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P Abida
PG & Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India

A John De Britto
PG & Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India

Joshy Antoney
PG & Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India

T Leon Stephan Raj
PG & Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India

Correspondence
T Leon Stephan Raj
PG & Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India

Evaluation of *in vitro* anticancer activity of *Symplocos cochinchinensis* (Lour.) S. Moore bark

P Abida, A John De Britto, Joshy Antoney and T Leon Stephan Raj

Abstract
Symplocos cochinchinensis (Lour.) S. Moore is used in Indian system of traditional medicine to treat various kinds of diseases. Among the *Symplocos* species *Symplocos cochinchinensis* is very important species which is otherwise known as kabli-vetti or Lodh tree is widely distributed in tropical, subtropical areas in Asia, and America. The present study aims to evaluate the anticancer activity of *Symplocos cochinchinensis* bark. The plant material was collected from Wayanad district of Kerala and extracted with methanol. Three different cell lines (U87, Hep G2 and MCF7) used to investigate the anticancer potential of the plant. The result suggested that the anticancer potential of the bark was increased when the concentration of the plant material increases. Among the three cell lines U87 have the more activity. Further research required to isolate the pure compound from the bark in future for the preparation of the drug.

Keywords: Anticancer activity, Medicinal plant, *Symplocos cochinchinensis*, Cancer Cell lines

1. Introduction
Medicinal plants are used in India from prehistoric time. Most of the Indian Traditional medicinal systems like Siddha, Ayurveda and Unani mainly depending upon the medicinal plants. The medicinal practitioners of India used the plant source to formulate and dispense their own recipes [1]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [1]. Globally cancer is one of the threatening diseases of the human population. The disease is characterised by cells in the human body continually multiplying with the inability to be controlled or stopped. Consequently, forming tumours of malignant cells with the potential to be metastatic [2]. Recently the following treatments like chemotherapy, radiotherapy and chemically derived drugs are used to control the disease. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer [3]. The plant based drugs should not cause any side effects if it is used to treat the diseases. So many plant based anticancer drug preparation research is going on. Medicinal plants constitute a common alternative for cancer treatment in many countries around the world [4, 5]. Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products [6] and the plant kingdom has been the most significant source. These include *Vinca* alkaloids, *Taxus* diterpenes, *Camptotheca* alkaloids, and *Podophyllum* lignans. Currently, of 16 new plant-derived compounds being tested in clinical trials, 13 are in phase I or II and three are in phase III [7]. Among these compounds, flavopiridol isolated from the Indian tree *Dysoxylum binectariferum* and meisoindigo isolated from the Chinese plant *Indigofera tinctoria*, have been shown to exhibit anticancer effects with lesser toxicity than conventional drugs [7].
Symplocos cochinchinensis (Lour.) S. Moore. (SC) from the family Symplocaceae, is a medicinal plant with anti-inflammatory, antitumor, antimicrobial and anti-diabetic properties [8, 9]. The genus *Symplocos* comprises of 300-500 species of the *Symplocaceae* family is traditionally used to for the treatment of diarrhoea, dysentery, eye diseases, hemorrhagic gingivitis, uterine disorders, menorrhagia [10], bowel complaints, ulcers [11], snake bites, malaria, tumefaction and enteritis [12]. With the background of the medicinal properties of the *Symplocos cochinchinensis*, the present investigation aimed to evaluate the anti-cancerous potential of the methanolic bark extract against three selected cancer cell lines.

2. Materials and Methods

The bark of the *Symplocos cochinchinensis* was collected from Wayanad district of Kerala and the plant material was identified by the experts at M. S. Swaminathan Research Foundation and also by literature survey.

2.1. Preparation of plant extract

The collected bark of the *Symplocos cochinchinensis* was cleaned and shade dried for a week. 10 gm of pulverized material was mixed with 100 ml of methanol and kept in a rotary shaker at 100 rpm overnight and filtered with Whatman no. 1 paper and concentrated to dryness at 40 °C, lyophilized and stored at 4 e2°C until further use. Different concentrations of the methanolic extracts (0.4, 2, 10, 50 and 250 µg/ml) were prepared in 0.5% DMSO for determining cytotoxicity.

2.2. Cell line and culture condition

U87 (brain cancer cell line), Hep G2 (human liver cancer cell line) and MCF7 (breast cancer cell line) were used for the *in-vitro* cytotoxicity studies. The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

2.3. *In vitro* assay for Cytotoxicity studies (MTT assay)

The Cytotoxicity of samples U87 (brain cancer cell line), Hep G2 (human liver cancer cell line) and MCF7 (breast cancer cell line) were determined by the MTT assay [13-16]. Cells (1×10^6 /well) were plated in 1ml of medium/well in 24-well plates. After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations (0.4 µg/ml, 2 µg/ml, 10 µg/ml, 50 µg/ml and 250 µg/ml) of the bark extract of *Symplocos cochinchinensis* in 0.5% DMSO for 48h at 37 °C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. MTT assay is a quantitative colorimetric assay for measuring cellular growth, cell survival and cell proliferation based on the ability of living cells. The assay was carried out using (3-(4, 5-dimethyl thiazol-2yl) - 2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity [17-20]. Triplicate analysis of *in vitro* cytotoxicity bark extracts of *Symplocos cochinchinensis* was carried out with various concentrations. The effect of the samples on the proliferation of cell lines were expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells/A570 of control cells × 100%.

