Journal of
Cancer Science & Therapy

Kaladhar et al., J Cancer Sci Ther 2014, 6:6
DOI: 10.4172/1948-5956.1000271

Research Article Open Access

Evaluation of Anti-inflammatory and Anti-proliferative Activity of *Abutilon indicum* L. Plant Ethanolic Leaf Extract on Lung Cancer Cell Line A549 for System Network Studies

Kaladhar DSVGK[1]*, Swathi Saranya K[1], Varahalarao Vadlapudi[2] and Nagendra Sastry Yarla[1]

[1]*Department of Biochemistry/Bioinformatics, GITAM University, Visakhapatnam, India*
[2]*Department of Biochemistry, Dr Lankapalli Bullayya P G College, Visakhapatnam, India*

Abstract

Pharmaceutically important components in medicinal plant kingdom are useful to control and cure many diseases like lung cancer. In the present study, an evaluation was performed to know the anti-inflammatory and anti-proliferative activity of ethanolic leaf extract of Abutilon indicum for potential chemopreventive agent against lung cancer. Experimentation is also conducted on lung cancer cell line A549 along with interaction studies of Apaf-1 gene. The ethanolic leaf extract of A. indicum is showing good anti-inflammatory activity (IC_{50}: 8.89 µg/mL) based on 5-Lipoxygenase (5-LOX) inhibition assay. The standard compound, Curcumin has shown IC_{50} as 8.14 µg/mL based on study. The ethanolic leaf extract of A.indicum has also shown good response on human Caucasian lung carcinoma of A549 cell line (IC_{50}: 85.2 µg/mL) shows anti-proliferative activity. Further analysis has also shown the interaction of proteins that are involved in inflammatory and cancer response. Apoptosis-activating factor, Apaf-1 gene increases the sensitivity of A549 cell line through interaction with proteins like CASP9, CASP3, CYCS, BCL2L1, TP53, BCL2, CASP8, HSPA4, DIABLO and CASP7. The experimental work concludes that bioactive components present in the ethanolic leaf extract of A.indicum shows good anti-inflammatory and anti-proliferative activity by inducing Apaf-1 through CASP9, CASP3, CYCS, BCL2L1, TP53, BCL2, CASP8, HSPA4, DIABLO and CASP7 network.

Keywords: *Abutilon indicum;* Anti-inflammatory activity; Anti-proliferative activity; A549 cell line; Apaf-1

Introduction

Plants have invariably had typical constituents of medicaments used from ancient preparations or as pure active principles. Plant extracts contains important sources of drugs used in control of aging and diseases. There are specific plant structures used as crude drug substances as active or major chemical constituents against diseased proteins [1]. These chemical components show characteristic profile that may be used to control networks in a system. The drug constituents with scientific data measure delineate within the major chemical constituents on the efficacy, security, beneficiary and internal control is used from plants which are associated with protein targets within a system.

Ayurveda is an ancient and applied research area used for the utilization of plants in treatment of varied human ailments. India has regarding 45,000 plant species and among them many are claimed to possess healthful properties [2]. Medicinal plants of the world contain valuable species that have indigenous, naturalized or cultivated plants [3]. Many Indian and Chinese healthcare plants historically employed in medicines were subjected to preliminary medicinal drug screening against morbific and opportunist microorganisms [4,5].

Abutilon indicum

Abutilon indicum (Linn.) Sweet (Malvaceae) is a shrub distributed throughout India, Sri Lanka, topical regions of America and Malaysia [6]. It is known as Atibala in Sanskrit. It grows as weeds that are found abundant in wastelands and seashores.

Various parts of the plant like leaves, roots, seeds and seed oil are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments [7]. The plant contain components like alkaloids, saponins, hexoses, flavonoids, n-alkane mixtures (C22-34), alkanols, sterols, coumarins, vanillic, fumaric acid, caffeic acid, amino acids, sesquiterpene lactones, steroids and essential oil [8-10].

The plant has a large number of pharmacological activities and there are no reports on the exact mechanism of plant ethanolic leaf extract of *A.indicum* for activity against lung cancer A549 cell line. From the ancient times, leaves of *A.indicum* are used for lumbago, piles, toothache, and all kinds of inflammation. Decoction of *A.indicum* leaves is used in catarrhal bilious diarrhoea, bronchitis, gonorrhoea, fevers and inflammation of bladder [6]. The decoction is prescribed as a mouthwash in various cases of tender gums and toothache. Bark of *A.indicum* is used in strangury and urinary complaints and is valued as a diuretic. Seeds are used as tonic. Unani systems of medicine suggest use of seeds in piles, chest troubles, bronchitis, and gonorrhea. Rectum of children affected with thread worms can be cured by exposure to the smoke of seeds burned on charcoal. According to the Chinese from Hong Kong, the seeds are employed as an emollient and demulcent [5].

