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# Effect of Various Extracts of *Dryopteris Cochleata* against Cancer Cell Line

**Ruchi Singh Thakur[1]* and Bharti Ahirwar[2]**

*[1,2]Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.)*
*E-mail: [1]ruchi_singh1260@yahoo.co.in, [2]ah_bharti@yahoo.com*

**Abstract—***Cancer is the second largest cause of death in India. Among various cancer treatment, chemotherapy has shown a number of side effects. To overcome these side effects, various plant-based drugs have been explored by the researchers as they contain a variety of secondary metabolites which can have multi-targeted action on cancer cells. The present study is designed to investigate the anticancer activity of Dryopteris cochleata which is a pteridophyte belongs to family Dryopteridaceae and commonly known as jatashankari. Rhizomes of Dryopteris cochleata is traditionally used for the treatment of snake and dog bites. It also contains a high amount of flavonoids, protoflavones and polyphenolic content. Petroleum ether, chloroform, ethyl acetate and hydroalcoholic extract of Dryopteris cochleata was evaluated for anticancer activity against lung cancer cell lines using MTT assay.*

## 1. INTRODUCTION

Cancer is the major cause of death in the world. It is characterized by uncontrolled cell division coupled with the lack of apoptosis. It occurs due to the disturbance in various pathways of cell division which results in impaired DNA activity [1]. All cancer patients express disturbance in different signaling pathways, which raised the demand of tailored made treatment for the individual patient[2-3]. Lung cancer is one of the most common cancer which is caused due to inhalation of carcinogens present in smoke or it may occur secondary to other existing cancer [4-5]. The combination of therapy given to patient has decreased the mortality rate but at the cost of inevitable side effects. Plant-derived drugs are proved to be a boon in cancer therapy. Many studies have established the plant derived novel biomolecules in cancer chemotherapy viz. taxol, vincristine and vinblastin etc [1]. Since thousands of secondary metabolites present in plants can be useful in targeting multiple pathways of cancer cell which can cause cancer cell death and prevent metastatis. Various categories of secondary metabolites such as diterpenoids, lignans, and some polyphenols have proven their cytotoxic activity.

*Dryopteris cochleata* is a pteridophyte belongs to family dryopteridaceae. In Chhattisgarh, it is mainly found in the achanakmar-amarkantak biosphere reserve. Phytochemically, it is rich in phenolic compounds especially, flavonoids and protoflavones. Traditional healers of Chhattisgarh use it in the treatment of snake bite and dog bite as an antidote and in gonorrhea. Leaves are reported to exhibit antioxidant activity[6,7]. Here, we have investigated anticancer activity of various extracts of *Dryopteris cochleata* rhizomes against lung cancer cell line.

## 2. MATERIAL AND METHODS

### 2.1. Collection, authentication and drying of plant material

The rhizomes were collected from Kabir chabutra, Chhattisgarh. The plant was identified and authenticated by Botanical survey of India, Allahabad, U.P. Rhizomes were air dried, coarsely powdered and stored in air tight container. Extraction of rhizomes was performed by cold maceration using hydroalcoholic solution (1:1). The brown colored extract was air dried and weighed. Further, fractionation of the hydroalcoholic extract was carried out using solvent-solvent fractionation by, petroleum ether, chloroform and ethyl acetate respectively.

### 2.2. Phytochemical analysis

Phytochemical analysis of hydroalcoholic extract of *Dryopteris cochleata* was performed using qualitative test for various categories of phytoconstituents.

### 2.3. Reagents

Cancer cell line was obtain from National Cancer Institute (NCI), Bethesda, United States of America (USA). The cancer cells were grown in RPMI-1640 medium enriched with 10% heat-inactivated foetal bovine serum (FBS), 0.37% sodium bicarbonate, penicillin (100 units/ml), pyruvic acid (0.11 mg/ml), L-glutamine (0.3 mg/ml), and streptomycin (100 µg/ml) at 37ºC temperature, 95% air, 5% CO2 and 98% humidity. All the extracts were dissolved in dimethylsulfoxide.

### 2.4. Cell culture

Lung cancer cells i.e. A549 cells were grown and centrifuged at 100g for 10 minutes. Resulting, cell pellets were mixed with RPMI medium; $4\times10^5$ cells were relocated to all the wells of 24-well tissue culture plate containing 1 ml complete medium.

