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ANTICANCER ACTIVITY OF LEAVES EXTRACT OF PLECTRANTHUS AMBOINICUS (KARPOORAVALLI) AGAINST LIVER CANCER CELL LINE

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ABSTRACT

In this present investigation, we have used very important karpooravalli (Plectranthus amboinicus) for the biomedical applications like anticancer activity against liver cancer cell line (HepG2). The In-vitro anticancer activity against Hep G2 Liver cell line culture analyzed using Assay for cytotoxic activity (MTT assay). The study proves the very good biomedical action of karpooravalli plant leaves extract.

KEYWORDS: *P. Amboinicus, Anticancer Activity, Hep G2 Liver Cell Line*

INTRODUCTION

Plant-derived compounds have been an important source of several clinically useful anti-cancer agents. These include vinblastine, vincristine, paclitaxel, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, homoharringtonine and elliptinium.

The isolation of the vinca alkaloids vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* introduced a new era of the use of plant material as anticancer agents and were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005). The discovery of paclitaxel from the bark of the Pacific Yew, *Taxus brevifolia* is evidence of the success in natural product drug discovery and used in the treatment of ovarian cancer, advanced breast cancer, small and non-small cell lung cancer (Wani et al., 1971; Rowinsky et al., 1992). In the Indian ayurvedic medicine Taxus baccata was used for the treatment of cancer. Camptothecin, isolated from the Chinese ornamental tree *Camptotheca acuminate* was advanced to clinical trials by NCI during1970 (Potmeisel and Pinedo, 1995). Topotecan and irinotecan are semi-synthetic derivatives of camptothecin and are used for the treatment of ovarian, small cell lung cancer and colorectal cancer (Creemers et al., 1996; Bertino, 1997).

Epipodophyllotoxin, the active anti-tumor agent isolated from the roots of podophyllum species was reported to possess anticancer activity against various cancers. Etoposide and teniposide are two semi-synthetic derivatives of epipodophyllotoxin and are used in the treatment of lymphomas, bronchial and testicular cancers. Homoharringtonine isolated from the Chinese tree Cephalotaxus harringtonia is another plant-derived agent in clinical use and has been used successfully for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Elliptinium, a derivative of ellipticine, isolated from medicinal plant Bleekeria vitensis is used for the treatment of breast cancer. Plant extracts are playing a very major role in the anticancer activity (Ponnanikajamidin et al., 2015, Rajeshkumar et al., 2015)

In this present study, we have carried out the anticancer activity against liver cancer cell line (Hep G2) by MTT Assay

MATERIALS AND METHODS

Preparation of Leaves Extract

Plant Material

Leaves of *Plectranthus amboinicus* (Lour.) was collected locally in and around Vandavasi and confirmed and authenticated by Mr.Raja, Botanist Santhimalai research foundation.

Extract Preparation

Leaf of *Plectranthus amboinicus* (Lour.) was washed with water to remove soil particles and other impurities. A 20 gram of plant leaves were cut into small pieces and extracted by boiling with water for 30 min at 90 °C. Then the boiled solution was filtered through Whatman No. 1 filter paper. Collect the filtrate and allowed to evaporate to obtain crude extract. For further experiment crude extract was diluted with water at the concentration of 1mg/1 ml and stored for further use.

In-Vitro Anticancer Activity against Hep G2

Liver Cell Line Culture

Normal and Liver cancer (Hep G2 cell lines were obtained from the Center for Research Faculty, Animal Sciences University and Ramachandra University, Chennai, Tamilnadu. The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fatal Bovine Serum (FBS), Penicillin (100 U/ml), and Streptomycin (100 U/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

Assay for Cytotoxic Activity (MTT Assay)

The cytotoxicity effect of aqueous extract in normal and liver cancer cell line Hep G2 was determined by the MTT assay (Demir et al., 2016). Briefly, cancer cells were seeded onto 96- well microplates at a density of 1×10^4 cells/100µL per well were incubated with the extract at the concentrations of 10 to 100 µg/mL for 48-hours.

The medium was then removed, and 100µL of MTT solution (0.5mg/mL MTT in PBS)wasadded. Then the cells were incubated for 4 hours in CO_2 incubator and the solutions turn into purple color indicates the formation of formazan. The MTT-purple formazan productions were dissolved in 0.1N isopropanol/hydrochloric acid (HCl) and optical densities of the solutions were measured by absorbance at 570nm in an ELISA plate reader. Cell viability was expressed as the optical density ratio of the treatment to the control (% of control) as described previously.