Lung cancer and A549 cell line

Lung cancer is mostly associated with cigarette smoking where

***Corresponding author:** Kaladhar DSVGK, Assistant Professor, Department of Biochemistry/Bioinformatics, GIS, GITAM University, Visakhapatnam-530045, Andhra Pradesh, India, Tel: 9885827025; E-mail: dr.dowluru@gmail.com

Received March 27, 2014; **Accepted** May 16, 2014; **Published** May 20, 2014

Citation: Kaladhar DSVGK, Saranya KS, Vadlapudi V, Yarla NS (2014) Evaluation of Anti-inflammatory and Anti-proliferative Activity of *Abutilon indicum* L. Plant Ethanolic Leaf Extract on Lung Cancer Cell Line A549 for System Network Studies. J Cancer Sci Ther 6: 195-201. doi:10.4172/1948-5956.1000271

the cellular system in lungs will be damaged and mutations may occur leading cancer [11,12]. Most of the cases for the cancer deaths worldwide are due to lung cancers that are associated with cigarette smoking. Human lung cancer cell line A549 may provide a molecular understanding of ethanolic extract of *Abutilon indicum* that blocks cell cycle progression in the G1 phase. The association and networks may be due to activating partner cdk2, 4 and 6 that induce p21/WAF1/CIP1, p300/CBP and Apaf1. The effect is also due to up-regulation of Bax, Fas ligand and Fas/APO-1 and in down-regulation of NF-κB, Bcl-2, and Bcl-X (Figure 1).

MAPK pathway

Controlling apoptosis in cancer development can be a major barrier to provide effective treatment using phytocompounds. Figure 2 shows Mitogen-Activated Protein Kinase (MAPK) cascade is involved in various cellular functions like cell proliferation, differentiation, apoptosis, migration and cancer [13]. This is a highly conserved module that expresses extracellular signal-related kinases like (ERK)-1/2, Jun amino-terminal kinases (JNK1/2/3), p38 proteins and ERK5 that are activated by specific MAPKKs. The MEK1/2, ERK1/2, MKK3/6, p38, MKK4/7 (JNKK1/2), JNKs, MEK5, ERK5 forms networks in mammals are linked to inflammation and cancer. Each MAPKK in turn be activated by more than one MAPKKK showing increase in complexity and diversity of MAPK signalling. MAPKKKs may activate ERK1/2 in response to pro-inflammatory stimuli (Figure 2).

The present investigation attempted to reveal the anti-inflammatory and anti-cancer activity of *A.indicum* which was used as an ethnomedicine in India and several other parts of the world. Understanding of the Apaf1 protein network can be helpful in cracking the role of Apaf1 in cancer development and conventional therapy. Protein interaction networks can be performed to know interaction between cancer and inflammation causing proteins to establish the relationship within and outside the cell.

Materials and Methods

Preparation of extracts

The *A. indicum* leaves were collected from Visakhapatnam regions during rainy season. The plant is authenticated by Dr. P.V. Arjun Rao, Ethanobotanist, Dept. of Botany, Phytopharma Technology Laboratory, GITAM University, Visakhapatnam (No. Res/3). The leaves were dried and the powder was prepared. Nearly 20 grams of the powder was soaked in 200 ml of ethanol for 24 hours in rotary shaker, filtered and centrifuged for 30 min at 4000 rpm. The supernatant solution was collected after centrifugation and was dried in the sunlight for getting powder. After drying 2 grams (equal to 10% yield) has been obtained. The powder was used for the present experimentation.

Anti-inflammatory activity

5-Lipoxygenase (5-LOX) is an important enzyme involved in inflammation. The anti-inflammatory effect refers to the property of a treatment or a substance to reduce inflammation that inhibits 5-LOX. The standard used in the present experiment is Curcumin. The test sample used in the experiment is ethanol leaf extract of *A. indicum*. Appropriate dilutions (70 µg/mL-1 µg/mL) of test items were prepared to assess the 5-LOX activity.

Chemicals and reagents

All the chemicals acquired from Sigma chemicals and the reagents are prepared as per the below given protocol.