Cells were mixed with 100 µg/ml of extracts and placed for 48 hrs in the $CO_2$ incubator. Cells were mixed and 40µl suspension relocated to 96-well plate along with 140µl

**Table 1: Phytochemical analysis**

| S. No | Phytoconstituents | *Dryopteris cochleata* |
|---|---|---|
| 1 | Alkaloids | - |
| 2 | Glycosides | ++ |
| 3 | Tannins | +++ |
| 4 | Flavonoids | +++ |
| 5 | Steroid | - |
| 6 | Proteins | ++ |
| 7 | Carbohydrate | + |
| 8 | Reducing sugar | +++ |
| 9 | Monosaccharides | - |
| 10 | Pentose sugars | + |
| 11 | Fats and oils | + |

of the medium. Subsequently, 20 µl of MTT solution was added and culture was incubated for 2 hrs at 37°C. after that, plates were spun for 15 min at 3000 rpm thereafter, the supernatant aspired and MTT-formazan crystals were dissolved in DMSO. Lastly, plate was stirred and cell growth was measured by comparing absorbance of test with control cells.

## 3. RESULTS

### 3.1. Phytochemical screening

Phytochemical analysis of rhizomes of *Dryopteris cochleata* extract showed the presence of tannins, flavonoids, glycosides, amino acids, proteins, reducing sugar, pentose sugar, and fats and oils (See Table 1).

### 3.2. Anticancer activity

We have investigated the cytotoxic activity of rhizomes of *Dryopteris cochleata* extract against A549 lung cancer cell line using MTT assay. Growth inhibition of human lung cancer cells was observed to determine the cytotoxic potential of extracts ( See Fig. 1).

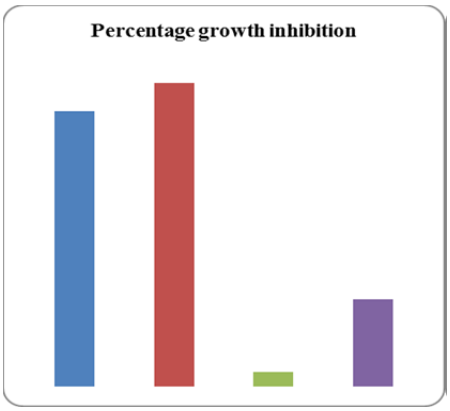


**Fig. 1: Percentage growth inhibition of A549 lung cancer cell line by *Dryopteris cochleata***

## 4. DISCUSSION

The rhizomes of *Dryopteris cochleata* was powdered and macerated by 50% ethanol. Phytochemical screening of the *Dryopteris cochleata* rhizome extract was performed which showed the presence of tannins, flavonoids, glycosides, amino acids, proteins, reducing sugar, pentose sugar, fats, and oils. The Hydroalcoholic extract was further fractionated into four different fractions viz. petroleum ether, chloroform, ethyl acetate, and the hydroalcoholic fraction. Anticancer screening of all the fractions was performed using MTT cytotoxic assay as described in earlier studies [8-9]. Chloroform fraction showed highest growth inhibition among all the other fractions followed by petroleum ether and the hydroalcoholic fraction. It indicates that nonpolar compounds present in *Dryopteris cochleata* extract possess cytotoxic activity, although they are not sufficient to inhibit cancer cell growth significantly. These results indicate that variety of flavonoids present in *Dryopteris cochleata* are not responsible for cytotoxicity. Thus, all flavonoids may not cause cytotoxicity which is contrary to the report that flavonoids are good cytotoxic agents. Secondly, the synergistic or antagonistic effect of various phytoconstituents does not allow cancer cell apoptosis. It may also possible that cell line selected for the anticancer activity does not respond to the selected plant.

## 5. CONCLUSION

Rhizomes of *Dryopteris cochleata* was found to be rich in flavones, flavonols, flavanones, chalcones and aurones. The presence of these phytoconstituents made it highly attractive to be searched for anticancer activity. However, various extracts of *Dryopteris cochleata* did not show significant cytotoxicity against A549. Among various extract of *Dryopteris cochleata*, chloroform extract inhibited maximum cancer cell growth.

## 6. ACKNOWLEDGMENTS

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