

Anti-Cancerous Activity of Leaves of *Cadaba fruticosa*, Druce using Human Lung Cancerous Cell Line A549 by *In Vitro* Methods***Sundara Vadivel D¹, Gopinath P¹, Jayakar Santhosh D², Balakumaran P³.**

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ABSTRACT

Lung cancer is a disease characterised by uncontrolled cell growth in tissues of the lung and is the commonest form of death worldwide due to cancer. Medicinal plants are considered to be the exclusive source of life saving drugs for majority of world's population. The present study was carried out to evaluate the anticancer activity of various extracts of leaves of *Cadaba fruticosa* in human lung cancerous cell line A549 and 5-fluorouracil, by *in vitro* methods. Cytotoxicity of leaf extracts were assessed by MTT assay. Ethyl acetate extract was found to be more cytotoxic with a median inhibitory concentration (IC₅₀) of 56.23 µg/ml when compared to Ethanol extract (72.44 µg/ml) and aqueous extract (81.28 µg/ml). Ethyl acetate extract treated A549 cells showed characteristics of Apoptosis like cell shrinkage, chromatin condensation and nuclei fragmentation. Expression levels of proteins were studied using bio analyser and expression of C- reactive protein and Phospho-histidine phosphatase was found to be decreased in ethyl acetate extract treated cells, when compared to control cells. Real time PCR was used to determine the gene expression levels. Expression of p53 and TNF-α was found to be high in cells treated with ethyl acetate extract than with control cells, but was low in comparison to standard 5 – Fluorouracil treated cells. Expression of Bcl-2 and IL-6 was low in cells treated with ethyl acetate extract and 5 – Fluorouracil, when compared with control cells. These results reveal the potential anticancer activity of ethyl acetate extract of *Cadaba fruticosa*.

Keywords: A549, Apoptosis, *Cadaba fruticosa*, Cytotoxicity, Ethyl acetate extract, RT-PCR.

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INTRODUCTION

Among different types of cancer, lung cancer contributes to be the commonest form of cancer worldwide in terms of both incidence and mortality [1]. In developing countries like China and India, the incidence of lung cancer is expected to increase drastically in the next few years. During the past five decades, the incidence of lung adeno carcinoma, a type of non small cell lung carcinoma started to rise relatively when compared to other types of lung cancer [2,3]. Over expression of epidermal growth factor receptor, a member of tyrosine kinase receptor has been identified as therapeutic target of several human carcinomas, including non small cell lung cancer. Recently tyrosine kinase inhibitors

have been approved for treatment of lung cancer, but the success rates to these drugs are very low [4]. Even though modern drug therapies has improved cancer patients care to wide extent, some cases of advanced metastasized cancer still continues to remain untreatable. With limited amount of success pertaining to available treatment options for cancer and considering the toxicities which arise from cytotoxic drugs like bone marrow depression, alopecia, lymphocytopenia and occurrence of secondary cancers like leukaemia and lymphoma, alternative and complimentary strategies are in need to be developed which exerts its action by without interfering with body's natural healing

process [5,6]. Medicinal plants play a major role in health care system and are exclusive source of life saving drugs for majority of world's population. Anti cancer activity of medicinal plants are mainly due to presence of phenolic compounds, flavanoids and phenolic diterpenes. Natural products have long been a rich source of cure for cancer. Some of them used in treatment of cancer include taxol, etoposide, topotecan, irinotecan, vincristine, vinblastine, colchicines and ellipticine [7,8].

Cadaba fruticosa is a commonly available shrub or a small tree, belonging to family Cappariaceae and its leaves are used to relieve rheumatic pain and used in the treatment of boils. It is also used in the treatment of cough, fever, dysentery and as antidote against poisoning [9]. Its medicinal properties like antipyretic, anti-diabetic, antifungal, cytotoxic, antimicrobial, hepato protective and antioxidant properties were scientifically evaluated and reported [10-15]. In spite of its potent antioxidant and

cytotoxic properties, there was no report for the evaluation of its anticancer activity focussing on gene expression levels and protein levels in human cancerous cells along with apoptosis study. So the study was carried out using *Cadaba fruticosa* to evaluate its anticancer effect in human lung cancerous cell line A549 using various *in vitro* models.

MATERIALS AND METHODS:

Plant Material:

The fresh, disease resistant leaves of *Cadaba fruticosa*, Druce was collected from Sattur, Virudunagar district and was identified and authenticated by Professor V. Chelladurai, Research officer-Botany (Retd.), Central council for research in Ayurveda and Siddha, Government of India. The leaves were washed thoroughly with tap water, followed by sterile distilled water and were shade dried at room temperature. It was subjected to size reduction to a coarse powder by using dry grinder and was used for extraction.



Figure 1: Leaves of *Cadaba fruticosa*

Preparation of Plant Extract:

50 grams of coarse powder of leaves of *Cadaba fruticosa* was packed into the thimble of soxhlet apparatus and was subjected to extraction sequentially with 500ml of petroleum ether, ethyl acetate, ethanol and water. The extraction was continued until the colour of the solvent in the siphon tube became colourless. Ethyl acetate, Ethanol and Aqueous extracts were

subjected to lyophilisation with the help of lyophiliser to a semisolid mass.