RESULTS

Anticancer Activity against a Liver Cancer Cell Line

In vitro assay of anticancer activity of aqueous extract of *P. amboinicus* leaves against liver cancer cell lines at different concentrations was evaluated by MTT assay. MTT assay is based on the metabolic reduction of MTT into formazan crystals on treatment with cancer cell lines. The inhibitory activity of the aqueous extract was compared with the standard drug doxorubicin for liver cancer cell lines (Hep G2). The cell growth inhibition percentage was found to be at different concentration of extracts. Anticancer activity at the different concentrations of 10 µg, 20µg, 40µg,

60µg, 80 µg and 100 µg/ml showed effective inhibition against liver cancer cell lines. The extract was active against liver cancer cell lines. Increased percentage of Cell line inhibition by suppressing viability was observed from Figure 1 and 2 that a gradually increase in percentage in all the treatments. However, at 100 µg/ml of tested extract sample and doxorubicin shows 34.69±1.13 and 44.92±1.33µg/ml cell inhibition against liver cancer cell lines.

The aqueous extract showed pronounced anticancer activity compared than standard (Figure 1 and 2). Aqueous extract inhibits the cell growth by up to 98.82%. The aqueous extracts show higher inhibition activity and this may be due to the greater stability of the water-soluble active phytochemicals present in the aqueous over a longer time.

In this study, the anticancer activity of aqueous extract of *P. amboinicus* leaves against liver cancer cell lines in invitro condition. The extract shows significant activity compared withcommercial drugs. Extracts of *P. amboinicus*reduce the risk of cancer due to the presence of flavonoids. Similarly, the results of this study are in accordance with these findings of Ponnanikajamidin et al., 2015 claimed that flavonoids would induce apoptosis by DNA fragmentation, nuclear condensation, and cell shrinkage.

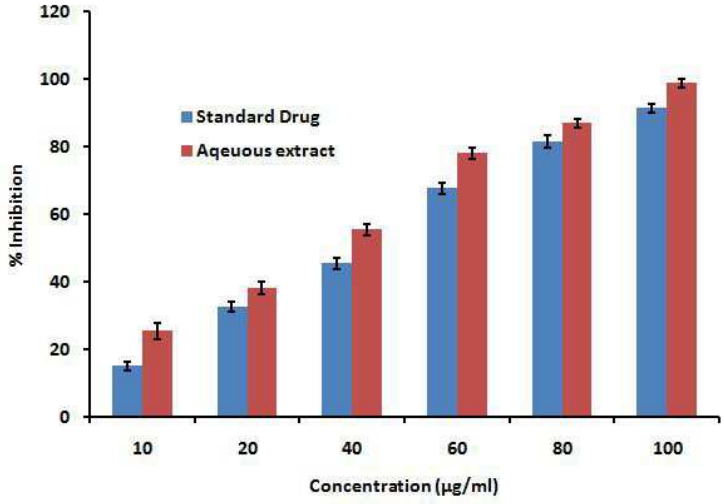

Figure 1: Anticancer Activity of Aqueous Extract of P. Amboinicus Leaves

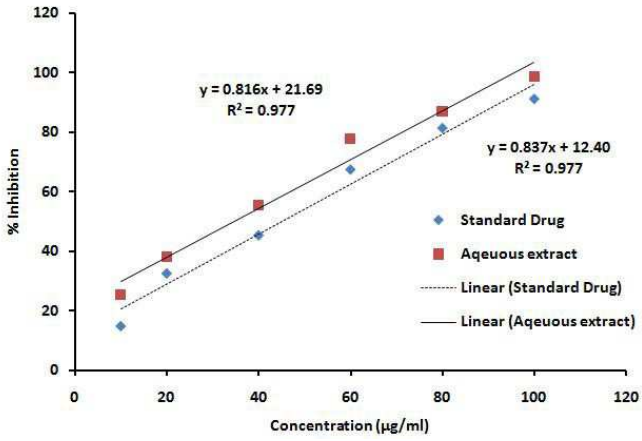

Figure 2: Linear Regression of Anticancer Activity of Aqueous Extract of P. Amboinicus Leaves

