such as reduced intellectual capacity, osteonecrosis and growth deficiencies, infertility, and an increased risk of secondary tumor development later in life. Hence, there is an imperative need for targeted therapy in cancer management to increase the quality of life for pediatric T-ALL survivors (Goossens and Vlierberghe, 2016).

Recently evidences and knowledge has accumulated in the literature to show immense potential of medicinal plants used in various medical, pharmaceutical, cosmetic and agrochemical applications. For thousand years, plants have been the subject of human curiosity. Traditional people relied on medicinal plants to combat various ailments. Plants and herbal extracts have acquired important place in modern medicine, due to their chemical and medicinal contents. Their secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities (Ladan *et al*., 2014).

Secondary metabolites are produced by plants which include alkaloids, terpenes, flavonoids, lignans, steroids, saponins, phenolics and glucosides. These phytochemicals neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. Phytochemicals also reduce the risk of coronary heart disease by preventing the oxidation of lipoprotein, cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity. Phytochemicals have also been promoted for the prevention and treatment of diabetes, high blood pressure, and macular degeneration. An individual compound may have more than one biological function serving as both an antioxidant and an antibacterial agent (Saxena *et al.*, 2013).

Bioactive natural products from plant sources have enormous economic importance as they can be used as drugs, lead compounds, biological or pharmaceutical tools, feed stock products, excipients and nutraceuticals. An advantage of natural bioactive molecule is that they have a milder side effect on the body in comparison to a chemically synthesized drug. With the increasing acceptance of herbal medicines as alternative

form of health care delivery, the screening of medicinal plants for bioactive compound is imperative (Devanaboyina *et al*., 2013).

Annona species commonly known as 'Custard-Apple' belongs to the family Annonaceae and was cultivated in many tropical countries all over the world, for its edible fruits. Among these, *Annona muricata* L. (Soursop or Graviola) is a naturally occurring plant seen in Central America and in Southern part of India, traditionally used to treat various ailments. Fruits and fruit juice of *A. muricata* were taken internally to treat worms and parasites, treat fever, to increase mother's milk after child birth and as an astringent for diarrhea and dysentery. The leaves of the plant are found to be anti-spasmodoic (George *et al*., 2015). With this background the hydroalcoholic extract of *Annona muricata* leaf was subjected to the phytochemical screening and its anticancer effect was also studied using Molt-3 T cells of Acute Lymphoblastic Leukemic origin.

Materials and Methods:

Collection and Preparation of Sample: The fresh leaves of *Annona muricata*, was collected from local area of Coimbatore, Tamilnadu, India. The leaves were washed in running tap water till the dirt was removed. About 10g of leaf was weighed and extracted with 100ml of 70% ethanol. The extracts were concentrated by incubating at 60˚C in a water bath and the residue was weighed and dissolved in dimethyl sulphoxide at a concentration of 10mg/ml and stored at 4˚C.

Qualitative Phytochemical Analysis: The hydro alcoholic extract was prepared and tested for the presence of alkaloids, flavonoids, phenolics, saponins, tannins, glycosides, steroids and terpenoids as per Khandelwal (2002).

Quantitative Determination of Phytochemical Constituents:

Determination of Total Alkaloids: Fresh leaves (1g) were extracted with 20ml of 95% ethanol: 28% NH_4OH (95:5) at room temperature overnight. The extract was filtered and concentrated under reduced pressure to a fuzzy residue, which was extracted twice with 1N HCl (10ml each) and then filtered. Alkaloids were liberated at a pH of 9.8 by the addition of 0.7M Na_2CO_3. The organic extract was dried over anhydrous sodium sulphate and its weight was estimated to obtain the yield of total alkaloids fraction.

Determination of Total Phenolics: Leaves (1g) were crushed using a mortar and pestle and extracted with 20ml of 80% ethanol at 80˚C for 15minutes. The leaves were extracted respectively till a clarified fraction was obtained and its dry weight was calculated.