Cell Culture:

Human lung cancerous adherent cell line A549, an adenocarcinomic alveolar basal epithelial cell line was procured from King Institute of Preventive Medicine, Chennai. The cells were grown in culture flask using Minimum Essential Medium supplemented with 3% L- Glutamine, 10% Foetal bovine

serum, Penicillin (100 IU/ml), Streptomycin (100 µg/ml) and Amphotericin B along with 7.5% sodium bicarbonate in a humidified atmosphere of 5% CO₂/95% O₂ incubator at 37°C. The cells were subjected to passaging for required number of flask for further studies.

Chemicals and Reagents:

5 - Fluorouracil (Sigma), MTT dye (Sigma), Acridine orange (Sigma), Ethidium bromide (Sigma), GeNei TRI solution, cDNA kit (Qiagen), forward and reverse primers, SYBR green RT mix (Qiagen), RNase and TPVG (Qiagen).

Preparation of Stock solution:

Dried plant extracts were dissolved in one ml of DMSO (0.1% v/v) and volume was made up to 10 ml with minimum essential medium to give a stock solution of 10 mg/ml. Standard drug 5- Fluorouracil were dissolved in one ml of DMSO (0.1% v/v) and volume was made up to 10 ml with minimum essential medium to give a stock solution of 1 mg/ml.

Dilution of Plant extract and standard drug:

Stock solution of plant extracts were diluted with minimum essential medium to obtain the concentration of 10, 20, 40, 60, 80 and 100 µg/ml. Stock solution of 5- Fluorouracil were diluted with minimum essential

medium to obtain the concentration of 1, 2.5, 5 and 10 µg/ml. All the diluted extracts were stored in air tight container for further use.

Cytotoxicity test by MTT Assay: [16]

Cytotoxic activity of *Cadaba fruticosa* in A549 cells were studied by MTT assay. Cells were seeded into 96 well plates, at a plating density of 10000 cells/well and the plate was incubated for 24 hours at 37°C, 5% CO₂ for attachment of the cells. After 24 hours the seeded cells in the 96 well plates were treated with various concentrations of leaf extracts and standard drug 5FU and incubated again for 48 hours at 37°C and 5% CO₂. After 48 hours of incubation, medium was removed from the 96 well plates and it was replaced with 100 µl of phenol red free medium and 15µl of MTT (5 mg/ml) solution was added to the well plate and wrapped with aluminium foil and the plate was incubated again for 4 hours. After incubation the medium was carefully removed without disturbing the formed formazan crystals and the crystals were solubilised in 100 µl of 0.1% v/v DMSO and the absorbance was measured at 570nm using ELISA reader. The Percentage cell growth inhibition was calculated using the formula,

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Optical density of treated cells}}{\text{Optical density of control cells}} \times 100$$

Microscopic Studies:

Light microscopic study:

Sub cultured flask containing A549 cells without any contamination were observed under inverted light microscope to study the morphological features, which served as control. The medium inside the flask was decanted and the cells were treated with IC₅₀ concentration of ethyl acetate extract and the flask was incubated for 48 hours at 5% CO₂ and 37°C. The flask was taken and observed under inverted light microscope. Cells were considered to be apoptotic if they displayed characteristics of cell shrinkage, reduction in cell population, chromatin condensation and nuclei fragmentation [17].

Fluorescent Microscopic study:

Two culture flasks with fully grown or 90% confluence reached A549 cells were taken, one serving as control and the other for plant extract treatment. The medium was decanted and treated with IC₅₀ concentration of ethyl acetate extract and incubated for 48 hours at 5% CO₂ and 37°C. Cells were trypsinised from both control and extract treated flask and subjected to centrifugation. Pellet of cells were resuspended in phosphate buffer saline of pH 7.4. 100 µl of this cell suspension was introduced into microscopic slide along with equal mixture of acridine orange and ethidium bromide for staining. The cells were then viewed under fluorescent microscope. The viable cells (green colour) and dead cells (red colour) were identified

by differential uptake of these two fluorescent DNA binding dyes.

Extraction of DNA, RNA and Protein:

Extractions of DNA, RNA and protein were carried out using GeNei TRI solution as per manufacturer's instructions. Three sub-cultured flask containing A549 cells were subjected to drug treatment. Flask 1 served as Control, flask 2 and 3 were treated with IC₅₀ concentration of ethyl acetate extract and 5-fluorouracil respectively. After 48 hours incubation at 37°C and 5% CO₂ adhered cells were disturbed by treatment with TPVG solution and the cells were pelleted by centrifugation. One ml of TRI solution was added to the pellet of cells and incubated for 5 minutes. 0.2 ml of chloroform was added, incubated and centrifuged at 12000 rpm for 15 minutes at 2-8°C, which resulted in upper aqueous phase containing RNA, interphase containing DNA and lower phenol chloroform phase containing protein.

Aqueous phase obtained was treated with 0.5 ml of isopropanol and centrifuged at 12000 rpm for 10 minutes at 2-8°C. RNA was obtained as a pellet, which was washed with 75% ethanol and centrifuged at 10000 rpm for 10 minutes at 2-8°C. RNA pellet was air dried and re-suspended in 100 µl of RNase free water.

