An Elsevier Indexed Journal ISSN-2230-7346

**Journal of Global Trends in Pharmaceutical Sciences**

*Research Article*

# *IN-VITRO* CYTOTOXIC ACTIVITY OF VARIOUS EXTRACTS FROM WHOLE PLANT OF *POLYCARPAEA CORYMBOSA* LAM

**Sakthi Abirami. M[1*], Muthusamy.P[2]**

[1]*Institute of Pharmacology, Madras Medical College, Chennai-600 003, India.*
[2]*Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600 003*

**ARTICLE INFO**

***Key words:***

*P. corymbosa*
*In-vitro*
cytotoxicity
MTT Assay

## ABSTRACT

The present study was aimed to evaluate *in-vitro* cytotoxicity activities of various extracts of *Polycarpaea corymbosa* on different cell lines. The *in-vitro* cytotoxicity activity of various extracts of *P.corymbosa* was studied against MCF-7, HL-60, HepG2, HT-29 and PC-3 cell lines, using the thiazolyl blue test (MTT) assay. Ethanolic extract of *P.corymbosa* exhibited prominent inhibitory effect against MCF-7, HL-60, HepG2, HT29 and PC3 cell lines under *in-vitro* condition. Similar result was not observed in other two extracts. From the result it can be found that the *P.corymbosa* extract has potent *in-vitro* cytotoxic activity

## INTRODUCTION

The World Health Organization (WHO) estimates that almost 75% of world's population has therapeutic experience with herbal drugs. Today 85000 plants have been documented for therapeutic use globally[1]. Cancer is the ultimate end stage of carcinogenesis characterized by abnormal cell and tissue proliferation, which eventually leads to invasion and metastasis[2].

***Address for correspondence**

**Dr. P. Muthusamy***
*Assistant Professor*
*Department of Pharmacognosy,*
*College of Pharmacy,*
*Madras Medical College,*
*Chennai-600 003, Tamilnadu, India*

Medicinal plants present a potential source of drugs or molecular models for new drugs, in fact some species provided many effective anticancer agents in current use such as vinblastine, irinotecan, topotecan, vincristine, taxanes[3] etc, the imminent strategy of the World Health Organization (WHO) lead scientists to improved herbal remedies and the discovery of a natural chemotherapy drug which guarantees the safety, efficacy and quality as a chemical drug. *In-vitro* cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work[4].

*Polycarpaea corymbosa* Lam. is a herb of annual or perennial, small shrubs with taproots slender to stout, stems erect, branched, leaves opposite, sometimes appearing whorled belonging to the family Caryophyllaceae. Flavonoids and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including antioxidant, anti-inflammatory, anti

carcinogenic, etc. Leaves, flower heads of *P.corymbosa* are used in reducing fever; anti-inflammatory and as a poultice for boils and other swellings; antidote for snakebite, leaves were reported to posses potent antioxidant property and are used for treatments of jaundice, demulcent and astringent in Indian folk medicine**.** The whole parts of *P.corymbosa* are used in Indian traditional medicinal system in inflammatory swellings and in treatment of ulcer, jaundice[5], liver diseases[6]. Antimicrobial activity reported different extracts of *P.corymbosa* against human pathogens[7].In this study, we have explored various extracts of *P.corymbosa* for their cytotoxic activity on the human MCF-7, HL-60, HepG2, HT-29 and PC-3 cell lines.

## MATERIALS AND METHODS

### Chemicals

Glutamine, gentamicin, trypsin, non-essential amino acid, fetal calf serum, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)-reagents were obtained from HIMEDIA, Mumbai, India. All other chemicals used were of analytical grade.

### Plant Material

Whole plant of *P.corymbosa* was collected from thirunelveli District, Tamil Nadu, India and plant authentication were done by the Botanical survey of India. The whole plant of *P.corymbosa* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

### Preparation of the extracts

The dried powder was packed in Soxhlet[8] apparatus and successively extracted with petroleum ether, ethyl acetate, ethanol extraction. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

### Cancer cell lines

Human breast cancer (MCF-7) and human leukemia (HL-60) cell lines were provided by Deshpandey Laboratory, Bhopal, India. HepG2 (human cancerous liver cell lines), HT29 (human colon cancer cell lines), PC3 (human prostate cancer cell lines) were obtained from Department of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad, India.

