RESEARCH ARTICLE

Ethanolic Stem Extract of *Excoecaria Agallocha* Induces G1 Arrest or Apoptosis in Human Lung Cancer Cells Depending on Their P53 Status

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ABSTRACT: *Excoecaria agallocha* L. is a mangrove widely used as folklore medicine but its anticancer properties have not been evaluated yet. In this study, the ethanol stem extract of this plant exhibited remarkable cytotoxic activities on human lung cancer cell lines in a dose-dependent manner after 48 h of incubation. Analysis of the mechanism of action demonstrated that the extract exerted apoptotic cell death in $p53^{+/+}$ cells and G1 arrest in $p53^{-/-}$ cells. Morphological observation using phase-contrast microscope also displayed apoptotic characteristics in treated $p53^{+/+}$ cells such as nuclear blebbing and chromatin condensation. Cell cycle analysis showed G1 arrest caused by the extract in $p53^{-/-}$cells. Furthermore, acute exposure to the extract produced a significant regulation of p21, Bcl-2 and Bax protein expression in both these cell lines. Due to its potent cytotoxic effect on lung cancer cell lines, it is strongly suggested that the extract could be further developed as an anticancer drug.

KEY WORDS: Anticancer activity, apoptosis, *E. agallocha*, lung cancer, mangrove, p53.

INTRODUCTION

Lung cancer remains a major global health problem and accounting for more than a million annual deaths worldwide (Leong et al., 2009). It is the leading cause of cancer mortality in most of the countries in the world (Magarth and Litak, 1993). Clinically, lung cancer is classified into two groups, small cell (SCLC) and non-small cell lung cancer (NSCLC). The latter is more prevalent, accounting for almost 80% of lung cancers. Non-small cell lung cancer is composed of several subtypes, including lung adenocarcinoma, which is the most common lung cancer in the western world. The incidence of lung cancer can be correlated with the age of both males and females and there is still lack of effective drugs to treat this disease. Chemotherapy is the standard method of treatment. Although only modest increase in survival rate can be achieved, it significantly improves symptoms and the quality of life of patients with lung cancer.

Plant derived compounds have played an important role in the development of several clinically useful anticancer agents. These include vinblastine, vincristine, the campothecin derivatives, topotecan and irinotecan, etoposide and paclitaxel (Cragg and Newman, 2005; Shoeb, 2006; Cochrane et al., 2008). Paclitaxel and docataxel derived from *Taxus* species, Vinorelbine, Carboplatin, Gemcitabine are primarily used in the treatment of NSCLC (Schwartsmann et al., 2002).The anticancer properties of these are related to the regulation of cancer-related gene expression, induction of apoptosis, cell cycle arrest and/or DNA fragmentation and inhibition of different cellular enzymes (Upur et al., 2008). Hence, screening and isolation of active components from the herbs possessing anticancer potential appears to be a promising way of discovering novel therapeutic compounds.

Mangroves are salt-tolerant evergreen forests found along sheltered coastlines, shallow-water lagoons, estuaries, rivers or deltas in 124 tropical and sub-tropical countries of the world. Out of the total 15.2 million hectares global mangrove habitat, India is known to contribute about 448000 hectares (FAO, 2007). Mangroves have a variety of economical and ecological uses. Many species of them have also been used traditionally in folklore medicine to treat various diseases since centuries.

Excoecaria agallocha L., belonging to the family Euphorbiaceae, is widely distributed on seashores and edge-mangroves throughout tropical Africa, Asia, and northwest Australia. The plant is known to play an important economical, ecological as well as medicinal role. A series of diterpenoid, tritepenoids derivatives and alkaloids were isolated from *E. agallocha* by several workers (Prakash et al., 1993; Konishi et al., 1998; Anjaneyulu and Rao, 2000 and 2003; Konishi et al.,

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2003; Wang et al., 2005; Wang et al., 2006; Zou et al., 2006) and some diterpenes extracted from the plant were also found to possess anti-tumour promoting activity (Konoshima et al., 2001). It is commonly used in folklore medicine to treat swollen hands and feet in leprosy, flatulence, epilepsy, and as an aphrodisiac agent. The milky latex exuded from the bark of *E. agallocha* has been used as a poison for fish by adding it to water and to poison arrowheads.

Modern clinical trials show that the plant may have anti-HIV, anti-cancer, anti-bacterial and anti-viral properties (Peter and Sivasothi, 1999; Subhan et al., 2008). But the cytotoxic potential of *E. agallocha* has not been evaluated yet. Hence the present investigation was undertaken to elucidate the anticancer effects of crude ethanol stem extract of *E. agallocha* and the initial molecular mechanisms responsible for it on cultured human lung cancer cell lines. We assessed the efficacy of stem ethanol extract of *E. agallocha* on cell growth, cell cycle progression and apoptotic cell death in human lung carcinoma cell lines. The results obtained clearly demonstrate that *E. agallocha* inhibits human lung cancer cell growth, causes a marked increase in p21 and G1 arrest in $p53^{-/-}$ cells. p53 overcomes this arrest and induces apoptosis in $p53^{+/+}$ cells. Hence, *E. agallocha* induces cell cycle arrest or apoptosis in lung cancer cells depending upon their p53 status.