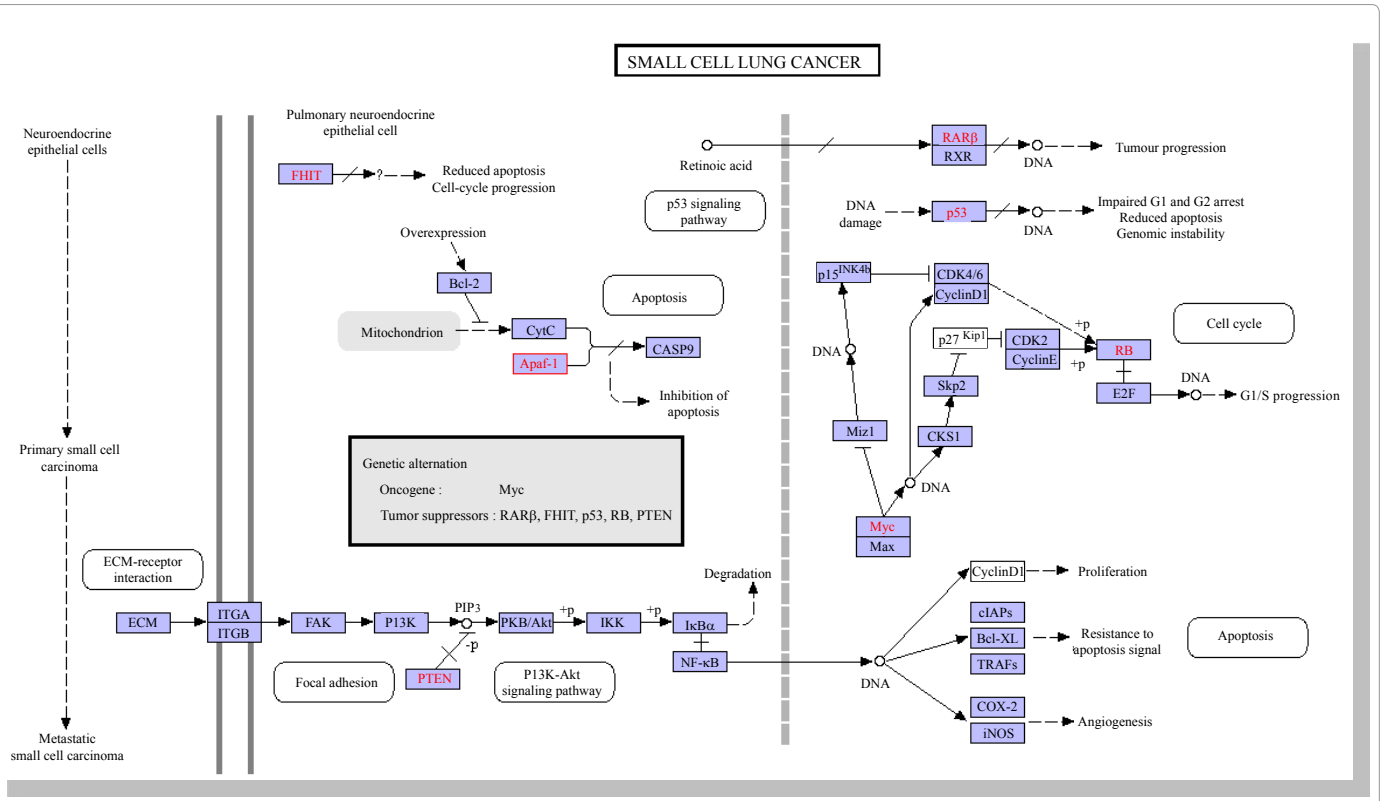


Figure 1: Small cell lung cancer pathway from KEGG.

