

Research Article**Anticancer activity of *Asparagus racemosus* root extracts in non-small cell lung cancer A549 cells****Debaashish Biswas^a, Manisha Mathur^c, Hemant Malhotra^b, Shipra Bhargava^a, Bharti Malhotra^{a*}**^aDepartment of Microbiology, S.M.S. Medical College, Jaipur (Rajasthan) India^bDivision of Medical Oncology, Department of Medicine, S.M.S. Medical College, Jaipur (Rajasthan) India^cGovernment G.D. Girls College, Alwar (Rajasthan) India

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Abstract

Background: Lung cancer is the leading cause of cancer-related mortality worldwide. Non-small-cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases. Recently emerged molecular targeted therapies have improved the treatment of lung cancer patients. But still, the problems like drug resistance, cancer recurrence and drug side effects persist with the current therapies. Therefore, the development of new chemotherapeutic agents has become an urgent need for patients to benefit and better survival. *Asparagus racemosus* is an important medicinal plant exhibiting the anti-cancer activity against various cancers; however, its activity against lung cancer remains to be addressed. **Objective:** In this study, we have assessed the anti-cancer activity of *Asparagus racemosus* root extracts in human lung adenocarcinoma cell line A549. **Material and methods:** The cytotoxic activity of the chloroform: methanol and methanol extract was determined by using MTT (3-[4, 5-dimethyl-2-thiazolyl]-2,5 diphenyltetrazolium bromide) assay, change in morphology by morphological analysis and reduction in migration was done by cell migration assay. **Results:** Significant cytotoxic effect of methanol extract (IC₅₀ 100.5 µg/ml) in comparison to chloroform: methanol extract (IC₅₀ 136.5 µg/ml) was observed. Treatment with the extracts changed the morphology of the cells, as cells become round in shape and migration reduced after treatment with root extracts. **Conclusions:** This study showed that *Asparagus racemosus* root extracts can cause cytotoxic effects, change the morphology and induce growth inhibition in A549 cells. Therefore, it can be developed as a plant-derived drug to treat NSCLC patients in the future.

Keywords: Non-small-cell lung cancer, MTT, *Asparagus racemosus*, lung adenocarcinoma

Introduction

Lung cancer is one of the most life-threatening cancers with high mortality and morbidity (Ellis et al., 2015). Lung cancer caused 1.6 million of all cancer-related deaths worldwide (Torre et al., 2015). On the basis of histology, lung cancer is divided into two categories- Non-small-cell-lung cancer (NSCLC) and small-cell lung cancer (SCLC). NSCLC is the most common and prevalent type of lung cancer which is further divided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Most of the patients diagnosed at an advanced stage survive for only 9-12 months (Bai et al., 2016). However, the

overall cure and survival rate of NSCLC patients remains low (Herbst et al., 2018).

In Ayurveda, *Asparagus racemosus* is regarded as 'Rasayana' (Goyal et al, 2003). *Asparagus racemosus* belongs to family Asparagaceae and is also known by name Shatavari. It is a woody climber that is found in low forest area around India, mainly in tropical and subtropical areas (Gomase and Sherkhane, 2010). The roots are cylindrical, fleshy and tuberous having creamy yellow colour. The plant is useful as lactagogue (Sharma et al., 1996), possesses anticandidal activity (Uma et al., 2009), is used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown and aphrodisiac (Rajeshwar et al., 2014). There have been several studies which had proved anti-cancer activity of *Asparagus racemosus* root extracts against cancer of colon (Bhutani et al., 2010; Mitra et al., 2012), breast (Mitra et al., 2012), kidney (Mitra et al., 2012), liver (Agrawal et al., 2008) and lung (Kongkanermit et al., 2011). However,

***Address for Corresponding Author:**

Dr. Bharti Malhotra

Senior Professor, Department of Microbiology and Immunology, S.M.S. Medical College, J.L.N. Marg, Near Albert Hall Museum, Ram Niwas Garden, Jaipur, Rajasthan- 302004 India

Email: drbhartimalhotra@gmail.com

Telephone: 09414042040; 08107351672

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the effect of root extracts on more aggressive lung adenocarcinoma cells A549 cells is still not known. Therefore, in this study, we investigated the anticancer activity of root extracts in A549 cell line in order to evaluate the potential of extract for the treatment of NSCLC.