MATERIALS AND METHODS

Preparation of Plant extract for cytotoxic activity assay

Stem of *E. agallocha* was collected near Ratnagiri coast (Latitude 17° 08' 44" N longitude 73° 19' 67" E) from the state of Maharashtra, India. The plant material was identified and authenticated by Dr. B. L. Jadhav, an expert taxonomist. The collected plant material was brought to the laboratory, washed thoroughly under the running tap water in order to remove dirt, germs and other contaminants from the sample. Stems of *E. agallocha* were then oven dried at a constant temperature of 40°C to remove the moisture content, ground thoroughly with help of a mortar and pestle. The resultant powder was carefully sieved through the muslin cloth. This stem powder (10 g) was then used to prepare hot extract of *E. agallocha* using soxhlet extraction method using solvent ethanol (200 ml). The extract was then evaporated on a rotary evaporator to completely remove the solvent and reduced to a volume of 20 ml (50%w/v). The resultant dark reddish green gummy mass was then stored in air tight bottles and kept at 4°C till further use.

Chemicals and reagents

RPMI-1640, Penicillin-Streptomycin solution, Flavopiridol, dimethyl sulfoxide (DMSO), trypsin, EDTA, propidium iodide, Cell lytic reagent, protease inhibitor cocktail, Bradford reagent, Enhanced chemiluminescence detection kit for Western blotting, anti-β-actin monoclonal antibody were purchased from Sigma. All the other primary antibodies, secondary antibodies and non-fat dry milk powder were purchased from Santa Cruz Biotechnology (USA). Immobilon-P (PVDF) membrane was procured from Millipore, USA. MTS reagent was purchased from Promega Co. (WI, USA). Fetal bovine serum (FBS) was from Hyclone (Logan, UT).

Cell lines and culture maintenance conditions

The human lung carcinoma cell lines A549, H460, H1299, H358 (NSCLC), normal cell line WI-38 (normal lung fibroblast) were purchased from American Type Culture Collection (MD, USA). All the cell lines were routinely grown in RPMI- 1640 culture medium supplemented with 10% heat inactivated FBS, 2% L-glutamine (200 mM) and 1% penicillin-streptomycin as a monolayer culture at 37°C in a humidified 5% CO_2 incubator.

***In vitro* cytotoxicity assay (MTS assay)**

Cells were used for cytotoxicity studies when 80% confluence was reached in T-75 flasks. Cells were harvested with 0.025% trypsin/EDTA, washed with PBS and counted using trypan blue dye exclusion method. Lung carcinoma cells and WI-38 cells were seeded into 96-well flat bottom microtiter plates at a density of 3000 cells/well, and 10,000 cells/well respectively and left to attach to the plates for 24 h. Next day, 20 µl of culture medium or medium containing the test compound was added to the wells. Each concentration was plated in triplicate. The ethanol extract of stem of *E. agallocha* was dissolved in DMSO (dimethylsulfoxide) and was applied in five concentrations (100, 30, 10, 3, 1 µg/ml) and flavopiridol (negative control) was applied in four concentrations (0.03, 0.1, 0.3 and 1 µM). Following 48 hours of drug exposure, the effect on the cell viability was measured using MTS cell proliferation assay (Cory et al., 1991), which measures the reduction of MTS tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] to formazan, in the presence of phenazine methosulfate (PMS) in the metabolically active cells. 20 µl of CellTiter$^{96®}$ AQueous non- radioactive MTS reagent was added to each well. Cells were incubated further to allow for color development before the absorbance values were read at 490 nm using a microplate reader. (Spectramax-Molecular devices, USA).

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A blank control was included by mixing media and MTS reagent in the absence of cells. The yellow colored MTS is converted to the reddish brown colored formazan product by the mitochondrial enzyme succinate-dehydrogenase. This conversion takes place only in the living cells. The amount of colored product formed was directly proportional to the number of cells and the time of incubation of the cells with MTS/PMS. The lower the absorbance values, the fewer cells survive and the lower viabilities of tested cells. The highest DMSO concentration was 0.5%. At this concentration, DMSO did not alter cell viability (data not shown). Growth stimulation/inhibition was calculated as treated/control X 100 (%T/C). IC_{50} values were determined by plotting concentration of the compound versus viability.

Cell cycle analysis

The human lung cancer cell lines A549 and H1299 were seeded in T-25 tissue culture flask at a density of 0.5×10^6 cells per flask. After 24 h, cells were treated with about thrice IC_{50} of the extract for cancer cells (i.e. for 24 hours). Both detached and adherent cells were then harvested. After washing in PBS, cells were fixed in ice-cold 70% ethanol and stored at 4°C overnight. Cells were washed twice with PBS to remove fixative and resuspended in PBS containing 50 µg/ml PI and 50 µg/ml RNase A. After incubation in dark at room temperature, fluorescence emitted from the propidum iodide-DNA complex was quantitated after the excitation of the fluorescent dye FACScan cytometry (Becton Dickinson, San Jose, USA).