3. Results and Discussion

In the present study, anti-cancerous property of selected medicinal plant *Symplocos cochinchinensis* was carried. For that various concentration of bark extract was used against three cancer cell lines U87 (brain cancer cell line), Hep G2 (human liver cancer cell line) and MCF7 (breast cancer cell line). The bark of the plant was collected from Wayanad district of Kerala and shade dried, powdered and extracted with methanol solvent. Five different concentrations (0.4 µg/ml, 2 µg/ml, 10 µg/ml, 50 µg/ml and 250 µg/ml) of bark extracts were used to study the anticancer potential of the plant. The percentage of growth inhibition of various concentrations of *Symplocos cochinchinensis* against selected cell lines were displayed in Table 1 and figure 1. Among the selected three cell lines, U87 showed the more activity followed by MCF7 and HepG2. The results suggested that the anticancerous potential of the *Symplocos cochinchinensis* was increase when the cell lines treated with high concentration. The U87 cell line exhibited the highest percentage (99.2± 1.93) of cell growth inhibition against high concentration (250 µg/ml) of plant extract. But the HepG2 cell line showed very less growth inhibition (5.75± 2.11) in the lowest concentration (0.4µg/ml). Ethyl acetate and chloroform extract of *Symplocos racemosa* exhibited cytotoxicity against human hepatocellular carcinoma (Hep3B) cells *in vitro* withIC_{50} value (µg/ml) of 63.45 and 75.55 respectively and not affected the normal liver (BRL-3A) cells [21]. The plant exhibited the potent anticancer activity due to the presence of many phytochemicals present in the plant. Lakshmi and Vadivu [22] reported that the methanolic extract of leaf showed the presence of carbohydrates, flavonoids, phenols, saponins, tannins, proteins, glycosides and alkaloids. In *in vitro* cytotoxic study, both ethyl acetate and methanol extracts showed the activity, but methanol extract showed significant cytotoxic effect than ethyl acetate extract. Methanol extract exhibits greater cytotoxic effect against colon cancer cell lines SW 620 and HepG2 (liver cancer cell) with a GI50 value of 20 mcg/ml. So the present study also revealed that the methanolic extract of bark showed the maximum activity against U87 (brain cancer cell line), Hep G2 (human liver cancer cell line) and MCF7 (breast cancer cell line).

Table 1: Anti-cancerous potential of various concentrations of *Symplocos cochinchinensis* bark extract against three cell lines.

Cell Lines	0.4 µg/ml	2 µg/ml	10 µg/ml	50 µg/ml	250 µg/ml
U87	39± 2.89	41.7± 1.98	65.0± 2.75	78.4±1.06	99.2± 1.93
HepG2	5.75± 2.11	6.8± 2.01	33.7± 3.07	41.3± 2.89	89.7± 1.96
MCF7	10.1± 2.43	38.2± 3.02	48.7±1.56	75.8± 2.92	91.0 ± 1.96

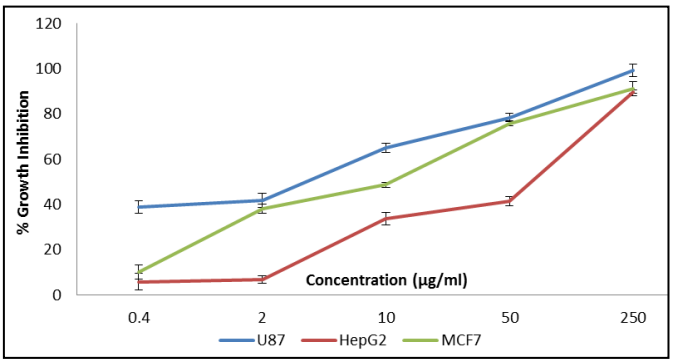

Fig 1: Percentage of cell growth inhibition of various concentrations of *Symplocos cochinchinensis* bark extract against three cell lines.

4. Conclusion

In the present study *in vitro* anticancer activity of *Symplocos cochinchinensis* exhibited potent activity against three cell lines like U87, Hep G2 and MCF7. The pure compound of the bark should be isolated to design the drug against cancer diseases in pharmaceutical industries. So the current research also helpful to find out the solution to cure the cancer disease by alternate method over the conventional method.

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