Material and methods

Chemicals and Reagents

Chemicals used for cell viability assay MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was obtained from Invitrogen and Dimethyl sulfoxide (DMSO) was obtained from Himedia. Cell-culture material including Dulbecco's Modified Eagles Medium (DMEM), Phosphate Buffered Saline (PBS) and Trypsin-EDTA solution was obtained from Himedia. Fetal bovine serum (FBS) was obtained from Gibco, Life Technologies.

Plant extract preparation

Asparagus racemosus roots were collected and washed thoroughly with tap water. Roots were then cut into small pieces and were allowed to dry in the shade until all moisture evaporated. Small pieces of roots were finally ground into powder form in a mixer grinder. The powder obtained was weighed and mixed with organic solvents [Chloroform: methanol (2:1) or Methanol] until the whole powder was completely covered with it. Soxhlet apparatus round bottom flask was kept on the water bath with the temperature at 50 °C for 4 hours per day for 6 days continuously, making it a total of 24 h. After completion of 24 h, the extract was filtered with Whatman filter paper 1 and filtrate was collected in Petri plates. Then Petri plates were kept in a water bath for evaporation of organic solvents. When the solvent got completely evaporated, the extract was scratched from petriplates (Mitra et al., 2012).

Cell line and cell culture

The human NSCLC cell line A549 was purchased from NCCS, Pune. The cell line was cultured in Dulbecco's modified eagles medium, supplemented with 10% (v/v) FBS and 1% antibiotic-antimycotic solution incubated in a humidified atmosphere of 5 % CO₂ at 37 °C (Wang et al., 2013).

Cell viability assay

Cytotoxicity of the *Asparagus racemosus* root extracts was checked on A549 cell line using MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5 diphenyltetrazolium bromide) assay as per Chen et al. (Chen et al., 2013). In this assay, 4 x 10⁴ cells were seeded into the flat bottom 96-well plate and next day incubated with the chloroform: methanol and methanol extract of roots for 24 h in a CO₂ incubator with concentration ranging from 7.81 µg/ml to 500 µg/ml for each extract. At the end of the extract treatment, 20 µl of MTT was added to each well and plate was incubated for 4 h at 37°C. The supernatant was removed, blue formazan produced was dissolved in 100 µl of DMSO. OD was measured at 570 nm using a plate reader. The experiment was performed in

triplicates (n=3). Cell viability percentage was calculated with formula O.D. of drug-treated sample/ O.D. of non treated sample × 100.

Morphological analysis

Morphological analysis was done to determine the morphological changes, like cell shrinkage, membrane blebbing etc, as per (Srivastava et al., 2011). Briefly, A549 cells were seeded and next day the cells were treated with chloroform: methanol and methanol extract for 24 h. The effect of the extract on the morphology of A549 cells was assessed under the inverted phase-contrast microscope after 24 h (at 10 X magnification). The cells which were not treated with plant extract served as control.

Cell migration assay

Cell migration assay was done to access cell-cell interaction *in-vitro* migration studies as per Wang et al (Wang et al., 2013). Briefly, A549 cells were allowed to grow till 80% confluency was reached. On the next day, the monolayer was scratched with the help of 200 µl pipette tip and then washed twice with PBS to remove suspended cells. Cells were then treated with different concentrations of extract for 24 h.

After incubation, cells that migrated to wound surface were observed under an inverted microscope at 24 h. In the end, the level of wound closure was observed. Experiments were done in triplicates.

Statistical analysis

The data were expressed as the mean of three replications with standard deviation (±SD). GraphPad Prism 5 was used for analysis of IC₅₀ value.