Western blotting Assay

A549 and H1299 cells were treated with extract at 10 and 20 µg/ml for 24 h. Cells were lysed with lysis buffer (Cell lytic reagent plus protease inhibitor cocktail) and the cell lysates were centrifuged at 14,000 rpm at 4°C to collect the supernatant. The protein concentration was determined using Bradford reagent. The cell lysates (30 µg) were separated on SDS-PAGE and transferred to PVDF membrane (Millipore, MA, USA). After blocking for 1 h at room temperature with a solution composed of 20 mM Tris-HCl, (pH 7.4), 125 mM NaCl, 0.1% Tween-20, 5% non fat dry milk, immunodetection was carried out by probing with appropriate dilutions of specific antibodies overnight at 4°C. Anti-PARP, anti-p53, anti-Bcl-2 and anti-Bax monoclonal antibodies (Santa Cruz Biotechnology) were used at a dilution of 1: 1000 (Chen et al., 2004; Lien et al., 2009). Anti- β-actin monoclonal antibody was used at a dilution of 1: 20,000. The secondary antibodies, HRP coupled anti-mouse and anti-rabbit antibody (Santa Cruz, USA) were incubated at room temperature for 1 h at dilutions of 1: 10,000 and 1: 5000 respectively. These proteins were detected by chemiluminescence (ECL, Sigma).

Statistical analysis

Significance of difference between control and *E. agallocha* treated samples was calculated by using GraphPad Prism (versions 3.0 and 210 5.0, GraphPad Software, USA) software in which one-way analysis of variance and Tukey's multiple comparison post tests were used to determine significant differences between several treatment groups. Statistical significance was evaluated by calculating *P*-values. *P*-values < 0.05 were considered statistically significant differences. All the data shown in the study are representative of two or three experiments.

RESULT

Cytotoxic effect of the ethanol extract of *E. agallocha* on lung cancer cell lines

In order to characterize cytotoxic effects, we performed MTS assay. Cytotoxicity was observed in cultures exposed to 1-100 µg/ml of crude ethanol stem extract of *E. agallocha* for 48 h. The extract significantly inhibited viability of all four lung carcinoma cell lines tested in a dose-dependent manner. Among the four lung cancer cell lines used, *E. agallocha* exhibited the strongest potency of cytotoxicity in A549 at a dose of 100 µg/ml. At the highest concentration (100 µg/ml), mean reduction of 81% cell viability was observed in A549 compared with a mean reduction of 58% cell viability in H1299 (Fig. 1). Hence, inhibition of the cancer cell lines H358 and H1299 only occurred at higher concentrations of the extract (>50 µg/ml). These results suggest that the $p53^{+/+}$ cancer cells (A549 and H460) were more sensitive to the treatment than $p53^{-/-}$ cancer cells (H358 and H1299). To ascertain whether the extract has any selectivity for normal versus cancer cells, human normal lung fibroblasts WI-38 were treated with the extract. The extract did not show significant growth inhibition of normal lung fibroblast WI-38 upto 100 µg/ml concentration. Hence, the IC_{50} value for normal fibroblast cell line was significantly higher than those obtained for human lung cancer cell lines. Flavopiridol demonstrated IC_{50} values in the range of 0.12- 0.16 µM (i.e. 0.05-0.06 µg/ml) on all the cell lines. The IC_{50} values obtained for each cell line are shown in Table 1.

***E. agallocha* stem extract as a growth suppressor and inducer of apoptosis: microscopic observation**

Lung cancer cell line A549 seeded (0.5×10^6) in T-25 flasks with or without various concentrations (IC_{50}, $2 \times IC_{50}$ and $3 \times IC_{50}$) of ethanolic stem extract of *E. agallocha* were photographed under a phase-contrast

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Table 1. Mean IC_{50} values of crude extract in human lung cancer cell lines. Significant dose effects were observed in four lung carcinoma cell lines as compared to the normal cell line ($P < 0.001$). $p53^{+/+}$ cells (A549 and H460) showed significantly lower IC_{50} values as compared to $p53^{-/-}$ cancer cells (H358 and H1299) ($P < 0.001$) with a lower cell viability observed at the highest treatment concentrations.