Determination of Total Flavonoids: The phenolic extract was further extracted with petroleum ether, while the flavonoids were present in the aqueous fraction. The aqueous fraction was dried and the weight was estimated.

Determination of Total Saponins: Fresh leaves (1g) were crushed, transferred to a conical flask and 200ml of aqueous ethanol was added. The mixture was filtered and re-extracted with another 200ml of 20% ethanol. The combined extract was reduced to 40ml over a water bath at about 90˚C. The concentrate was transferred into a 250ml separating funnel, 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. The extraction was repeated twice with the addition of n-butanol. The combined n-butanol extract was washed with 10ml of 5% NaCl. The remaining solution was heated in a water bath, evaporated and dried in an oven and its weight was calculated.

Determination of Total Steroids: Fresh leaves of about 2g was weighed and added to 10ml of methanol. It was kept in a water bath for 15 min. The mixture was filtered, condensed and the dry weight was observed.

Determination of Total Tannins: The plant material was suspended in methanol and allowed to stand overnight. It was refluxed for 4 hours, then filtered and the residue was washed with methanol. The filtrate was allowed to cool down, observed for any modification and an aliquot of this was used to calculate the weight and to determine the tannin content.

Determination of Terpenoids: The plant material was suspended in petroleum ether and filtered. The filtrate was condensed and an aliquot of the filtrate was quantified.

HPLC Analysis: The ethanol extract of *Annona muricata* leaf was dissolved in an appropriate volume of HPLC grade methanol and 20µl of the sample was injected using Hamilton syringe into the reverse phase C18 column of the HPLC system (Sigma-Aldrich equipped with PDA detector). The sample analysis was performed at room temperature in the wavelength ranging between 220-800nm at 1000psi and the mobile phase used was 100% HPLC grade methanol 60 minutes at a flow rate of 0.5ml/minute.

Free Radical Scavenging Assays: The free radical field has undergone massive expansion in recent years because free radicals and oxidants are now implicated in physiological responses and in several diseases (Forman *et al*., 2015). Hence, the following free radical assays were performed to study the scavenging potential of the extracts.

DPPH Radical Scavenging Activity: The ability of the extracts to scavenge the stable free radical DPPH and convert it into diphenyl picryl hydrazine was determined by the method described by Mensor *et al*. (2001). The plant extract was added to 3ml of 1.0mM DPPH in methanol solution. After 30 minutes, the discoloration from deep violet to yellow color was measured at 515nm in a spectrophometer. The % inhibition was calculated by the formula,

DPPH Scavenging activity (%) = [(Abs control −Abs sample)] / (Abs control)] ×100

Where Abs control is the absorbance of DPPH; Abs sample is the absorbance of sample.

Hydrogen Peroxide Scavenging Activity: The ability of the ethanol extract of *Annona muricata* to scavenge hydrogen peroxide radical was determined by measuring the decrease in absorbance at 230 nm spectrophotometrically, explained by Ruch *et al*. (1989). A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Plant extract was added to 0.6ml H_2O_2 solution. The total volume was made upto 3ml. The absorbance of the reaction mixture was recorded at 230nm. The solution containing phosphate buffer with H_2O_2 acts as blank. The percentage of H_2O_2 scavenged by the plant extract was calculated using the formula,