### *In vitro* cytotoxicity

The cytotoxic effect various extracts of *P.corymbosa* was evaluated by MTT assay using MCF-7, HL-60, HepG2, HT-29 and PC3 tumor cell lines. This MTT assay was performed according to a slight modification of the procedure reported by Mosman[9]. Cells were cultured in minimum essential medium (MEM) supplemented with glutamine (0.6 g/L), gentamicin (25 mg/ mL) and 10% fetal calf serum at 37°C and in humidified 5% $CO_2$. For experiments, cells were plated in 96-well plate ($10^5$ cells/ well for adherent cells for suspended cells in 100 µL of medium). After 24 hour, the extracts (0.01, 0.1, 1, 10 and 100 µg/mL) dissolved in DMSO (1%) was added to each well and incubated for 96 hr. The control groups received the same amount of DMSO. Doxorubicin (0.01, 0.1, 1, 10, 100 µg/mL) was used as positive control. Growth of tumor cells was quantified by ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 96 hr incubation, the medium in each well was replaced by fresh medium containing 0.5 mg/mL of MTT. Four hour later, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured at 550 nm. Drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm. Percentage inhibitions [100 - (absorbance of test wells/absorbance of control wells) X 100] were calculated and plotted against the concentrations used to calculate the IC50 values[10, 11]. The experiments were performed in triplicate.

### Statistical analysis

Data were presented as mean ± SEM. The $IC_{50}$ values were obtained by nonlinear regression using the statistical package for social sciences (SPSS) version 14.

## RESULTS

### *In-vitro* cytotoxicity

In order to evaluate the cytotoxic effect of various extracts of *P.corymbosa*, an antiproliferative assay with five human cell lines (MCF-7,HL-60 , HepG2, HT-29 and PC3) was performed.

**Table 1. Cytotoxic activity (IC50 values) of plant extracts that are used in the treatment of Human breast cancer (MCF-7)**

| Extracts | Human breast cancer (MCF-7)* | |
|---|---|---|
| | $IC_{50}$ µg/mL | Status |
| Petroleum ether extract | >100 | Inactive |
| Ethyl acetate extract | 28.05±0.02 | Moderately active |
| Ethanol extract | 6.80±0.05 | Active |

*Mean ± SEM; n=3.

**Table 2. Cytotoxic activity (IC50 values) of plant extracts that are used in the treatment of Human Leukemia (HL-60)**

| Extracts | Human Leukemia (HL-60)* | |
|---|---|---|
| | $IC_{50}$ µg/mL | Status |
| Petroleum ether extract | >100 | Inactive |
| Ethyl acetate extract | 91.55±0.01 | Inactive |
| Ethanol extract | 11.50±0.45 | Active |

*Mean ± SEM; n=3.

**Table 3. Cytotoxic activity (IC50 values) of plant extracts that are used in the treatment of Human Cancerous Liver Cell Lines (HepG2)**

| Extracts | Human Cancerous Liver Cell Lines (HepG2)* | |
|---|---|---|
| | $IC_{50}$ µg/mL | Status |
| Petroleum ether extract | >100 | Inactive |
| Ethyl acetate extract | 85.55±0.71 | Inactive |
| Ethanol extract | 10.00±0.02 | Active |

*Mean ± SEM; n=3.

**Table 4. Cytotoxic activity (IC50 values) of plant extracts that are used in the treatment of Human Colon Cancer Cell Lines (HT29)**

| Extracts | Human Colon Cancer Cell Lines (HT29)* | |
|---|---|---|
| | $IC_{50}$ µg/mL | Status |
| Petroleum ether extract | 97.55±0.75 | Inactive |
| Ethyl acetate extract | 82.69±0.05 | Inactive |
| Ethanol extract | 7.05±0.60 | Active |

*Mean ± SEM; n=3.

**Table 5. Cytotoxic activity (IC50 values) of plant extracts that are used in the treatment of Human Prostate Cancer Cell Lines (PC3)**

| Extracts | Human Prostate Cancer Cell Lines (PC3)* | |
|---|---|---|
| | $IC_{50}$ µg/mL | Status |
| Petroleum ether extract | >100 | Inactive |
| Ethyl acetate extract | 98.02±0.05 | Inactive |
| Ethanol extract | 17.20±0.85 | Active |

*Mean ± SEM; n=3.