Cell Line	Mean IC_{50} (µg/ml)	
	E. agallocha stem ethanol extract	Flavopiridol
A549	8	0.05
H460	11	0.05
H358	57	0.06
H1299	62	0.06
WI-38	100	0.06

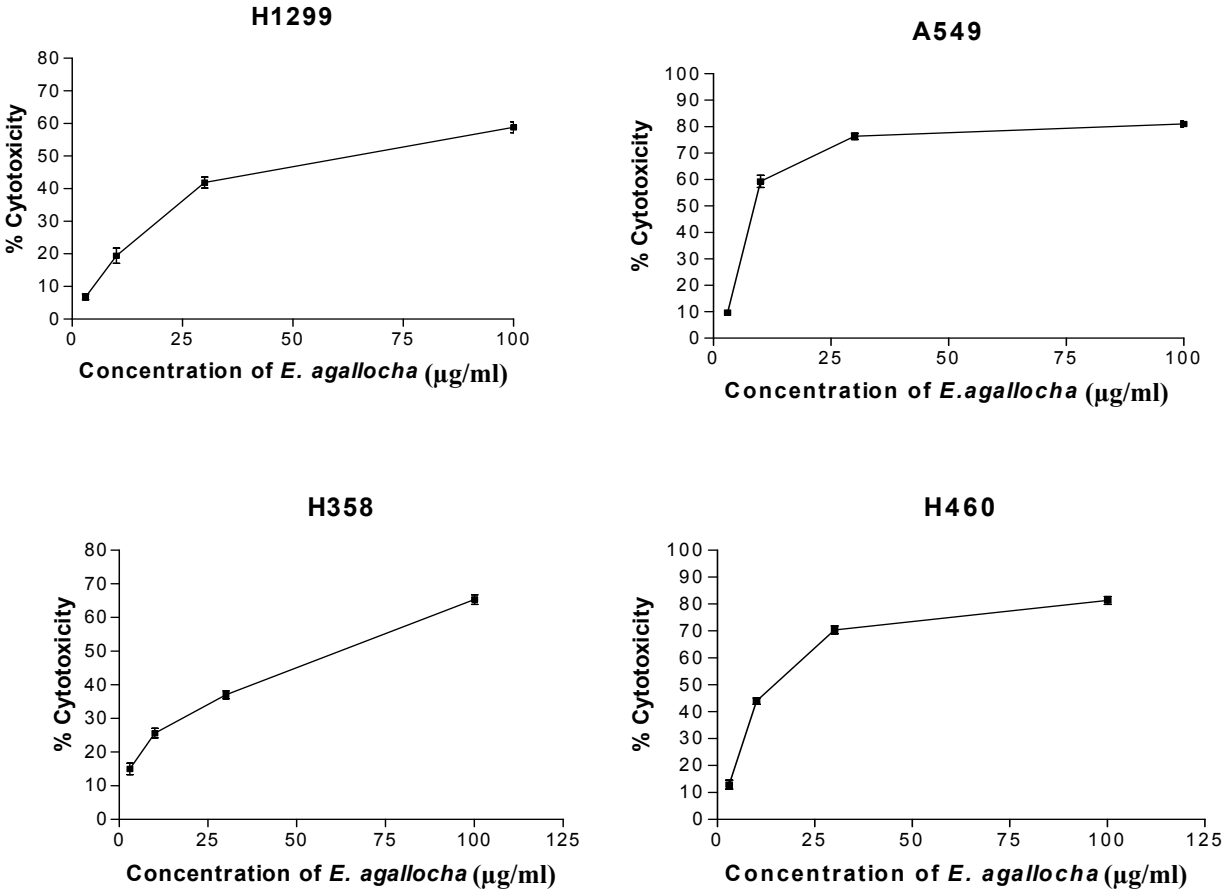


Fig. 1. Growth inhibitory effect of *E. agallocha* extract on lung cancer cells. Cells were treated with different concentrations of the extract for 2 days when the cell viability was determined by the MTS assay. The growth inhibition was calculated as percentage of inhibition compared with the control.

microscope after 24 h treatment (Fig. 2). Significant decrease of the number of cells treated with all the three concentrations of the extract for 24 h was observed compared with the control group. The control group cells showed a typical monolayer appearance. The treated cells began to have morphological changes; showing round-shaped cells poorly adhered to the culture flasks. The nucleus as well as the cell membrane appeared to shrink and the chromatin appeared brighter due to condensation. Nuclear blebbing was also apparent in cells treated with 2 × IC_{50} and 3 × IC_{50} concentrations.

Effect of *E. agallocha* on cell viability

We characterized the cytotoxic effects of *E. agallocha* on A549 and H1299 cells by conducting cell viability assay. A549 cells were treated with *E. agallocha* extract at various concentrations for 48 h. The results indicated that *E. agallocha* extract had obvious cytotoxicity on A549 and H1299 cells. After 48 h treatment with *E. agallocha* extract at concentrations