0.3ml of 100% ethanol was added to the inter phase and organic phase and centrifuged at 5000 rpm for 10 minutes at 2-8°C and DNA was obtained as a pellet. DNA pellet obtained was washed twice with wash buffer containing 0.1 N sodium citrate in 10% ethanol. It was suspended in 2 ml of 75% ethanol and centrifuged at 5000 rpm for 10 minutes at 2-8°C. DNA pellet was then air dried for 10 minutes and re-suspended in 300 µl to 600 µl of 8mM sodium hydroxide and incubated at room temperature for 15-20 minutes.

The supernatant obtained on treatment with 0.3ml of 100% ethanol to the inter phase and organic phase was treated with 1.5 ml of isopropanol and stored at room temperature to precipitate protein as a pellet. Protein pellet was washed with 0.3 M guanidium hydrochloride in 95% ethanol and centrifuged at 10000 rpm for 5 minutes at 2-8°C. The pellet was vacuum dried for 5-10 minutes and re-suspended in 1% SDS by

incubating at 50°C for 2-3 minutes. DNA, RNA and Protein obtained were quantified using Nanodrop 1000 spectrophotometer and used for DNA fragmentation, RT-PCR and protein analysis.

DNA Fragmentation study:

DNA isolated from control, extract and standard treated cells were mixed with TAE buffer and loading dye and loaded carefully in submerged wells of gel slab in the electrophoresis chamber along with 100 base pair standard DNA ladder and the gel was run. After gel electrophoresis, Gel tray was carefully taken out and the gel were placed inside the Gel document system and photographed under UV light [18].

Protein Assay:

Agilent high sensitivity protein 250 bio analyzer was used to identify and quantify the proteins isolated from control cancer cells and in cancer cells after treatment with standard drug and ethyl acetate extract. Equal quantity of protein and ladder were subjected to labelling with fluorescent dye and was loaded in the wells of the protein chip along with gel mix and destaining solution. The chip was inserted into the bio analyzer and the chip was run. All the procedures were carried out as described under manufacturer's protocol and the proteins were identified and quantified with the help of standard ladder using bio analyzer.

Real time Reverse Transcriptase Polymerase Chain Reaction:

It is a technique where expression of RNA is studied by converting it into cDNA and quantitatively measuring the amount of amplified target sequence from entire cDNA using fluorescent dye SYBR green in real time. Upon binding with DNA, SYBR green dye used will emit fluorescence and the fluorescence intensity is directly proportional to number of DNA copies or expression produced.

➤ **cDNA Synthesis:** Isolated RNA were treated with 5X buffer, 10mm DNTP's, hexamer primer and enzyme reverse transcriptase in an eppendorf tubes. Later the tubes were placed in a thermal cycler at 25°C for 5 minutes for binding of hexamer, 42°C for 45 minutes for cDNA synthesis, 85°C for 5 minutes for denaturation of remaining unconverted

RNA's and finally at 4°C for 5 minutes. cDNA was stored at -20°C for further use.

- **Primer Synthesis:** Primers for cancer DNA marker p53, Bcl-2, TNF- α and Immune response marker IL-6 were

synthesized along with housekeeping gene GPDH by using Primer express software with the available primer sequence as shown in (Table 1)

Table 1: Primer Sequence

PRIMER NAME	PRIMER SEQUENCE FROM 5' - 3'
P53	AGGGATACTATTTCAGCCCGAGGTG ACTGCCACTCCTTGCCCCATTC
Bcl-2	ATGTGTGTGGAGAGCGTCAACC TGAGCAGAGTCTTCAGAGACAGCC
TNF α	TCTCTAATCAGCCCTCTGGCC TGGGCTACAGGCTTGTCCTC
IL-6	GCCTTCGGTCCAGTTGCCTT GCAGAATGAGATGAGTTGTC
GPDH	ATTGACCACTACCTGGGCAA GAGATACACTTCAACTTTGACCT

- cDNA synthesized was used to study the expression levels of cancer DNA marker and immune response marker. For a total of 50 μ l reaction, 25 μ l of SYBR green RT mix, 5 μ l of cDNA, 2 μ l of 25 pM/ μ l forward primer, 2 μ l of 25 pM/ μ l reverse primer and 16 μ l PCR grade water were added into eppendorf tubes and were placed in real time PCR instrument and the program was set as follows: Step 1: Pre denaturation at 95°C for 1 minute, Step 2: Denaturation at 95°C for 15 seconds, Step 3: Annealing at 60°C for 15 seconds and Step 4: Extension at 72°C for 45 seconds. Step 2 to step 4 repeated for 40 cycles. The relative expression of genes were analyzed and interpreted by Applied Biosystem Software

RESULTS AND DISCUSSION

Anti Cancer activity of various extracts of leaves of *Cadaba fruticosa* was assessed by cytotoxicity test – MTT assay. Ethyl acetate extract of leaves of *Cadaba fruticosa* was found to be more cytotoxic, when compared to other extracts and ethyl acetate extract was further employed to test the apoptotic activity by microscopic and DNA fragmentation studies. Protein expression was studied using Bio Analyser and regulation of gene expression was studied by real time RT – PCR.