Scavenging activity (%) = [(Abs control –Abs sample)] / (Abs control)] ×100

Culturing of Molt-3 Cell Line and *Saccharomyces Cerevisiae:* Molt-3 T-cell Acute Lymphoblastic Leukemic cell line, was purchased from NCCS, Pune, India. It is originally derived from 19 year old male with acute lymphoblastic leukemia. It was cultured using RPMI 1640 medium supplemented with 10% FBS and incubated at 37˚C. The cell count and viability was tested with trypan blue using haemocytometer and 1×10^6 cells suspension were seeded onto 96 and 6 well plates and was treated with extracts for 24 hour and then cytotoxic and apoptotic assays were performed. Imatinib, a tyrosine inhibitor, is used to treat ALL (Fielding *et al*., 2014). Hence, in the present study it is used to compare the anticancer activity of the leaf extract. *Saccharomyces cerevisiae* is a primitive eukaryotic, non-pathogenic fungi whose complete genome has been sequenced. Yeast cells have remarkable similarities to mammalian cells at the molecular and organelle level, and several yeast proteins are functionally interchangeable with highly homologous human proteins. Thus, it is not surprising that using yeast cells as a model system provides relevant contribution to understand the molecular mechanisms underlying oxidative stress and apoptosis (Kiruthika and Padma, 2013). H_2O_2 is a simple but very important molecule in nature. It is extensively used as an oxidizing agent in the chemical and food industries and also as a vital mediator in food, pharmaceutical, clinical and environmental analysis (Chen *et al*., 2014). *Saccharomyces cerevisiae* cells/untransformed cells were inoculated and incubated at 37˚C for overnight. The cells were harvested and 1×10^6 cells suspension were seeded and treated with 200µM H_2O_2 along with various concentrations ranging from 10, 25, 50, 100 and 200µg of ethanol extract of *Annona muricata* leaves.

Cytotoxicity Assay:

MTT Dye Reduction Assay: MTT assay was performed as per Igarashi and Miyazawa, (2001). The treated Molt-3 cells and *Saccharomyces cerevisiae* cells were incubated with 50µl of MTT at 37ºC for 3 hours after centrifugation. After incubation, 200µl of PBS was added to all samples. The liquid was then carefully aspirated. Then 200µl of acid propanol was added and left overnight in the dark. The absorbance was read at 650nm in a micro titer plate reader (Anthos 2020, Austria). The optical density of the control cells were fixed to be 100% viabile and the per cent viablity of the cells in the different treatment groups were then calculated using

Percent cell viability = [(C− T)] / (C)] ×100

Where, T- Absorbance of test sample; C - Absorbance of control

SRB Assay: Sulphorhodamine B (SRB) assay was carried out as per Skehan *et al*. (1990). The treated Molt-3 cells and *Saccharomyces cerevisiae* cells were collected by centrifugation and washed with PBS. An aliquot of 350µl of ice-cold 40% TCA was layered on the top of the treated cells and incubated at 4ºC for one hour and it was washed 5 times with 200µl of ice cold PBS. The PBS was removed and SRB dye (350µl) was added to each tube and left in contact with the cells for 30 minutes at room temperature. After which they were washed 4 times with 1ml portion of 1 % acetic acid to remove the unbound dye, then 350µl of 10mM Tris (pH 10.5) was added to each tube to stabilize the protein bound dye. The pellet was shaken gently for 20 minutes on a gyratory shaker. The debris was spun down and the absorbance of the tris layer in each group was transferred to a 96-well plate and read in a microtiter plate reader at 490nm. The percent viability of the cells in the treatment groups were calculated using

Percent cell viability = [(C− T)] / (C)] ×100

Where, T- Absorbance of test sample; C - Absorbance of control

Determination of Apoptosis: The magnitude of apoptosis elucidated by extracts was determined using Annexin V/FITC Apoptosis detection kit (BD Biosciences). The procedure was followed according to the manufacturer protocol.

Results and Discussion:

Phytochemical Analysis of the Ethanol Extract of *Annona Muricata* Leaves: Preliminary phytochemical studies are helpful in finding out chemical constituents in the plant material that may well lead to their quantitative estimation. Thus, in the present study phytochemical screening for the candidate plant was carried out and the results of the preliminary phytochemical analysis of the ethanol extract of *Annona muricata* is presented in Table 1. It showed the presence of all the secondary metabolites including alkaloids, flavonoids, phenols, terpenoids, tannins, saponins and steroids thus showing that the leaf extract is rich in secondary metabolites which might be responsible for their medicinal potenial.