Results

Effect of *Asparagus racemosus* root extracts on cell viability

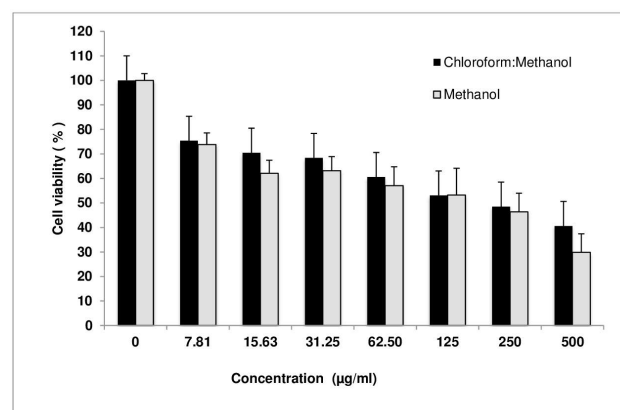


Figure 1. Effect of different concentrations of *Asparagus racemosus* root extracts on cell viability using MTT assay in A549 cell line. Black bars represent chloroform: methanol extract cell viability and grey bar show methanol extract cell viability.

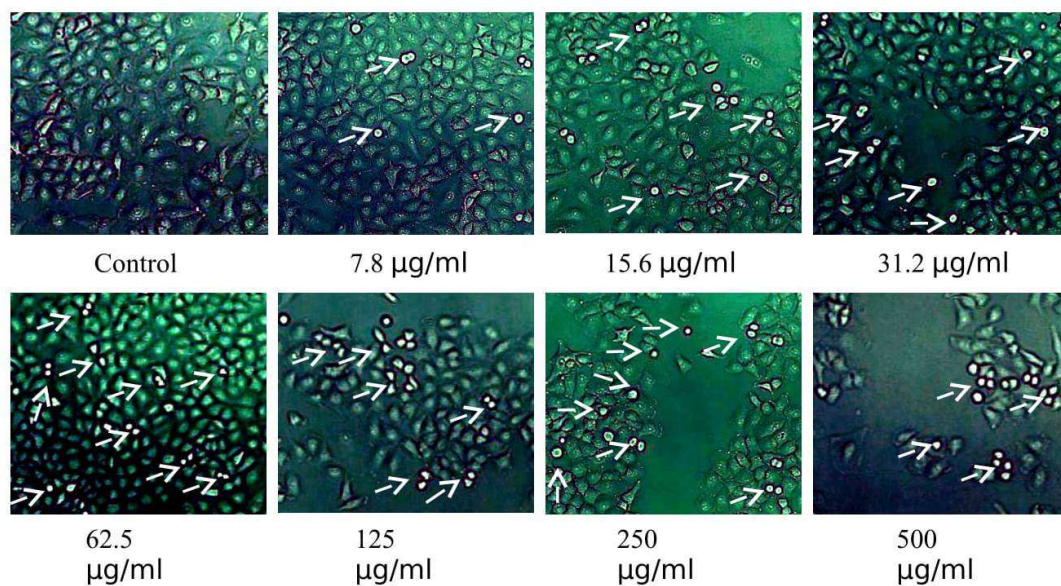


Figure 2. Effect of different concentrations of *Asparagus racemosus* methanol root extract on the morphology of A549 cells. White arrows indicate the cell shrinkage and cell rounding (Magnification 10x).