MTT Assay is an *in vitro* method used commonly for anticancer drug screening, which utilizes a colour reaction in measurement of viability of cells. MTT is a yellow water soluble tetrazolium salt. Succinate dehydrogenase, a mitochondrial enzyme present in living cells converts MTT to an insoluble purple formazan by cleaving the tetrazolium ring and the amount of formazan formed is directly proportional to number of viable cells. MTT assay is used to determine the IC₅₀ of the substance under study, with the capability of spontaneous scanning of hundreds of cell samples simultaneously [16]. Among the three extracts evaluated, the effective extract was found to be ethyl acetate extract with an IC₅₀ value of 56.23 μ g/ml, followed by ethanol and aqueous extract with a IC₅₀ value of 72.44 μ g/ml and 81.28 μ g/ml respectively. IC₅₀ value of 5 Fluorouracil was found to be 1.31 μ g/ml (Fig. 2 & 3).

Ethyl acetate extract of *Cadaba fruticosa* caused inhibition of cell growth and cell survival. A549 cells on treatment with increasing concentration of ethyl acetate extract showed decrease in number of viable cells and it has been depicted in (Fig. 4).

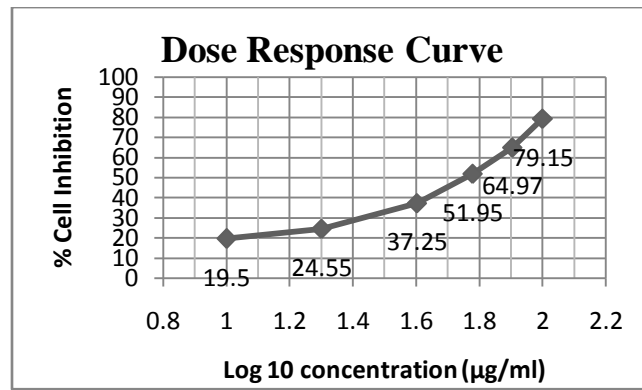


Figure 2: Graphical representation of %Cell inhibition vs Log₁₀ conc. of Ethyl acetate

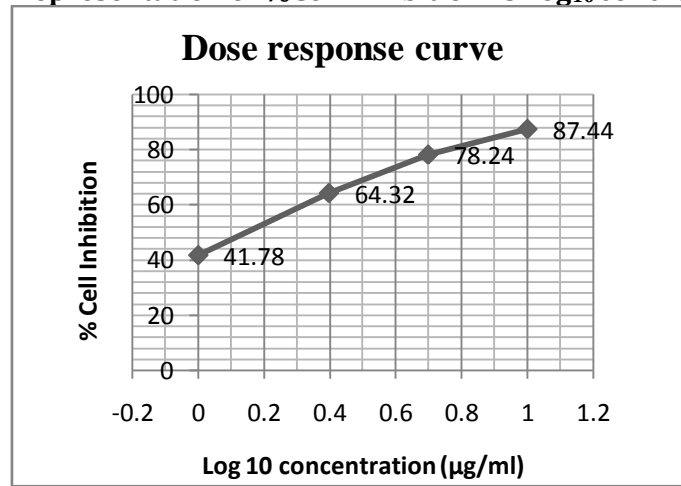


Figure 3: Graphical representation of % Cell inhibition vs Log₁₀ conc. of 5 Fluorouracil

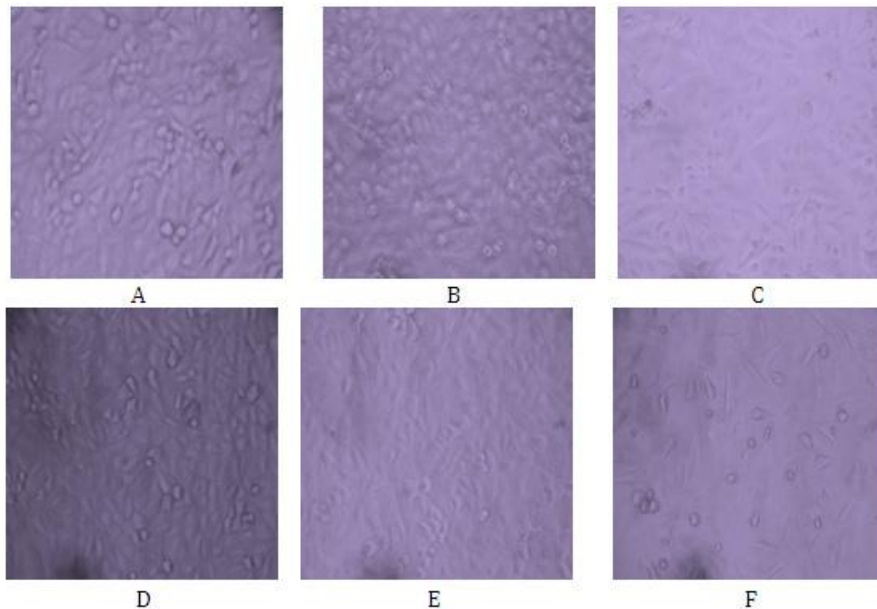


Figure 4: A549 cells treated with Ethyl acetate extracts- A.10 µg/ml, B.20 µg/ml, C.40 µg/ml, D.60 µg/ml, E.80 µg/ml and F.100 µg/ml