Table 1: Phytochemical analysis of the ethanol extract of *Annona muricata* leaves

S.No	Phytochemical constituents	*Annona muricata* leaf extract	Yield (g)/g leaf tissue
1.	Alkaloid	+++	0.65
2.	Phenolics	+	0.15
3.	Flavonoids	+	0.01
4.	Saponins	++	0.62
5.	Steroids	+	0.45
6.	Terpenoids	+	0.03
7.	Tannins	++	0.39

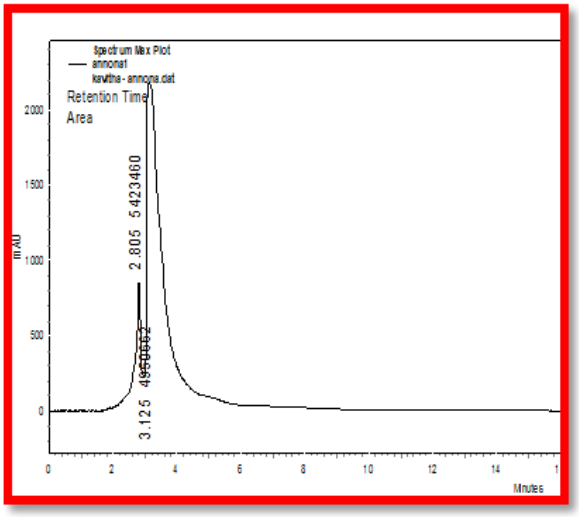
(+++) – present strongly; (++) – present moderately; (+) – present mildly

Quantitative phytochemical analysis was performed in order to determine the major phytoconstituents present in the ethanol extract of *Annona muricata* leaves. The concentration of the secondary metabolites present in the leaf extract was analyzed by calculating the dry weight of the alkaloids, flavonoids, phenols, terpenoids, tannins, saponins and steroids and it was found to be 0.65, 0.01, 0.15, 0.03, 0.39, 0.62 and 0.45g respectively. Among all the phytochemicals tested, alkaloids represent the predominant phytoconstituent of *Annona muricata* leaf as shown in Table 1.

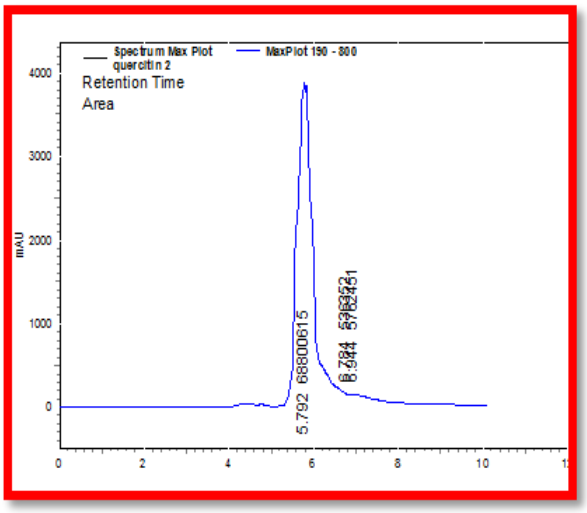
HPLC Analysis of the Ethanol Extract of *Annona Muricata* Leaves: The HPLC analysis of the ethanol extract of *Annona muricata* leaves showed the presence of one major peak and one minor peak with retention time of 2.80 and 3.12 as shown in Figure 1a. Figure 1b shows the HPLC chromatogram of the standard quercetin with retention time of 5.79. More spectral and chromatographic analysis need to be carried out to determine the nature of phytoconstituents present in the leaves of *Annona muricata.*

Figure 1: HPLC profile of the ethanol extract of *Annona muricata* leaves and Quercetin

1a. *Annona muricata* leaf extract



1b. Quercetin (standard)