Anticancer Activity of Karpooravalli Extract

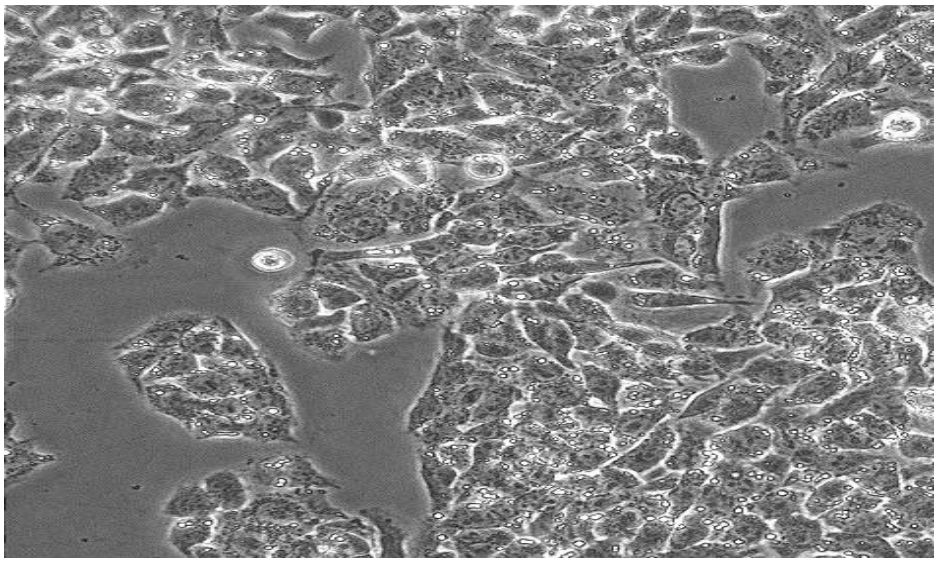

Figure 3: Liver Cancer Cell Line (Normal)

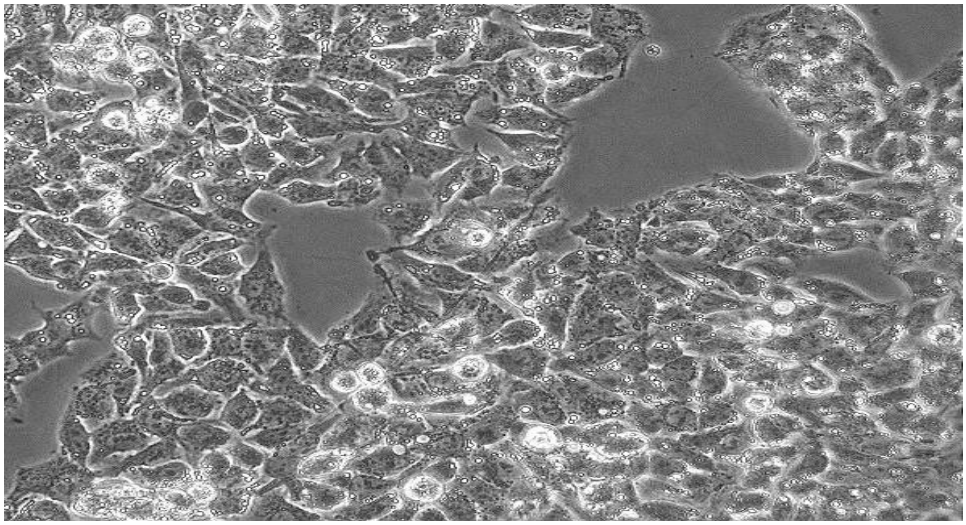

Figure 4: Anticancer Activity of Karpooravalli (Treated)

Table 1: Anticancer Activity of Aqueous Extract of P. Amboinicus Leaves

Concentration (µg/ml)	% Inhibition	
	Standard Drug	Aqueous Extract
10	15.06±1.34	25.55±2.33
20	32.75±1.52	38.33±1.85
40	45.58±1.74	55.51±1.59
60	67.63±1.64	78.05±1.64
80	81.54±1.81	87.06±1.25
100	91.33±1.33	98.82±1.44

CONCLUSIONS

Anticancer activity of aqueous extract of *P. amboinicus* leaves depends on the solvent used for extracting phytochemicals which present in the leaves. The aqueous extract shows more inhibition of cells due to the presence of

alkaloids and flavonoids. Minimum inhibitory concentration was observed based on the percentage of cell inhibition is 50% at 40 µg/ ml against liver cancer cell line (Hep G2). Based on this result, an aqueous extract of *P. amboinicus* leaves potentially to be developed as herbal medicine which replaces the chemotherapeutic agent against free radicals and cancer cells.

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