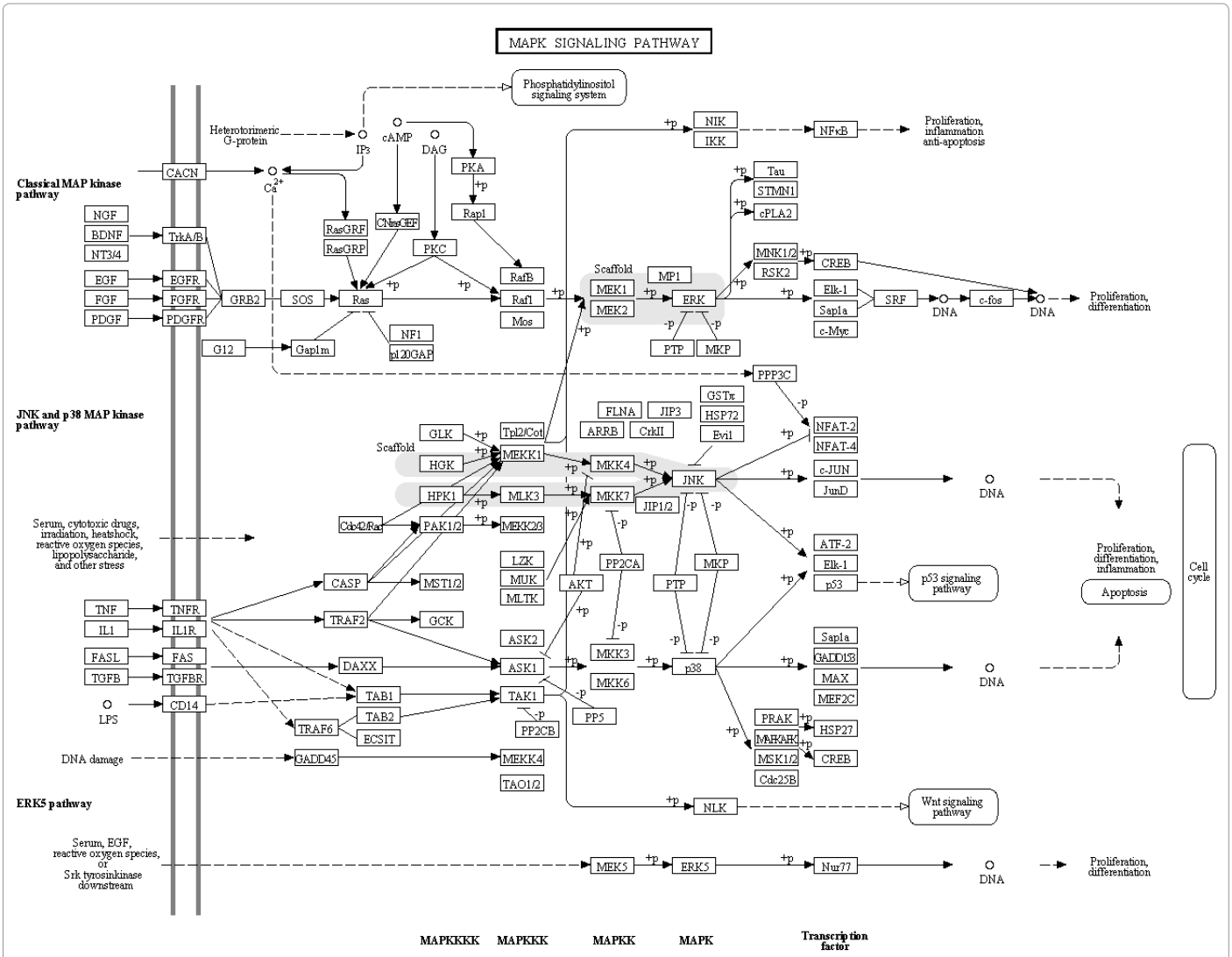


Figure 2: MAP Kinase pathway from KEGG.

FOX reagent (Dissolve 30mM H_2SO_4, 100 µM Xylenol orange, and 100 µM Ammonium ferrous sulphate and made upto 5 mL using distilled water), Tris-HCL Linoliec acid (80 mM): (80 mM solution was prepared from stock of 100 mg Linoleic acid in 990.6 µL ethanol (360 mM)), 5-LOX enzyme (Sigma), Tris-HCL Buffer (7.8 grams of Tris-HCL was added in 1 litre distilled water and adjust pH to7.4) has to be prepared.

Assay mixture

About 250 µL of assay mixture prepared that contains 20 µL test sample of different concentrations dissolved in 175 µL of 50 mM Tris-HCL buffer, 5 µL enzyme, 5 µL Lenoleic acid and 65 µL FOX reagent was prepared.

Experimental method

Approximately 5 µL of 5-LOX enzyme was added with 175 µL of 50 mM Tris HCL buffer (Pre-incubated with 20 µL test sample for 5 min at 25°C) is to be prepared. The reaction is then initiated by addition of 5 µL linoleic acid (final concentration 140 µM) in 50 mM Tris HCL buffer followed by incubation at 25°C in dark for 20 min. The above total volume contained 185 µL of reaction mixture is terminated by the addition of 65 µL freshly prepared fox reagent. After the termination the absorbance was taken at 595 nm upto 30 minutes at 25°C with Xmark Micro plate spectrophotometer (BIO-RAD).

Calculation

$$\%\text{inhibition of 5-LOX} = \frac{((\text{Absorbance of control} - \text{Absorbance in background}) - (\text{Absorbance of sample} - \text{Absorbance of background}))}{(\text{Absorbance of control} - \text{Absorbance of background})} \times 100$$

An IC_{50} value will be determined as the concentration that elicits the half maximal response.

In vitro anti-proliferative activity

Medicinal plants acts as useful source in research for finding new biologically active compounds, especially for ethno-medical data approach. The ethno-medical data is not always completely reliable as it is difficult to diagnosed and treat cancer.

Caucasian lung carcinoma of A549 cell line used in this study is procured from National Centre for Cell Science (NCCS), Pune. The cells were grown in Minimal essential medium (MEM, GIBCO) that is supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal

bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

XTT assay

The biochemical procedure is based on the activity of mitochondrial enzymes which are inactivated shortly after the cell death. The method is found to be very efficient in considering viability of the cells. Colorimetric based method based on the tetrazolium salt, XTT. The trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 24 hrs incubation, the supernatant was discarded and the cells were pretreated with growth medium. The media were subsequently mixed with different concentrations of test compounds (12.5,25,50,100 and 200 µg/ml) to achieve a final volume of 100 µl were cultured for 48 hrs. The compound is prepared as 1.0 mg/ml concentration stock solution prepared with in DMSO solution. Culture medium and solvent are used as controls. Each well then received 50 µl of fresh XTT (0.9 mg/ml in RPMI along with XTT activator reagent) followed by incubation for 2 hrs at 37°C. At the end of the incubation, shake the 96 micro well plates for 15 seconds on a shaker. The Optical Density (OD) of the culture plate was read at a wavelength of 490 nm (reference absorbance at a wavelength of 630 nm) on Anthos 2020 spectrophotometer.

Protein interaction network mapping

Apaf-1 is a vital factor responsible for activation of cytochrome **c**-driven caspase during the mitochondrial apoptosis. It is an important tumor suppressor like lung carcinoma of A549 cell line. Protein interaction network mapping was constructed and analysed for mechanism using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) 9.05 server used to know and predict protein interactions. In the present work, "Apaf-1" was selected as query protein to understand the interaction network between cancer and inflammatory causing proteins.

Results and Discussion

The plant products like glycosides in *A. indicum* may play an important role in the decreased risk of chronic diseases associated with a diet and a wide range of disease [14]. The studies on cytotoxic effects of some anti-cancer drugs suggest that phytocompounds has cancer chemo-therapeutic and chemo-preventive potential without side effects [15,16].