Alkaloids constitute a wide variety of nitrogenous compounds and found to inhibit auto oxidation in several biological systems. The derivatives of alkaloids are also exhibit antioxidant activity (Bhattacharya and Chakraborty, 2015). Flavonoids are the biggest class of antioxidants. The flavonoids are good scavengers of free radicals and prevent oxidative stress (Prakash and Suneetha 2014). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Wadood *et al.*, 2013). Phenolic compounds are a category of phytonutrients with strong antioxidant properties and the relationships between phenolic content and antioxidant activity have been reported in many studies. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals donate hydrogen atoms or electrons or chelate metal cations (Saravanan and Parimelazhagan, 2014). From the results of the phytochemical analysis, it is evident that the leaves of *A. muricata* are found to be a potent source of secondary metabolites which might be responsible for their antioxidant activity and their therapeutic potential. Thus it becomes imperative to study the radical quenching ability of the ethanolic extract of *Annona muricata* using an array of free radicals.

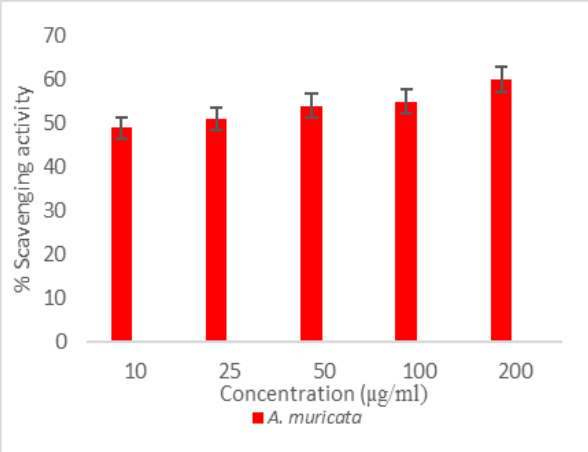
DPPH Radical Scavenging Activity of the Ethanol Extract of *A. Muricata* Leaves: From the methodological point of view, the DPPH method is recommended as easy and accurate with regard to measuring the antioxidant activity of extracts (Neelambika and Leelavathi, 2015). The results revealed that the ethanol extract of *A. muricata* leaves showed significant scavenging activity against DPPH radical. The maximum scavenging activity is seen at the concentration of 200µg/ml which is presented in the Figure 2a. The result demonstrates

that leaf extract possess good antioxidant potential in a concentration dependent manner. Adeosun *et al.* (2016) showed that *Phoenix dactilyfera L.* exhibited stronger scavenging activity against DPPH radical. Among the two plants tested for the antioxidant activity *Blepharis maderaspatensis* ethyl acetate extract shows highest antioxidant capacity followed by *Blepharis molluginifolia* alcoholic extract and also reported that these plants are rich in phenols, flavonoids, glycosides, alkaloids, saponins, diterpenes, triterpenes and phytosterols. The activity of these plants is because of their biologically active secondary metabolites (Neelambika and Leelavathi, 2015).

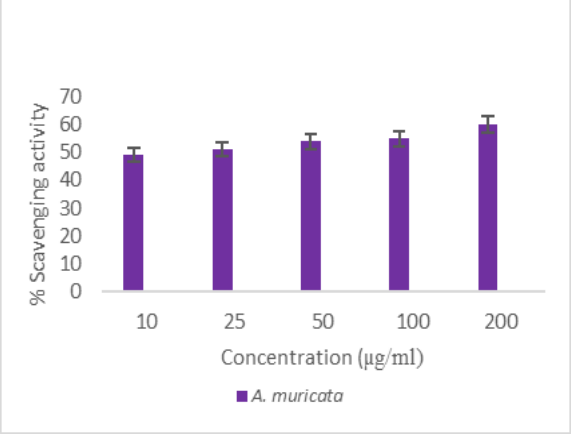
Hydrogen Peroxide Scavenging Activity of the Methanol Extract of *A. Muricata* Leaves: Hydrogen peroxide is a biologically relevant, non-radical reactive oxygen species and is inevitably generated as a by-product of normal aerobic metabolism. However, when concentration increases under stress conditions, H_2O_2 could be detrimental for cells and, furthermore, could be converted into other ROS such as hydroxyl radicals. Thus, elimination or neutralization of H_2O_2 is crucial for the cells. The ethanol extract of *A. muricata* leaf was subjected to oxidative stress by adding H_2O_2. The leaf extract was able to scavenge H_2O_2 to a considerable extent. At lower concentration of 10µg, the leaf extract was able to scavenge H_2O_2 moderately. When the concentration is increased, the per cent scavenging is also increased in a concentration dependent manner. The result is depicted in Figure 2b. The antioxidant activity and radical scavenging property of the extracts may be due to the presence of polyphenolic compounds such as flavonoids and tannins and this, in turn, may be attributable to the hydrogen or electron donating ability of the group present in the structure. Numerous reports have already proven that nutritive phenols play a significant role in protecting mammalian and bacterial cells from cytotoxicity induced by H_2O_2, indicating that the observed activity of plants extracts could be due to the presence of phenols (Gul *et al.*, 2013). Thus indicating that the phytoconstituents present in the *A. muricata* leaves might be responsible for H_2O_2 scavenging activity.