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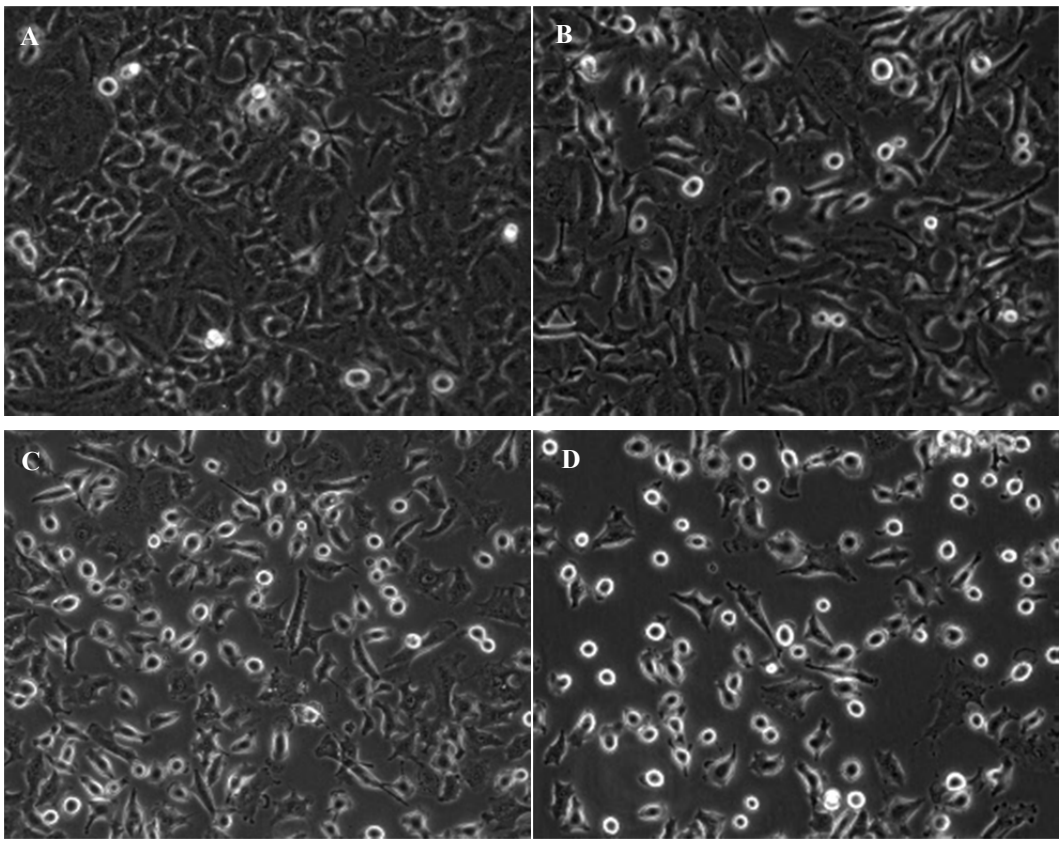


Fig. 2. Phase contrast micrographs of *E. agallocha* induced changes in cellular morphology. A549 cells were cultured for 24 h in T-25 flasks in FCS-containing medium, and then *E. agallocha* extract was added to the medium. After 24 h of treatment cells were still attached to the plates, whereas cell shape changed from flat to round in a dose-dependent manner. A. Untreated, B-D. Increasing concentrations of the extract were added to the cultures for 24 h i.e. IC_{50}, 2 × IC_{50} and 3 × IC_{50} respectively. Magnification, × 100.

of IC_{50}, 2 × IC_{50} and 3 × IC_{50} both the cell lines showed highly significant (** $P < 0.01$ and *** $P < 0.001$) dose-dependent decrease in cell viability which was determined by trypan blue dye exclusion method as shown in Fig. 3.

Effect of *E. agallocha* on cell cycle progression

To determine the effect of *E. agllocha* extract on the cell cycle progression, human lung carcinoma cell lines A549 ($p53^{+/+}$) and H1299 ($p53^{-/-}$) treated with *E. agallocha* extracts at a concentration of 2 × IC_{50} for 24 h were analyzed by flow cytometry. Representative DNA histograms of the various treatments are shown in Fig. 4. After treatment with the extract for 24 h, A549 cells showed increased DNA contents of the sub-G_1 phase as compared with the control. For H1299 cells, the fraction of cells with sub-G1 DNA content increased from 2% in control to 3% in the 24 h *E. agallocha* 2 × IC_{50} treated sample. The G_1 or G_0 fraction changed from 54% to 58% in H1299 cells indicating G1 arrest.

Effect of *E. agallocha* extract on apoptosis-related protein expression level of A549 and H1299 cells

Western blotting was used in order to determine the expression levels of cell cycle and apoptosis related proteins in *E. agallocha* treated cells. We evaluated the expressions of Bcl-2, Bax, p53, p21 and PARP by western blot analysis in A549 and H1299 cells. Unlike the majority of NSCLC cell lines, A549 carry a wild-type p53 gene. As shown in Fig. 5, significant up regulation of p21 could be seen after 24 h of treatment in both A549 and H1299 suggesting p53-independent up-regulation of p21. Interestingly, expression of p53 was not significantly up regulated in A549 cells undergoing apoptosis. Dose-dependent significant decrease in the Bcl-2 expression following treatment was observed in A549 cells, while in H1299, Bcl-2 levels remained unaltered at the same time point. The pro-apoptotic protein Bax levels were not altered and remained at the basal level in both the cell lines. The apoptotic response in A549 was confirmed by demonstration of PARP cleavage. H1299 cells did not exhibit PARP cleavage. The membranes were reprobed with anti-β-actin antibody to ensure equal loading.