5-LOX and cyclooxygenase (COX) inhibition may have anti-carcinogenic effects. COX and LOX inhibitors that are used for the treatment of inflammatory diseases act as potential anticancer drugs [17-19]. The ethanolic leaf extract of *A. indicum* has shown good anti-inflammatory Activity (IC_{50}: 8.89 µg/mL) based on 5-LOX assay. The IC_{50} values of test sample and the standard are 8.89 µg/mL and 8.14 µg/mL respectively (Figure 3 and Table 1). *A. indicum* extract has not shown much variation with standard Curcumin. The isolated samples may further show good phytochemical components for anti-inflammation and anti-cancer activity. These results have provided that the plant extract has shown good anti-inflammatory activity and may provide the involvement in control of disease like cancer.

Table 2 and Figure 4 shows good results for lung cancer cell lines (A549) treated with the ethanolic leaf extract of *A.indicum*. The percentage of cell inhibition is gradually increased to 72.1% at 200 µg/ml. Standard compound, Cisplatin showed percentage cell inhibition as 91.1% at 200 µg/ml.

Table 3 shows the IC_{50} values for *A.indicum* and cisplantin. Leaf extract of *A.indicum* in the present experimentation was shown good result as anti-proliferative source (IC_{50}: 85.2 µg/mL) but observed less effective compared to standard (IC_{50}: 24.9). Hence ethanolic leaf extract

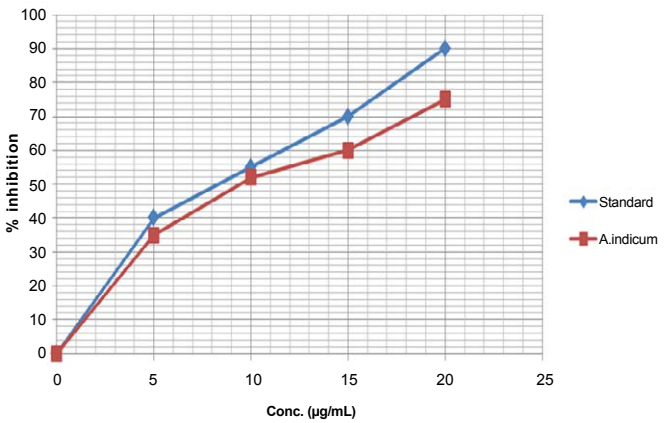


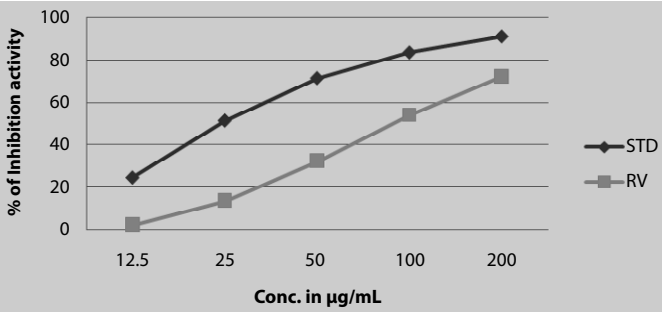
Figure 3: Anti-inflammatory activity of Curcumin and *A.indicum* ethanolic leaf extract.

Test sample	IC_{50}(µg/mL)
Abutilon indicum	8.89
Curcumin	8.14

Table 1: IC_{50} values of ethanolic crude extract of *A. indicum* for anti-inflammatory activity.

Conc (ug/ml)	Cisplatin			ethanolic leaf extract of *A.indicum* A.indicum		
	OD at 490 nm	% Cell Survival	% Cell Inhibition	OD at 490 nm	% Cell Survival	% Cell Inhibition
12.5	1.043	75.5	24.5	1.303	97.98	2.02
25	0.685	48.6	51.4	1.156	86.6	13.4
50	0.420	28.6	71.4	0.914	67.8	32.2
100	0.259	16.5	83.5	0.634	46.1	53.9
200	0.158	8.9	91.1	0.400	27.9	72.1

Table 2: Antiproliferative activity of ethanolic leaf extract of *A. indicum* on lung cancer cell line A549.



Note: STD: standard (Cisplatin); RV: ethanolic leaf extract of *A.indicum*

Figure 4: Anti-proliferative activity of ethanolic leaf extract of *A. indicum* on A549 cell line.

Test sample	IC50(µg/mL)
Abutilon indicum	85.2
Cisplatin	24.9

Table 3: IC_{50} values of ethanolic crude extract of *A. indicum* for anti-proliferative activity.

of *A.indicum* showed good anti-proliferative activity, less compared to standard at IC_{50}.

Figure 5 shows antiproliferative response of ethanol leaf extract of *A.indicum* against Lung cancer cells (A549) demonstrated in before and after treatment of extract. There is more number of lung cancer cells before the treatment with plant compound. After adding 200 µg/mL of ethanolic leaf extract of *A.indicum* has shown shrinkage/lysis in lung cancer cells.