Figure 2: DPPH and H_2O_2 scavenging activity of the ethanol extract of Annona *muricata* leaves

2a. DPPH



2b. H_2O_2

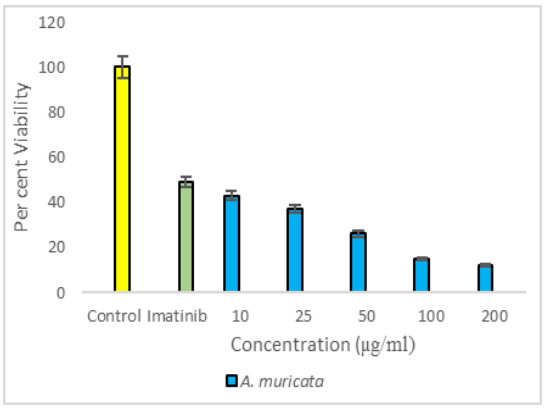


Cytotoxic Effect of Ethanol Extract of *Annona Muricata* on Molt-3 Cell Line as Determined by MTT Assay: In order to determine the cytotoxic effect of the leaf extract of *Annona muricata*, MTT assay was performed in leukemic cells (Molt-3 cell line) in comparison with the standard chemotherapeutic drug Imatinib at a concentration of 50µg/ml. The experiment was carried out using varying concentrations of leaf extracts ranging from 10, 25, 50, 100 and 200µg. The viability of the control cells were fixed as 100%. The results revealed that ethanol extract of *Annona muricata* leaves are able to induce cell death even at lower concentration of 10µg/ml. When Molt-3 cells are treated with increasing concentration of extract, the viability was found to be decreased in a dose dependent manner. The results showed that the leaf extract exhibited good cytotoxic effect towards the leukemic cell line. The data also revealed that 50% viability was observed at lower concentration (10µg/ml) while the viability was decreased to a greater extent of about 12% at the higher concentration 200 µg/ml as shown in the Figure 3.

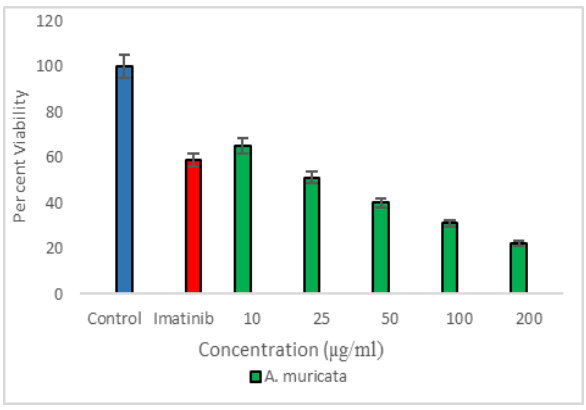
Cytotoxic effect of Ethanol Extract of *Annona Muricata* on Molt-3 Cell Line as Determined by SRB Assay: The anti-proliferative SRB assay was performed to assess growth inhibition by the *Annona muricata* leaf extract. The results demonstrated that the ethanol extract of *A. muricata* leaves showed significant cytotoxic effect against Molt-3 cell line. When the cells were treated with different concentrations of the leaf extract, cell death was observed in a dose dependent manner. It is evident that the leaf extract was able to induce cell death to a considerable extent. Upon treatment with leaf extract, the cytotoxic effect was observed even at lower concentration of 10µg/ml which was comparable with the standard drug imatinib as shown in Figure 3 and was increased gradually at the higher concentration (200 µg/ml).