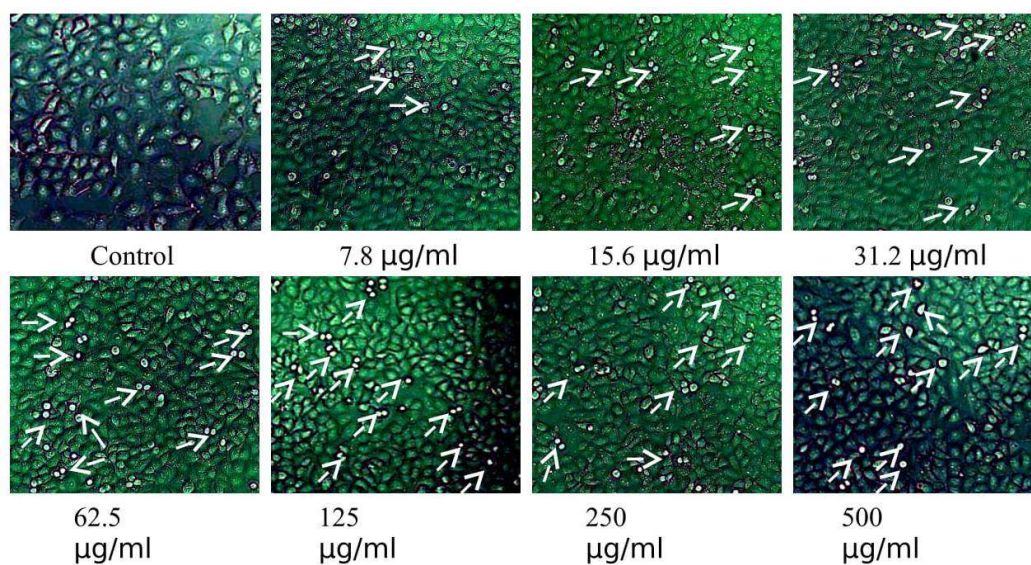


Figure 3. Effect of different concentrations of *Asparagus racemosus* chloroform: methanol root extract on the morphology of A549 cells. White arrows indicate the cell shrinkage and cell rounding (Magnification 10x).

Both chloroform: methanol and methanol extracts significantly reduced the cell number. The effect of methanol extract was better ($IC_{50} = 100.5\mu\text{g/ml}$) than chloroform:methanol extract ($IC_{50} = 136.5\mu\text{g/ml}$) (Figure 1).

Effect of *Asparagus racemosus* root extracts on cell morphology

Both the extracts induced cell shrinkage and rounding after 24 h of treatment (Figure 2 and 3). Control cells (untreated cells) showed no change in their morphology after incubation with plant extract.

Effect of *Asparagus racemosus* root extracts on cell migration assay

In control, where cells were not treated with extract, cells migrated from the wound edge, caused complete closure of the wound by 24 h. However, after 24 hours of incubation migration rate were significantly lower in treated cells versus untreated cells (Figure 4, 5 and 6).

Discussion

In spite of all the efforts for the development of the new therapeutic agents, lung cancer remains to be the major cause of cancer-related deaths worldwide. Most of the patients are diagnosed at an advanced stage which leads to poor prognosis and minimal overall survival rate. Currently, available radio- and/or chemotherapeutic