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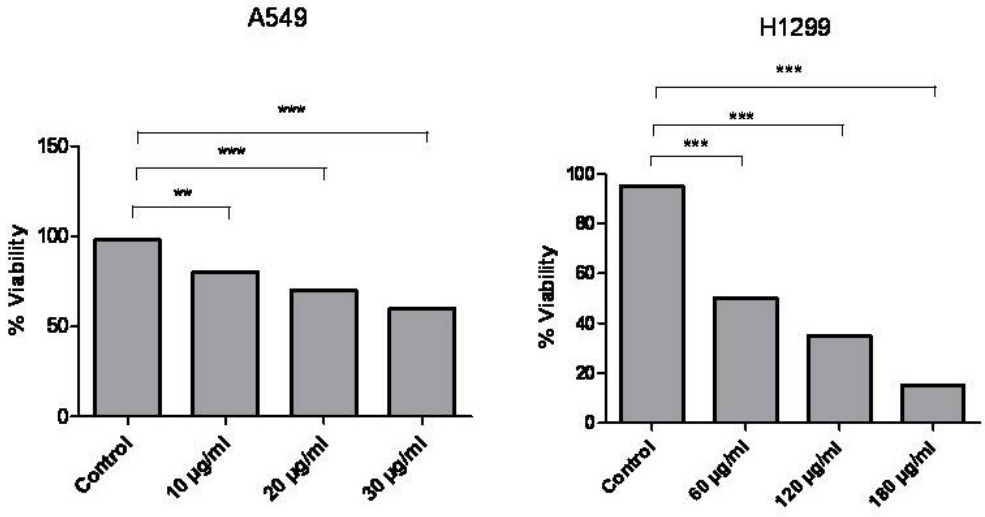


Fig. 3. Cytotoxic effects of *E. agallocha* extract on A549 and H1299 cells. Cells were seeded in 6-well tissue culture plates and treated with *E. agllocha* extract for 48 h. Cells were harvested by trypsinization, stained with trypan blue and the viable and dead cells were counted. Viable cells (%) = [(total cells- dead cells)/ total cells] X 100%. ** *P* <0.01, * *P* < 0.001.**

Discussion

A number of phytochemicals have been demonstrated to possess anticancer properties and hence have become one important source of newer chemotherapeutic agents. The use of complementary and alternative medicine such as herbal extracts is becoming increasingly popular among cancer patients (Wang et al., 2009). Several mangroves species such as *Acanthus illicifolius, Bruguiera sexangula, Morinda citrifolia*, *Terminalia catappa, Ecteinacidia turbindate* have been shown to produce compounds that show strong activity against a variety of carcinomas, melanomas and lymphomas (Jongsuvat, 1981; Goh and Jantan, 1991; Iinuma et al., 1994; Hirazumi and Furasawa, 1999; Bandarnayake, 2002). However, a thorough scientific screening of *E. agallocha* for its anticancer properties is still in infancy.

In this study, we revealed the potent cytotoxic action of *E. agallocha* stem ethanol extract on lung carcinoma cell lines. This was demonstrated by dose-dependent decrease in viable cell counts and concomitant increase in the percentage of cells with sub-G1 DNA content or cell cycle arrest at the G1 phase. The p53 protein is a potent transcription factor that is involved in the regulation of cell-cycle arrest and in the induction of apoptosis. The cyclin-dependent kinase inhibitor p21 is induced by both p53-dependent and p53-independent mechanisms (Gartel and Tyner, 2002). The p53-p21 pathway is activated in cells after DNA damage and activation of this pathway temporarily arrests cell at the G1 and G2 checkpoints of the cell cycle, and terminates DNA replication and cell division (Han et al., 2002). p53 gene status has been shown to modulate chemosensitivity in lung cancer cells (Li et al., 1995; Lai et al., 2000). Hence, we were further interested to confirm whether p53 has a role in *E. agallocha* induced apoptosis. For this purpose, we compared the effect of the extract in wild type p53 cells (A549, H460) and p53-null cells (H1299, H358). From our cytotoxicity and cell cycle analysis data, it was evident that treatment of $p53^{+/+}$ cells with the extract resulted in significant induction of apoptosis whereas $p53^{-/-}$ cells treated with higher concentrations of the extract exhibited G1 arrest. These results signify that p53 modulates the chemosensitivity of lung cancer cells for *E. agallocha*. Earlier work by Kagawa et al. (1997) shows that p53-mediated apoptotic pathway is dominant over the growth arrest pathway and p53 overcomes p21 mediated G1 arrest and induces apoptosis in human cancer cells. Interestingly, upon treatment with the extract there was no significant up-regulation of the p53 protein in A549 cells. Previously, it has been shown that treatment of intact cells with 1, 10- phenanthroline- a chelating agentincreases p53 transcriptional activation and DNA-binding activity without increasing p53 protein levels (Sun et al., 1997). Thus, possibility of presence of such a chelating agent in the crude extract of *E. agallocha* cannot be ruled out. Irrespective of the p53 status, p21 expression was induced by *E. agallocha* treatment in H1299 and A549. The G1 arrest in H1299 cells could be attributed to up regulated p21 expression. The extract had no significant cytotoxic effect on normal fibroblast cells, suggesting that this selective killing effect of the extract against actively proliferating cells could be exploited in developing this compound as a