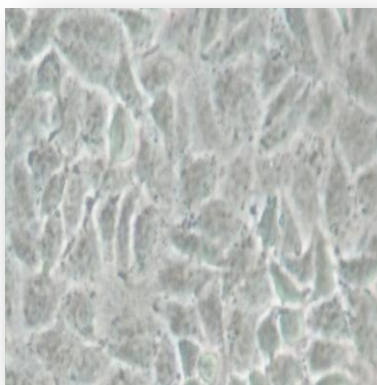
Apoptosis is also known as 'programmed cell death'. It is the elimination of unwanted

cells in an active, orderly and inherently controlled manner. Characteristic of

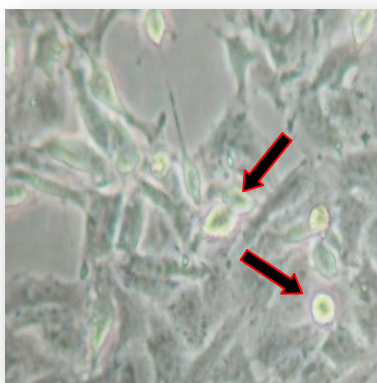
apoptotic process include cell and nuclear shrinkage, chromatin condensation, formation of apoptotic bodies and phagocytosis by adjacent cells. These morphological changes were observed using light microscope when ethyl acetate extract of *Cadaba fruticosa* treated A549 cells, showed typical morphological features

of apoptosis which includes destruction of monolayer, reduction of A549 cell population, reduction of cell volume, loss of integrity of membrane which resulted in crooked and vesicle shape of the membrane and chromatin condensation when compared to cells without any treatment (Fig. 5).

Figure 5: Light Microscopic study images



A) A549 cells - Control



B) A549 cells after treatment with ethyl acetate extract of *Cadaba fruticosa* indicating decrease in cell population, chromatin condensation and destruction of monolayer

Apoptotic effect of ethyl acetate extract of *Cadaba fruticosa* treated A549 cells were further confirmed with the help of fluorescence microscopy using acridine orange and ethidium bromide. Acridine orange is a vital dye capable of staining both dead and live cells, where as ethidium bromide will stain only cells that have lost their membrane integrity [17]. On examination of cells without any treatment under fluorescent microscope, the cells were stained green in colour representing viable or live cells, whereas examination of cells after treatment with ethyl acetate

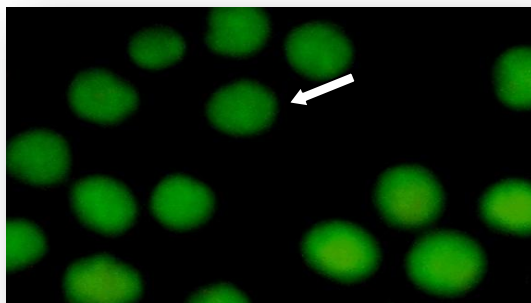
extract showed reddish or orange colour with loss of membrane integrity and leakage of cytoplasmic contents representing dead cells (Fig. 6).

Cleavage of chromosomal DNA into oligonucleosomal fragments is a hallmark of apoptosis and apoptotic cells often produce nucleotide fragments at an interval of 180-200 base pairs, which can be visualized by DNA agarose gel electrophoresis [18]. Fragmentation was observed in A549 cells treated with ethyl acetate extract of *Cadaba fruticosa* and standard 5 fluorouracil (Fig.7). In the cells without any treatment,

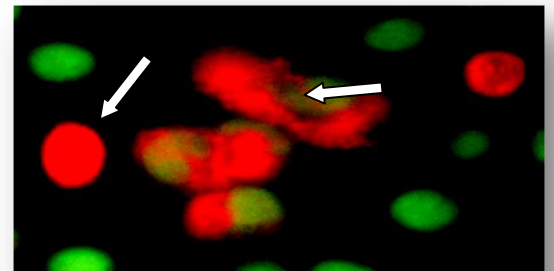
there was no fragmentation observed. Thus ethyl acetate extract of *Cadaba fruticosa*

causes DNA damage in A549 cells, thereby inducing apoptosis.

Figure 6: Fluorescent Microscopic Images



A) A549 cells – Control, indicating viable cells stained green in colour



B) A549 cells after treatment with Ethyl acetate extract of *Cadaba fruticosa* showing dead cells stained orange in colour.

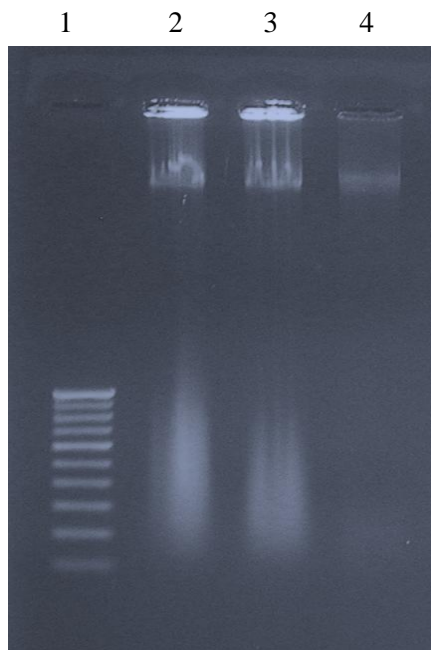


Figure 7: DNA Fragmentation in A549 cells. Lane 1: 100 base pair DNA marker, Lane 2: A549 cells treated with 5 Fluorouracil, Lane 3: A549 cells treated with Ethyl Acetate extract of *Cadaba fruticosa* and Lane 4: A549 cells without any treatment.