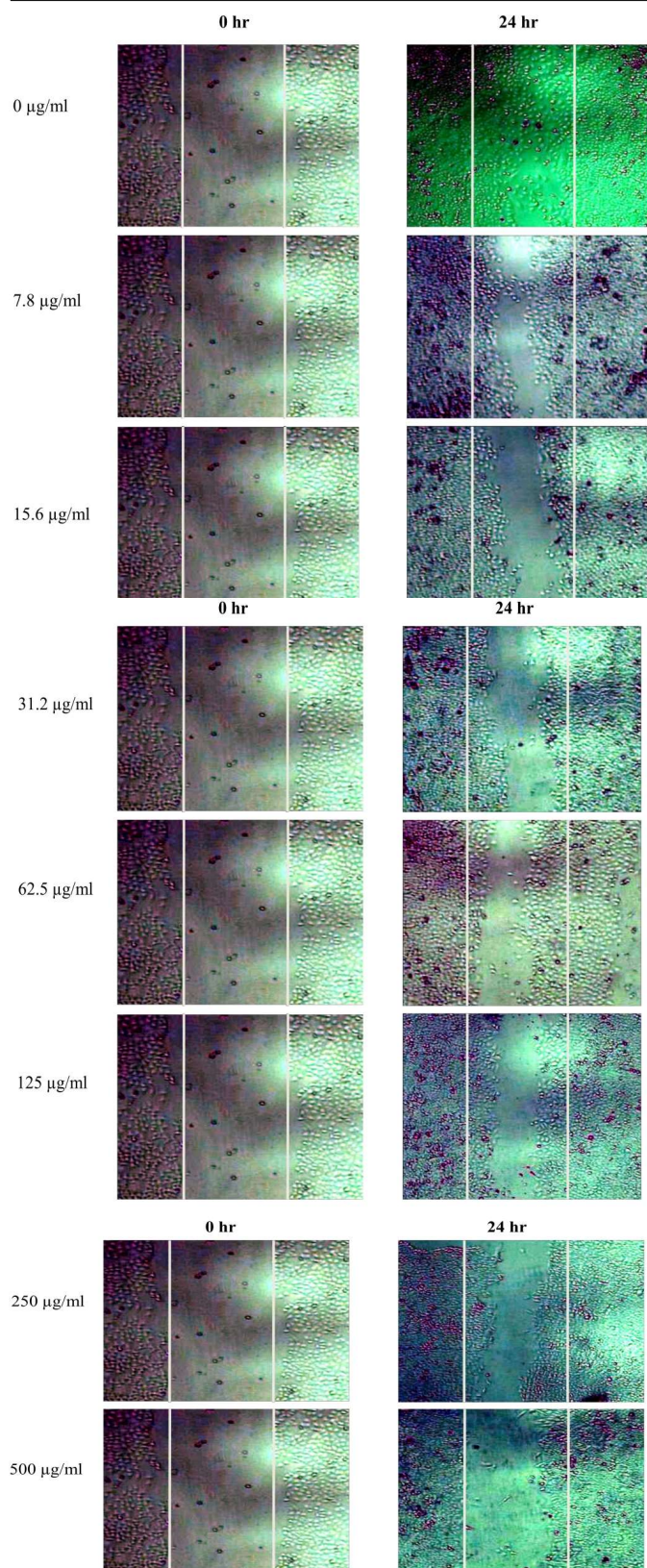


Figure 4. (h)Effect of different concentrations (0 to 500 µg/ml) of *Asparagus racemosus* root methanol extract on migration of A549 cells after 24 h of treatment

treatment are effective in treating cancer but all of them provides only meagre benefits to patients(Soengas and Lowe, 2003). Therefore, it becomes important to find and develop alternative