Cell growth inhibition (LD_{50}) on HeLa cells for an ethanolic extract of saffron (*Crocus sativus L.*) dry stigmas was shown as 2.3 mg/ml [20]. Ethanol extracts of S. barbata was inhibited A549 cell growth (IC_{50}) of 0.21 mg/ml [21]. An ethanol intake was not been linked with lung cancer [22] and was not having effect on NNK metabolism [23] in mouse lung microsomes. Hence the intake of ethanol may cause lung cancer but the ethanolic extracts contains phytocompounds that are non-toxic and shown antiproliferative activity on A549 (Lung cancer cell line).

Molecular interaction studies provide better understanding component stacks in lung cancer towards drug discovery and designing. Silencing of miR-155 and the over expression Apaf-1 greatly increased the sensitivity of A549 cells involved in lung cancer to cisplatin [24]. Figure 6 was provided the mechanism of Apaf1 interaction with other proteins in link to lung cancer using string database. Apaf-1 has shown interactions with the proteins like CASP9, CASP3, CYCS, BCL2L1, TP53, BCL2, CASP8, HSPA4, DIABLO and CASP7. These components has shown network with components relevant to MAPK signaling pathway that links to inflammation and lung cancer.

In the present decades, plant kingdom is considered as gold mines due to existence of many biologically active principles that show therapeutic value [25]. Cancer is a major public health problem in all around the world that does not contain complete cure [26]. The interfering lung epithelial cell death using phytocompounds is an important protective and therapeutic strategy [27]. The ethanolic leaf extract of *A.indicum* contains components that show antiproliferative response for lung cancer cell line (A549) through Apaf-1 gene by activation of cytochrome **c**-driven caspase like CYCS etc.

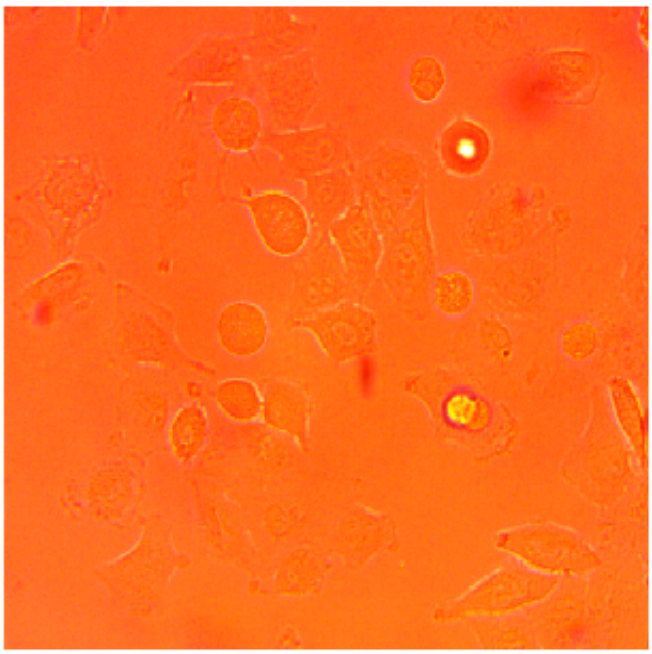
A. indicum leaves extract enhances the antioxidant potential due to free radical scavenging activity [28,29]. *A. indica* leaf extract was found to possess better antioxidant activity may be due to the phenolics and flavonoids present in the extract. The *F. hispida* and *A Scholaris* leaf extracts in ethanol exhibited more cytotoxicity when compared to other extracts in HeLa and MCF-7 cell lines [30]. The present experimentation has shown good anti-oxidant and antiproliferative activity of ethanol extract of *A.indicum* and shown interactive mechanism with MAP Kinase pathway. Protein network analysis provided molecular understanding showing network topologies in the biological systems that regulates anti-apoptotic molecules and cell cycle progression [31].

Conclusion

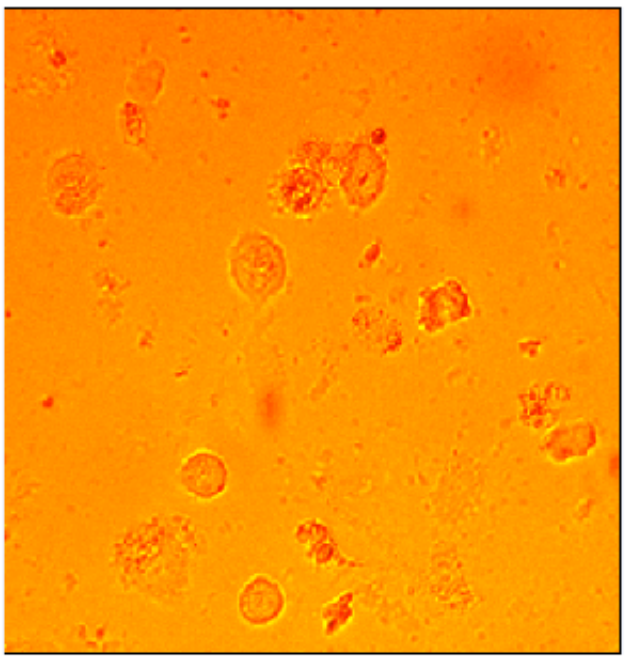
The ethanolic leaf extract of *A.indicum* has shown medicinal importance with anti-inflammatory and anti-proliferative properties. The study has shown interactive mechanism with MAP Kinase pathway. There may be future scope to discover the novel anti-inflammatory and anti-cancer drugs from ethanolic leaf extract of *A. indicum.*

Acknowledgment

Authors would like to thank management and staff of GITAM University, India for their kind support in bringing out the above literature and providing lab facilities.