Quantification of proteins, C reactive protein and Phosphohistidine phosphatase were analysed using Bio analyzer and results obtained were given in (Fig. 8-9). Concentration of C Reactive Protein after treatment with ethyl acetate extract and 5 fluorouracil were found to be 352.6 pg/μl and 322.9 pg/μl respectively when compared to control cells - 422.5 pg/μl. Concentration of Phosphohistidine phosphatase after treatment with ethyl acetate extract and 5 fluorouracil were

found to be 87.8 pg/μl and 64.3 pg/μl, when compared to control cells - 127.4 pg/μl. C Reactive Protein is a 21KDa protein, a systemic biomarker of inflammation whose levels were found to be high in malignancies indicating a close relationship between inflammation and malignancy. Elevated levels of CRP were significantly associated with disease progression such as increase in tumour size, depth of wall invasion, lymph node metastasis and distant metastasis. It was reported that C reactive protein levels

were high in patients suffering from lung cancer and there was a strong association of elevated levels of CRP with tobacco related lung cancer. CRP lowering agents may prove to play a promising role in prevention and therapy of malignancies in future [19]. Another protein associated with lung cancer is 14KDa phosphohistidine phosphatase

which was reported to play a role in lung cancer cell migration and invasion [20]. Marked reduction in protein levels which are said to be associated with lung cancer risk in cells after treatment with ethyl acetate extract shows that the ethyl acetate extract of *Cadaba fruticosa* posses the anti cancer effect.

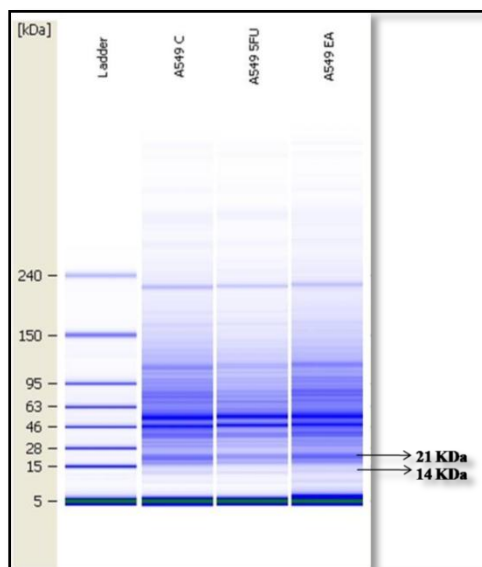


Figure 8: Gel Image of Protein analysis

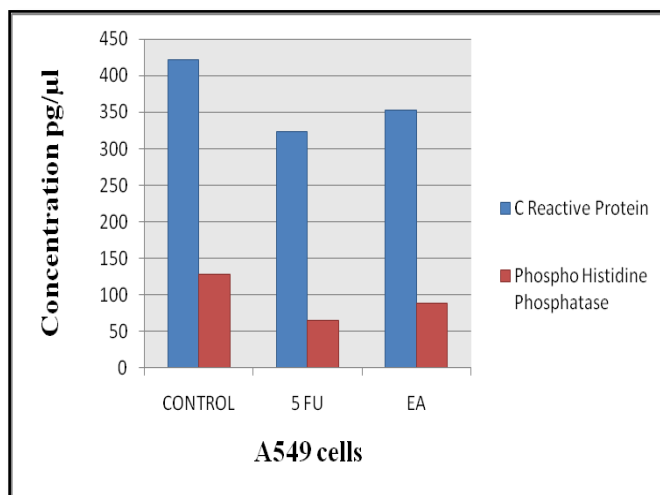


Figure 9: Graphical representation of concentration of C Reactive Protein and Phospho histidine phosphatase in A549 cells before treatment and after treatment with Ethyl acetate extract of *Cadaba fruticosa* and 5 fluorouracil.

Cancer DNA markers like p53, Bcl2, TNF- α and immune response marker IL-6 plays a major role in cancer pathology and their expression levels determine the progression of the disease. These gene

expression levels were studied in cells treated with ethyl acetate extract of *Cadaba fruticosa* and 5 Fluorouracil by RT-PCR methodology and the results are shown in (Fig. 10).

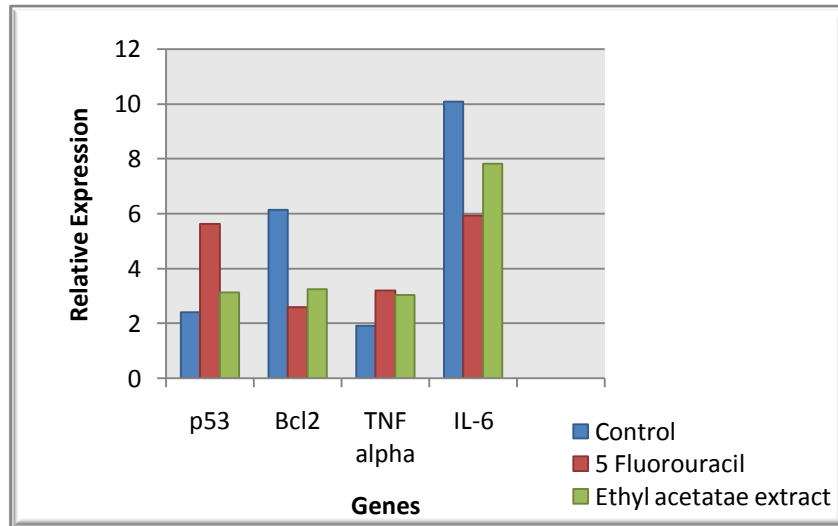


Figure 10: Comparative Gene expression levels of p53, Bcl-2, TNF- α and IL-6 in A549 cells treated with ethyl acetate extract of *Cadaba fruticosa* and standard 5 Fluorouracil.