Figure 3: Cytotoxic effect of ethanol extract of *Annona muricata* on Molt-3 cell line

3a. MTT



3b. SRB

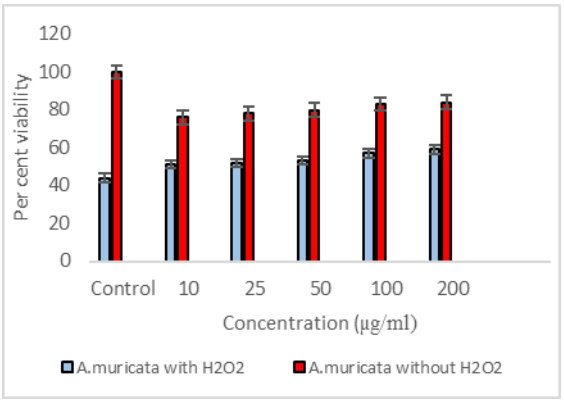


After analyzing the results of cytotoxicity assays, it becomes essential to check whether the extract elicits cytotoxicity to normal or untransformed cells which then may lead to different side effects as caused by the pharmaceutical drugs that are available commercially. Therefore, the cytotoxicity assays were performed in untransformed cells such as Yeast cells to understand the property of these *Annona muricata* extract.

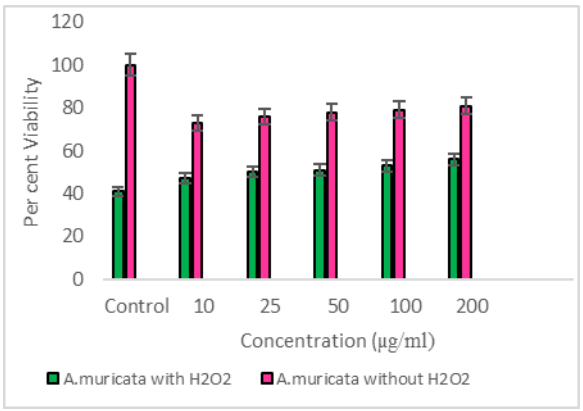
Cytotoxic Effect of Ethanol Extract of *Annona Muricata* on *S. Cerevisiae* as Determined by MTT Assay: In the present study, the normal/ untransformed cells are treated with varying concentrations of the extract and the results showed that the cell viability was found to increase with the increasing concentration. Further, treatment with H_2O_2 caused a steep decrease in the cell survival rate and upon administration of the ethanol extract of *A. muricata* leaves there is an improvement in the cell viability indicating its protective property in normal/ untransformed cells. As the concentration increases, the cell viability also increased in a dose dependent fashion as given in Figure 4.