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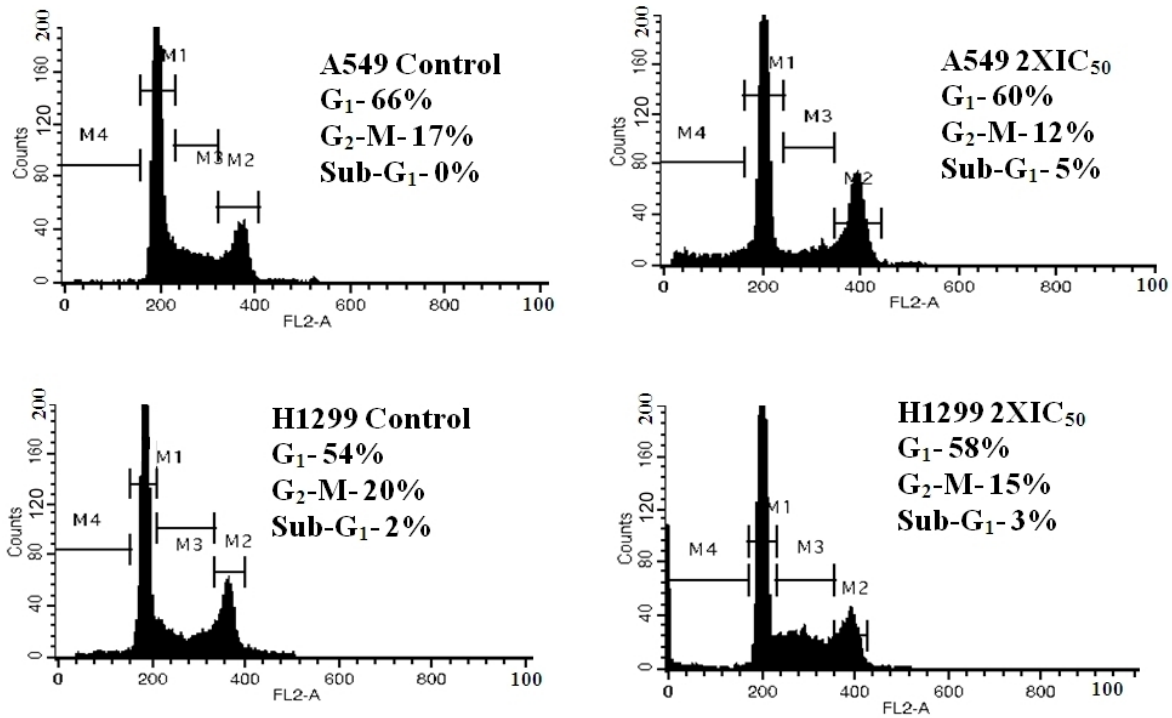


Fig. 4. *E. agallocha* crude extract induced apoptosis in A549 cells and G1 arrest in H1299 cells. Both the cell lines were treated with 2 × IC_{50} concentrations for 24 h and stained with PI as described in Materials and Methods.