p53 plays a role in apoptosis, maintaining the stability of the genome and in inhibition of angiogenesis. It plays a significant role in regulation of cell cycle and helps in preventing cancer. p53 protein has been mutated in 50% of cancers of lung, liver, prostate and breast. Many studies reported the up regulation of p53 paved way for apoptosis [21]. The expression levels of p53 was found to be increased in cells treated with ethyl acetate extract of *Cadaba fruticosa* (RQ-3.12) and in cells treated with 5 Fluorouracil (RQ-5.62) when compared to cells without any treatment (RQ-2.41), indicating the ability of ethyl acetate extract to up regulate p53 and promote apoptosis. TNF is an endogenous pyrogen which can induce fever, apoptotic cell death, sepsis and inflammation. Abnormalities or irregularities in production of TNF have lead to diseases like Alzheimer's disease, cancer, depression and inflammatory bowel disease. Apoptotic activity is attributed to increase in expression of TNF- α [22]. The expression levels of TNF- α was found to be increased in cells treated with ethyl acetate extract (RQ-3.04) and in cells treated with 5 Fluorouracil (RQ-3.21) when compared to cells without any treatment (RQ-1.92), indicating the ability of ethyl acetate extract to induce apoptosis by increasing the expression of TNF- α . The results obtained agreed with those obtained by Azizi et al [23] and Ali Alshehri [24].

Bcl-2 along with its family of proteins plays a major role in regulation of Apoptosis. Bcl-2 plays a significant role in cancer and its resistance thereby interfering with therapeutic action of chemotherapeutic drugs. High expression of anti-apoptotic members like Bcl-2 found in human cancers leads to neoplastic cell metastasis by interfering with normal cell death mechanism [25]. Decrease in expression of Bcl-2 leads to apoptosis. The expression levels of Bcl-2 in ethyl acetate extract treated cells (RQ-3.24) and 5 Fluorouracil treated cells (RQ - 2.58) was found to be decreased when compared to expression in cells without any treatment (RQ - 6.15) which implies that apoptosis in A549 lung cancerous cells may be due to decreased expression of Bcl-2. The results obtained were similar to those reported by Gul Ozcan Arican et al [26] in Hela cells.

Immune response marker IL-6 plays a role in cancer. Serum levels of IL-6 were detected in patients with lung, breast, prostate, colorectal, gastric, pancreatic, ovarian and renal cell cancers. High serum IL-6 levels were associated with progressive diseases and poor survival [27]. Expression of IL-6 in control cells were found to be high (RQ-10.08) when compared to cells treated with ethyl acetate extract (RQ-7.82) and cells treated with standard 5 Fluorouracil (RQ-5.92) indicating that apoptosis in cells of A549 may be due to decreased expression of IL-6. These gene expression

levels indicates that ethyl acetate extract of *Cadaba fruticosa* exhibits apoptotic effect by over expression of p53, TNF- α and down regulation of Bcl-2 and immune response marker IL-6.

CONCLUSION

In this present study, Ethyl acetate extract of *Cadaba fruticosa* was found to possess potent cytotoxic activity in human lung cancerous cell line A549 by causing inhibition of cell growth and inducing apoptosis. Anti cancer effect was further confirmed by reduced levels of proteins Phospho histidine phosphatase and C Reactive Protein in treated cells, whose elevated levels are said to possess a risk in cancer development. Its apoptotic and anti cancer effect may be due to up regulation of genes like p53 and TNF α and down regulation of genes like Bcl-2 and IL-6, which was confirmed by RT-PCR. These results show that ethyl acetate extract of *Cadaba fruticosa* possess anti cancer effect and for future perspective, the compounds responsible for the activity can be isolated and studied and the results can be confirmed using *in vivo* animal models, which may pave way for *Cadaba fruticosa* emerging as an effective plant source in treatment of cancer.