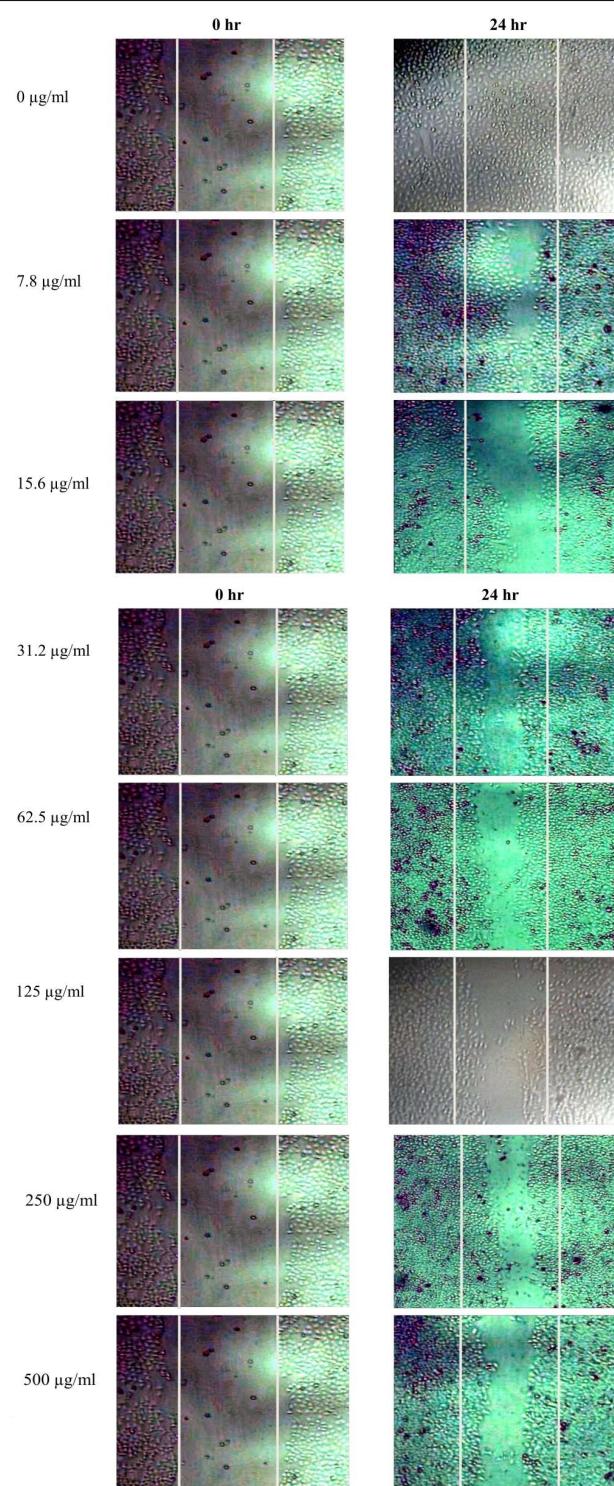


Figure 5. Effect of different concentrations (0 to 500 µg/ml) of *Asparagus racemosus* root chloroform:methanol extract on migration of A549 cells after 24 h of treatment

therapeutic agents. Medicinal plants have been the source of various anticancer agents including taxol, vinblastine, vincristine, etoposide, etc. They are more effective and safer and do not have side effects as compared to synthetic drugs (Gullett et al., 2010).

Increase in the death rate from cancer has resulted into the

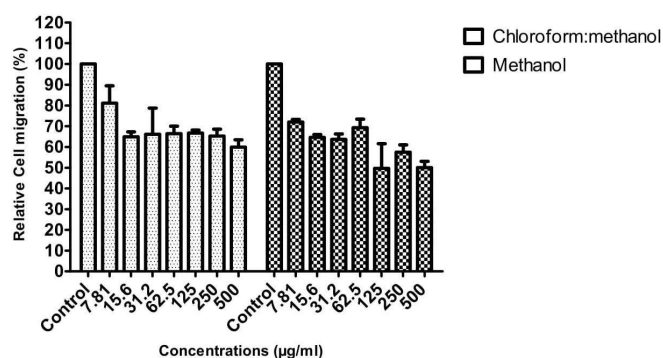


Figure 6. Effect of different concentrations of chloroform:methanol and methanol extracts on A549 cell migration

urgent search of natural products which can be developed for cancer therapy and many among them are in different phases of the therapeutic trial (Pendleton et al., 2008). According to a report by the World Health Organization, nearly 80% of the world population relies on herbal medicine for their primary health needs (Duraipandiyan et al., 2006; Willett, 1994). In India, herbs have been a part of traditional medicine since 4000 BC (Pandey et al., 2013). From ancient times, natural products have been in use for treatment of cancer (Banerjee et al., 2016).

Asparagus racemosus is one of the well-known drugs in Ayurveda and its root extract have several pharmacological activities including antiulcer, antioxidant, antidiarrhoeal, antidiabetic and immunomodulatory activities. Its roots have also got beneficial effects if taken in nervous disorders, dyspepsia, diarrhoea, dysentery, tumours, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases (Alok et al., 2013).