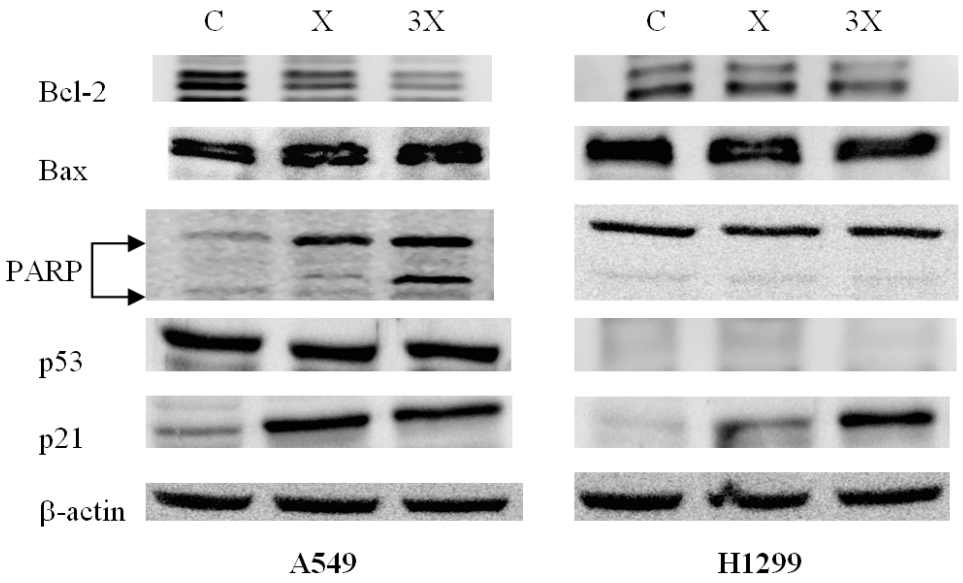


Fig. 5. Western blot analysis of cell cycle and apoptosis-related proteins in A549 and H1299 cells. C-Control. Cells were treated for 24 h with two concentrations of *E. agallocha* crude extract i.e. IC_{50} (X) and 3 × IC_{50} (3X). Cell lysates were prepared and subjected to SDS-PAGE .

potential antitumorigenic agent.

In recent years, evidence has suggested that apoptosis is important to most anticancer agents in tumor cells (Hickman, 1992; Fisher, 1994). Increased expression of the anti-apoptotic protein Bcl-2 is involved in the development and progression of many tumors (Rinner et al., 2004). An early event in the cell that sensitizes it to apoptosis is the down-regulation of the anti-apoptotic protein Bcl-2. Importantly, the ratio of pro- and antiapoptotic protein expression such as

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Bax/Bcl-2 is critical for the induction of apoptosis and decides a cell's susceptibility to undergo apoptosis (Butt et al., 2000; Tee and Azimahtol, 2005). Change in this ratio induces the release of cytochrome c from mitochondria into cytosol. Cytosolic cytochrome c can interact with Apaf-1 and leads to the activation of caspase-3 and PARP (Katiyar et al., 2005). In some models, Bcl-2 down-regulation alone has been seen to induce the commitment of a cell to apoptosis (Chen et al., 2002). Therefore, it is important to find new cytotoxic agents that increase or restore the ability of tumor cells to undergo apoptosis through reduction of anti-apoptotic factors like Bcl-2. Because Bcl-2 family proteins are not present in plants, certain phytochemicals that directly bind to Bcl-2 family proteins may act as natural pesticides selectively to induce cell death in insects and nematodes that are harmful for plants (Zi and Simoneu, 2005). *E. agallocha*-induced apoptosis was found to be associated with the downregulation of Bcl-2. Treatment of A549 cells resulted in significant down-regulation of Bcl-2 protein levels along with PARP cleavage, which is hallmark of apoptosis. No significant decrease in Bcl-2 protein levels was observed in $p53^{-/-}$ H1299 cells and this could be attributed to p21 mediated G1 arrest and lack of apoptosis in the absence of p53. These results provide evidence for the role of p53 in *E. agallocha* induced apoptosis.

In conclusion, this study could offer scientific basis for the further in-depth evaluation of the ethanolic stem extract of *E. agallocha*. It inhibited the proliferation of lung carcinoma cells in a dose-dependent manner and caused apoptotic programmed cell death in $p53^{+/+}$ cells and p21-mediated G1 arrest in $p53^{-/-}$ cells. The induction of apoptotic cell death was suggested to be mediated via p53, Bcl-2, Bax-dependent cell apoptotic pathways. Clearly, further studies are required to isolate and characterize the bioactive constituents of this extract and to determine the precise molecular mechanisms underlying the *E. agallocha* induced apoptosis in lung carcinoma cells.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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土沉香之莖乙醇萃取物依p53蛋白質之狀態對人類肺癌細胞誘發G1阻滯期效應或細胞凋亡作用

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摘要：土沉香 (*Excoecaria agallocha* L.) 是種具有廣泛民俗醫療用途的紅樹林植物，但其在抗癌上的價值還未被審慎評估過。本研究發現土沉香之莖萃取物在與人類肺癌細胞株共同培養48小時後，以劑量依存方式對人類肺癌細胞株展現了高度的細胞毒性。經實驗分析後，證明土沉香之萃取物會誘發$p53^{+/+}$之細胞進行細胞凋亡之程序，並使$p53^{-/-}$細胞停滯在G1期。在使用相位差顯微鏡觀察精萃取物處理後的$p53^{+/+}$細胞形態時，也發現了細胞凋亡的形態特徵，如細胞核出泡和染色質皺縮等現象。細胞週期分析也顯示了土沉香萃取物使$p53^{-/-}$細胞停滯在G1期。再者，觀察與細胞凋亡有關之蛋白質如p21、Bcl-2和Bax在處理後的細胞內之表現，也顯示萃取物對這類蛋白質有顯著的調控作用。由於土沉香萃取物對肺癌細胞的潛在細胞毒性作用，本研究強烈建議其未來作為抗癌藥物之可行性。

關鍵詞：抗癌作用、細胞凋亡、土沉香、肺癌、紅樹林植物、p53。

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