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REFERENCES

1. Ferlay J, Shin HR, Bray F et al. "Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008". J Clin Oncol. 2010 Dec; 127(12):2893-2917.
2. Charloux A, Quoix E, Wolkove N. "The increasing incidence of lung adenocarcinoma: reality or artefact? A review of the epidemiology of lung adenocarcinoma". Int J Epidemiol. 1997;26(1):14-23.
3. Kadara H, Kabbout M, Wistuba II. "Pulmonary adenocarcinoma: a renewed entity in 2011". Respirology. 2012;17(1):50-65.
4. Hirsch F.R, Scagliotti G.V., Langer C.J., Varella Garcia M, Franklin W.A. Epidermal growth factor family of receptors in preneoplasia and

- lung cancer: perspective for targeted therapies. Lung Cancer. 2003;41:S29-S 42.
5. Toh CK. The changing epidemiology of lung cancer. Methods mol biol. 2009;472:397-411.
6. Tripathi K.D. Essentials of Medical Pharmacology, Ed 6. New Delhi. Jaypee Brothers Medical Publishers; 2008: 669-674.
7. Wanq H, Khor To, Shu L, Su Zy, Fuentus F, Lee JH etal. Plants vs Cancer: A review on Natural Phytochemicals in preventing and treating Cancers and their druggability. Anticancer agents Med Chem. 2012 Dec;12(10):1281-305.
8. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in Cancer therapy with plant based Natural products. Curr Med Chem. 2001;8(12):1467-86.
9. Nadkarni K.M. Indian Materia Medica. 3rd ed. Panvel: Dhootapapeshwar Prakashan Pvt ltd; Elsevier saunders; Page 225.
10. Selvamani.P, Latha.S. Studies on Antimicrobial activity of *Cadaba indica* Lam. Indian J Pharm Sci. 2005;Sep-Oct:637-638.
11. Mohamed Al-Fatimi, Martina Wurster, Gudrun Schroder, Ulrike Lindequist. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol. 2007 Jan;111:657-666.
12. Mythreyi.R, Sasikala.E, Geetha.A, Madhavan.V. Antipyretic activity of leaves of *Cadaba fruticosa*(L.) Druce. Pharmacologyonline. 2008;3:136-142.
13. Siva.N, Ganesan.S, Banumathy.N, Muthuchelian. Antifungal effect of leaf extract of some medicinal plants against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. Ethnobotanical leaflets. 2008 Mar;12:156-163.
14. Arokiyaraj.S, Radha.R, Martin.S, Perinbam.K. Phytochemical analysis and anti-diabetic activity of *Cadaba fruticosa*. Indian J Sci Technol. 2008 nov;1(6).
15. Umesh.B.Telrandhe, Anuj modi, Vaibhav Uplanchiwar, Avinash Gahane, hemalatha.S, Goswami.D.V. Hepatoprotective and anti oxidant activity of root of *Cadaba farinose*, Forsk. against carbon tetrachloride induced hepatotoxicity in rats. Journal of pharmacy research. 2010 May;3(6):1412-1416.
16. Gupta S.K. Drug Screening Methods (Pre clinical Evaluation of New Drugs). 2nd ed. New Delhi (India): Jaypee Brothers Medical Publishers; 2009. 168-9.
17. Shahrul Hisham Zainal Ariffin, Wan Haifa Haryani Wan Omar, Zaidah Zainal Ariffin, Muhd Fauzi Safian, Sahidan Senafi, Rohaya Megat Abdul Wahab. Intrinsic anticarcinogenic effects of Piper

- sarmentosum ethanolic extract on a human hepatoma cell line. *Cancer Cell Int.* 2009 March;9(6).
18. Abhimanyu kumar Jha, Meenakshi Jha, Jagdeep kaur. Ethanolic extracts of *Ocimum sanctum*, *Azadirachta indica* and *Withania somnifera* causes apoptosis in SiHa cells. *Research journal of Pharmaceutical, Biological and Chemical sciences.* 2012 April-June;3(2):557-562.
 19. Chia siu wang, Chien feng sun. C reactive protein and malignancy: clinico pathological association and therapeutic implication. *Chang Gung Med J.* 2009 Sep-Oct;32(5):471-82.
 20. Xu A, Hao J, Zhang Z, Tian T, Jiang S, Hao J et al. 14KDa phosphohistidine phosphatase and its role in human lung cancer cell migration and invasion. *Lung cancer.* 2010 Jan;67(11):48-56.
 21. Philomena George. P53 how crucial is its role in Cancer? *Int J Curr Pharm Res.* 2011;3:19-25.
 22. Locksley RM, Killeen N, Lenardo MJ. "The TNF and TNF receptor superfamilies: integrating mammalian biology". *Cell.* 2001;104(4):487-501.
 23. Azizi E, Abdolmohammadi M.H, Fouladdel Sh, Shafiee A, Amin Gh, Ghaffari S.M. Evaluation of p53 and Bcl-2 genes and proteins expression in human breast cancer T47D cells treated with extracts of *Astrodaucus persicus*(Boiss.) Drude in comparison to tamoxifen. *Daru.* 2009 June;17(3):181-186.
 24. Ali Alshehri. Molecular and Biochemical evaluation of Antiproliferative effect of *Cichorium endivia* L. phenolic extracts on cancer cell line HCT-116. *Academic journal of Cancer Research.* 2012;5(2):53-60.
 25. Ziwei huang. Bcl-2 family proteins as targets for anticancer drug design. *Oncogene.* 2000; 19:6627-6631.
 26. Gul ozcan arican, Ozgur cakir, Ercan arican, Neslihan turgut kara, Ozlem dagdeviren, Sule ari. Effect of Geven root extract on proliferation of He La cells and Bcl-2 gene expressions. *African Journal of Biotechnology.* 2012;11(18):4296-4304.
 27. Culig Z. Interleukin-6 as a therapy target in oral squamous carcinoma. *Expert Opin Ther Targets.* 2013;17(1):53-59.