Before treatment with ethanolic leaf extract of A.indicum



After treatment with ethanolic leaf extract of A.indicum

Figure 5: Dose response of ethanolic leaf extract of *A.indicum* on A549 (Lung cancer cell line).

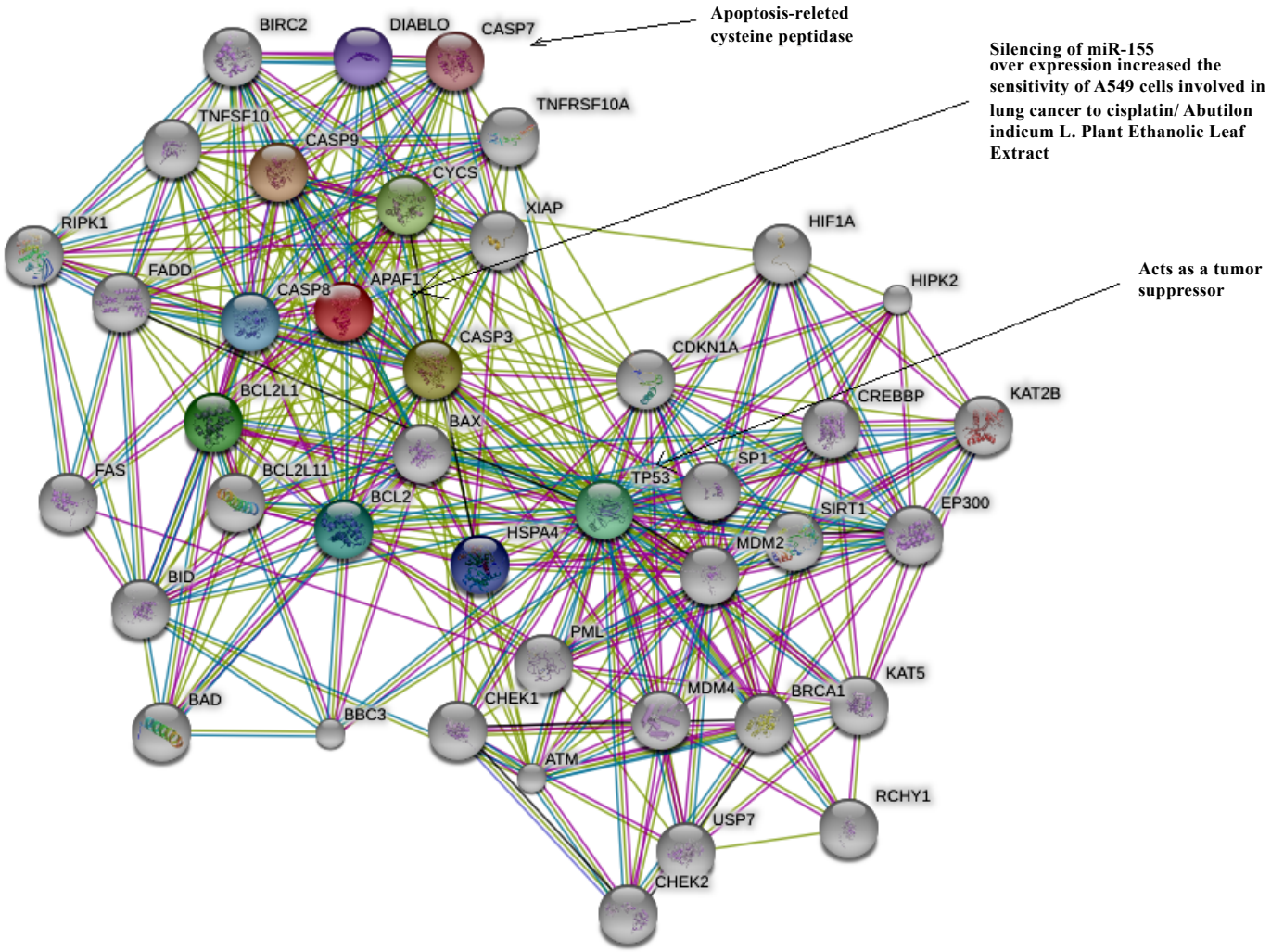


Figure 6: Protein interaction of Apaf-1 involved in A549 cell system.