Tables 1-5 were showed the cytotoxic activity of various extracts of *P.corymbosa* against various cell lines. The ethanolic extract of plant extract was active on all cell lines (MCF-7, HL-60, HepG2, HT-29 and PC3). The $IC_{50}$ value of ethanolic extract *P.corymbosa* on various cell lines like MCF-7 (6.80±0.05), HL-60 (11.50±0.45), HepG2 (10.00±0.02), HT-29 (7.05±0.60) and PC3(17.20±0.85). The ethylacetate extract was found moderately active on MCF-7(28.05±0.02) cell line and inactive on HL-60, HepG2, HT-29 and PC3 cell lines. On the other hand petroleum ether extract were not found active on all cell lines.

## DISCUSSION

Plant substance continues to serve as viable source of drugs for the world population and several plant based drugs are in extensive

clinical use[12]. Agents capable of inhibiting cell proliferation, inducing apotosis or modulating signal transduction are currently used for the treatment of cancer[13]. The use of multiple chemopreventive agents with multiple targets on cancer cells are considered to be more effective in cancer treatment[14]. In the present study, the cytotoxic effect of various extracts of *P.corymbosa* on MCF-7, HL-60, HepG2, HT29 and PC3 cells was evaluated by MTT assay. MTT assay is a well-established *in-vitro* method for cytotoxicity against cancer cell lines and non-cancer cell lines[15], and here it was utilized to determine the selective activity of the extracts. Different dilutions of extracts were treated and $IC_{50}$ values were calculated. In our screening program, we adopted the criteria of the American National Cancer Institute to consider a crude extract promising for further purification based on the IC50 values lower than 30 µg/mL in order to discover and develop potential anticancer natural compounds[16,17]. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties for future work[18].

## CONCLUSION

From the result it shows that the ethanol extract of *P.corymbosa* has potent *in-vitro* cytotoxic activity against all cell lines. Further studies are also in process to evaluate the most potent fraction of the active plant.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest

## REFERENCES


1. Liu Y , Botanical drugs: Challenges and opportunities: Contribution to Linnaeus Memorial Symposium. Life Sci. 2008; 82: 445-449.
2. Kubmarawa D, Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr J Biotech, 2007; 6: 1690-6.
3. Suffness M, Assays related to cancer drug discovery. In: Hostettmann, K. (Ed). Methods in Plant Biochemistry: Assays for Bioactivity Academic Press London 1990; (6): 71-133.
4. Cardellina et al. The selection dereplication and prioritization of antitumor and HIV inhibitory natural products extracts. In: Bohlin, L., Bruhn, J.G. (Eds.), Bioassaay Methods in Natural Product Research and Development. Kluwer Academic Publishers Dordrecht, 1999; 25–36
5. Madhava chetty K, Sivaji K, Tulasi Rao K. "Polycarpaea corymbosa L." Flowering Plants of Chittoor District, Andhra Pradesh, India. 2008.
6. R. Varadharajan and D. Rajalingam. Hepatoprotective effect of *Polycarpaea corymbosa l*. against CCl4 induced hepatotoxicity in rats. Inter. J. of Pharmacotherapy, 2012; 2(1): 18-23.
7. Sindhu. Antimicrobial activity of Polycarpaea corymbosa Lam. Journal of Chemical and Pharmaceutical Research, 2012; 4(8):4014-4019.
8. Harborne JB. Phytochemical methods 11 Edn. In Chapman &, Hall. New York, 1984: 4-5.
9. Mosmann T. application to proliferation and cytotoxicity assays. J Immunol Methods, 1983; 65(1-2): 55-63.
10. Freshney RI. Cytotoxity in culture of animal cells: a manual of basic techniques. USA: Wiley-Liss; 2000; 329-345.
11. Park JG, et al. MDR1 gene expression: its effect on drug resistance to doxorubicin. J Natl Cancer Inst 1994; 86(9): 700-705.
12. Heinrich, Ethnobotany and ethnopharmacy their role for anticancer drug development. Curr Drug targets, 2006; 7: 239-245.
13. De Flora, Overview of mechanisms of cancer chemopreventive agents. Mutat Res, 2005; 591: 8-15.
14. Howells, Prospects for plant derived chemopreventive agents exhibiting multiple mechanisms of action. Curr Med Chem, 2005; 5: 201-213.
15. Abu-Dahab, Antiproliferative activity of Jordan against a breast adenocarcinoma cell line (MCF7). Sci Pharm 2007; 75: 121-136.
16. Mesquita et al. Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines. J Ethnopharmacol 2009; 123(3): 439-445.
17. Steenkamp V, Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. S Afr J Bot 2006; 72(4): 630-633.
18. Baskar A, Chemopreventive potential of β-sitosterol in experimental colon cancer model-an in vitro and in vivo study. BMC Complement Altern Med.