There are several reports suggesting the anticancer activity of *A. racemosus* in different cancer cell lines (Agrawal et al., 2008; Bhutani et al., 2010; Kongkanermit et al., 2011; Mitra et al., 2012). However, only one to two studies report its activity against lung cancer. In one of the first study, the aqueous root extract was found to be effective by inhibiting lipofectamine induced apoptosis in a human lung large cell carcinoma cell line H460 (Kongkanermit et al., 2011). The molecular mechanism of apoptosis induced by *A. racemosus* has not been fully understood. In the present study, we determined the *in vitro* anticancer activity of methanol and chloroform:methanol root extract of *Asparagus racemosus* on A549, a human lung adenocarcinoma cell line.

The cytotoxicity of root extracts was primarily examined by MTT assay with the A549 cell line. There are several studies where plants of different *Asparagus* family showed cytotoxic effects against different types of cancers (by MTT assay) (Huang et al., 2008; Ji et al., 2012; Liu et al., 2009; Liu et al., 2016; Maro et al., 2013; J Wang et al., 2013; Xiang et al., 2013;

Zhang et al., 2018). Mitra et al. (2012) found that steroidal compounds present in the insoluble fraction of ethyl acetate of chloroform:methanol extract (2:1) showed cytotoxicity towards breast cancer cell line (MCF-7), colorectal carcinoma cell line (HT-29) and kidney carcinoma cell line (A498) (Mitra et al., 2012). But interestingly, our study showed that the inhibition of cell growth was better in methanol extract in comparison to chloroform:methanol extract with IC_{50} of 100.5 µg/ml and 136.5 µg/ml respectively. Similar results were also obtained by two of the previous studies in which methanolic extract showed better anti-tumour activity against acute promyelocytic leukaemia cell line (HL-60) and colon carcinoma cell line (SW480 and SW620). Steroidal saponins extracted from methanolic extract were cytotoxic to HL-60 at a concentration greater than 200 µg/ml (Shao et al., 1996) while 80% of cell growth inhibition occurred after exposure with 80 µg/ml of white asparagus shoots methanolic extract against SW480 and SW620 (Bousserouel et al., 2013).

Morphological changes in cells are an indicator of apoptosis. In the previous study, it was found that renal cell carcinoma (UOK 146) cells after treatment with 1mg/ml leaf extract have induced cell rounding and detaching from the cell surface. Similar morphological changes like cell rounding, shrinkage and floatation were obtained in our study also.

Migration of cancer cell is one of the most important features for development of malignancies. One of the molecular event responsible for the same is Epithelial to Mesenchymal Transition (EMT), occurs frequently in metastatic tumour cells. During EMT, cell loses their epithelial characteristics and junctions and gains mesenchymal characteristics to outreach newer locations (Batlle et al., 2000). To investigate whether *Asparagus racemosus* root extracts can inhibit metastasis, cell migration assay was performed. In the previous study, saponins from the stem of *Asparagus* reduced migration of MDA-MB-231 breast cancer cells at an inhibitory concentration of 400µg/ml. In this study, when cells were treated with different doses of the root extracts they exhibited reduced migration of A549 cells in comparison to control cells.

The limitations of our study are that we have not done Column Chromatography (CC), Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) to find out the active compounds in each extract. Further, evaluation of individual compound in each extract which shows significant anticancer activity is also not done. It is important to know the signalling pathways being involved and affected by the plant extract. *In-vivo* studies were also not done.

Conclusion

In this study, we found that both methanol and chloroform: methanol (2:1) extracts successfully inhibited the cell growth, migration and induced changes in the cell morphology attributing apoptosis. But methanol extract was found to be effectively better in showing anticancer activity in comparison to chloroform: methanol extract. However, the detailed mechanism and signalling pathways involved in extract induced apoptosis needs to be studied. This was only a preliminary study to find out the activity of *Asparagus racemosus* root extract against lung cancer. Therefore, *Asparagus racemosus* can prove to be a potent candidate to be developed as new and plant-based therapeutic drug against lung cancer.

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Conflict of interest

The authors report no conflict of interest in this work.

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