References

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z (1985) Medicinal plants in therapy. Bull World Health Organ 63: 965-981.
2. Grover JK, Yadav S, Vats V (2002) Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 81: 81-100.
3. Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology 4: 685-688.
4. Ahmad I, Mehmood Z, Mohammad F (1998) Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 62: 183-193.
5. Buriani A, Garcia-Bermejo ML, Bosisio E, Xu Q, Li H, et al. (2012) Omic techniques in systems biology approaches to traditional Chinese medicine research: present and future. J Ethnopharmacol 140: 535-544.
6. Kaladhar DSVGK (2012) Traditional and Ayurvedic medicinal plants from India: Practices and Treatment for human diseases. LAP LAMBERT Academic Publishing: 176.
7. Pradhan BK, Badola HK (2008) Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering Khangchendzonga Biosphere Reserve, in North Sikkim, India. J Ethnobiol Ethnomed 4: 22.
8. Dashputre NL, Naikwade NS (2011) Evaluation of anti-ulcer activity of methanolic extract of Abutilon indicum Linn leaves in experimental rats. International journal of pharmaceutical sciences and Drug research 3: 97-100.
9. Kuo PC, Yang ML, Wu PL, Shih HN, Thang TD, et al. (2008) Chemical constituents from Abutilon indicum. J Asian Nat Prod Res 10: 699-703.
10. Seetharam YN, Chalageri G, Setty SR, Bheemachar (2002) Hypoglycemic activity of Abutilon indicum leaf extracts in rats. Fitoterapia 73: 156-159.
11. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, et al. (2002) Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 21: 7435-7451.
12. Pleasance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, et al. (2010) A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 463: 184-190.
13. Kaladhar DSVGK, Chandra SP (2012) MAPK Signaling in Human Diseases: in silico analysis. LAP LAMBERT Academic Publishing, Germany.
14. MatÅ‚awska I, Sikorska M (2002) Flavonoid compounds in the flowers of Abutilon indicum (L.) Sweet (Malvaceae). Acta Pol Pharm 59: 227-229.
15. Rajalakshmi PV, Senthil KK (2009) Direct HPLC analysis of quercetin in exudates of Abutilon indicum (Linn). Malvaceae. Journal of Pharmaceutical Science and Technology 1: 80-83.
16. Vadlapudi V (2010) In vitro antimicrobial activity of methanolic extract of selected Indian medicinal plants. Pharmacophore 1: 214-219.
17. Goossens L, Pommery N, Hénichart JP (2007) COX-2/5-LOX dual acting anti-inflammatory drugs in cancer chemotherapy. Curr Top Med Chem 7: 283-296.

18. Cianchi F, Cortesini C, Magnelli L, Fanti E, Papucci L, et al. (2006) Inhibition of 5-lipoxygenase by MK886 augments the antitumor activity of celecoxib in human colon cancer cells. Mol Cancer Ther 5: 2716-2726.

19. Pidgeon GP, Lysaght J, Krishnamoorthy S, Reynolds JV, O'Byrne K, et al. (2007) Lipoxygenase metabolism: roles in tumor progression and survival. Cancer Metastasis Rev 26: 503-524.

20. Escribano J, Alonso GL, Coca-Prados M, Fernandez JA (1996) Crocin, safranal and picrocrocin from saffron (Crocus sativus L.) inhibit the growth of human cancer cells in vitro. Cancer Lett 100: 23-30.

21. Yin X, Zhou J, Jie C, Xing D, Zhang Y (2004) Anticancer activity and mechanism of Scutellaria barbata extract on human lung cancer cell line A549. Life Sci 75: 2233-2244.

22. Le Marchand L, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN (2000) Intake of flavonoids and lung cancer. J Natl Cancer Inst 92: 154-160.

23. Smith TJ, Guo ZY, Thomas PE, Chung FL, Morse MA, et al. (1990) Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mouse lung microsomes and its inhibition by isothiocyanates. Cancer Res 50: 6817-6822.

24. Zang YS, Zhong YF, Fang Z, Li B, An J (2012) MiR-155 inhibits the sensitivity of lung cancer cells to cisplatin via negative regulation of Apaf-1 expression. Cancer Gene Ther 19: 773-778.

25. Kundu JK, Surh YJ (2008) Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Lett 269: 243-261.

26. Govinda RD, Sastry YN, Kaladhar DSVGK, Ajay BP, Kamalakara RK, et al. (2011) Preliminary studies on in vitro anti-tumor activity of tender seed coat extract of Borassus flabellifer L. ON HELA CELL LINE. International Journal of Current Research 3: 075-077.

27. Zhang X, Shan P, Sasidhar M, Chupp GL, Flavell RA, et al. (2003) Reactive oxygen species and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. American journal of respiratory cell and molecular biology 28: 305-315.

28. Dharmendra S, Radhey SG (2008) Modulatory Influence of Abutilon Indicum Leaves on Hepatic Antioxidant Status and Lipid Peroxidation against Alcohol-Induced Liver Damage in Rats. Pharmacologyonline 1: 253-262.

29. Harini R, Sindhu S, Sagadevan E, Arumugam P (2012) Characterization of in vitro antioxidant potential of Azadirachta indica and Abutilon indicum by different assay methods. Journal of Pharmacy Research 5: 3227-3231.

30. Sunil D, Anil KNV, Anand SN, Sachin RU, Deepa P, et al. (2013) Cytotoxicity studies of microwave assisted natural product extracts in HeLa and MCF-7 cell lines. Int J Med Arom Plants 3: 27-31.

31. Kaladhar DS, Sai PC, Rao PV, Chaitanya AK, Rao DG, et al. (2012) Towards an understating of signal transduction protein interaction networks. Bioinformation 8: 437-439.