Figure 4: Cytotoxic effect of ethanol extract of *Annona muricata* on *S. cerevisiae*

4a. MTT



4b. SRB

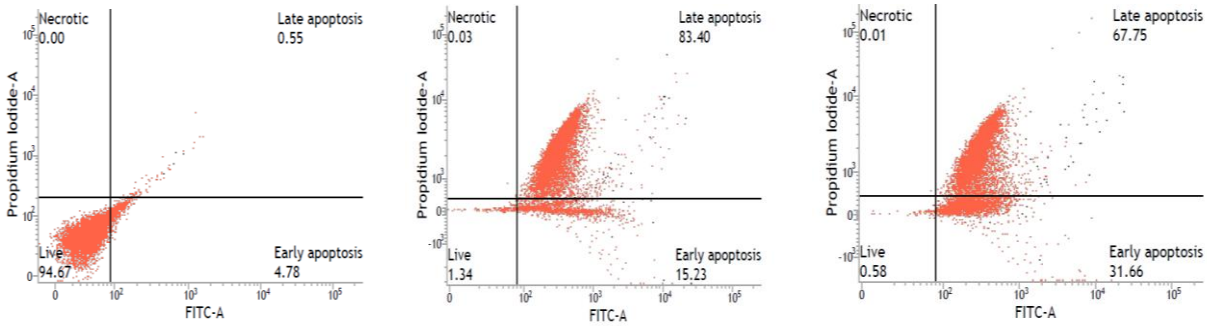


MTT and SRB assays were carried out using yeast cells. Treatment with the standard oxidant H_2O_2 reduces the cell survival rate whereas administration of *A. muricata* plant leaf extracts could reverse the cytotoxic effect exerted by H_2O_2. No significant change observed in the plant extract alone treated group indicates that the leaf extract by itself is not toxic to the normal/untransformed yeast cells. However, these extracts were able to cause significant cell death when tested in Molt-3 cell lines. Thus, the results evidenced a differential response (ie) the leaf extract is decreasing the rate of cell survival when administered in Molt-3 leukemic cells along with imatinib. Whereas, rendering protection to yeast cells in the presence of the standard oxidant hydrogen peroxide. This disparity in response evoked by the leaf extract of *A. muricata* may be due to the presence of phytoconstituents such as alkaloids, flavonoids, phenols, tannins and saponins as evidenced by the results of phytochemical analysis. Llagas *et al*. (2014) reported that the ethylacetate, chloroform, and crude ethanol extracts of *Ficus pseudopalma* (FP) leaf exhibited cytotoxicity against human prostate cancer PRST2 cell line. They also have stated that the anticancer activity of *Ficus* species have been attributed to its naturally occurring compounds such as terpenoids and flavonoids. *Nardostachys jatamansi* extract showed significant antioxidant and anticancer activity due to the presence of phenols (Chaudhary *et al*., 2015). Report of Medini *et al*. (2015) suggest that *Limonium densiflorum* has the strong anticancer potential because of the source of phenolic compounds. Thus, it can be concluded that, the leaf extract exhibit apoptosis inducing property against the oxidant induced damage only in leukemic cells. As the yeast/untransformed cells were readily protected by the leaf extract of *A. muricata* even in the presence of H_2O_2.

Determination of Apoptosis: The results of the present study showed that the *A. muricata* leaf extract were able to induce apoptosis in the Molt-3 cell line which was analyzed by Annexin V/FITC staining by flow

cytometry. The results indicate that after treating with the leaf extract, most of the Molt-3 cells were in the late apoptotic stage of cell death and only very few cells were undergone early apoptosis. When treated along with standard drug imatinib, the percentage of apoptosis was reduced to some extent. These results clearly indicate that *A. muricata* leaf extract were able to induce apoptotic cell death in Molt-3 leukemic cell line.

Control *A. muricata* Imatinib



Conclusion:

In the present study, phytochemical analysis was carried to out identify the predominant secondary metabolites present in the ethanol extract of *Annona muricata* leaves. Further its antioxidant and anticancer property was analyzed by various parameters. The results showed that alkaloid is one the phytoconstituents predominantly present in the *Annona muricata* leaf extract and it is able to scavenge free radicals effectively and also exhibited good cytotoxicity towards Molt-3 cell line while rendering protection against the normal/untransformed *S. cerevisiae* cells from oxidative damage. Thus, the ethanol extract of *Annona muricat*a leaves can serve as potential drug candidate for the free radical mediated diseases such as cancer and leukemia.

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