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Short Communication

EVALUATION OF *IN VITRO* ANTICANCER ACTIVITY OF S*YMPLOCOS RACEMOSA* BARK AGAINST HEPATOCELLULAR CARCINOMA

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ABSTRACT

Objective: To investigate *in vitro* anticancer activity of different extracts of bark of *Symplocos racemosa* against hepatocellular carcinoma.

Methods: Different successive extracts of *Symplocos racemosa* bark were prepared using hexane, chloroform, ethyl acetate, n-butanol and water and were tested *in vitro* for cytotoxicity using (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay in rat normal liver cells (BRL-3A) and human hepatocellular carcinoma (Hep3B) cells.

Results: Ethyl acetate and chloroform extract of *Symplocos racemosa* exhibited cytotoxicity against human hepatocellular carcinoma (Hep3B) cells *in vitro* with IC_{50} value (µg/ml) of 63.45 and 75.55 respectively and not affected the normal liver (BRL-3A) cells.

Conclusion: S*ymplocos racemosa* bark extracts showed potential cytotoxic effects on human hepatocellular carcinoma cells. The anticancer activity exhibited by ethyl acetate and chloroform extract might be due to presence of phenolics and flavonoid constituents present in the bark. Ethyl acetate extract can further be explored for possible cytotoxic activity using *in vivo* models of liver cancer.

Keywords: S*ymplocos racemosa*, Hepatocellular carcinoma, Cytotoxicity, Hep3B, MTT assay.

Malignant tumors are the second leading cause of death and hepato cellluar carcinoma is the one of the world's third deadliest cancer with very high morbidity and mortality rates and poor prognosis. Considering the continuing need for effective anticancer agents, medicinal plants play an inexhaustible source of anticancer agents in term of both variety and mechanism of action [1, 2]. Epidemiological studies revealed that consumption of food and beverages rich in polyphenols is associated with low risk of cancer due to presence of various phyto constituents. Over 50% of anticancer drugs approved by United States Food and Drug Administration have been originated from natural resources especially from terrestrial plants [3, 4].

Symplocos racemosa Roxb. belongs to a unigeneric family Symplocaceae and is a small evergreen tree with the height of 6 to 8.5 m and diameter of 15 cm. It is found commonly in the plains and hills of northern India and other Asian countries up to a height of 1400 m. The bark of *S. racemosa* has many glycosides, terpenoids and flavonoids with various pharmacological effects. Ethnobotanical literature indicates the utility of the bark in liver, uterine and bowel complaints such as diarrhea, dysentery and dropsy. It is also used in skin diseases, ear & eye disease, vaginal and menstrual disorders, tumors, fever, ulcers and scorpion string bite [5-11]. In Ayurveda pittaja arbuda and medoja arbuda tumors are reported to be treated with bark of *S. racemosa* in combination with other drugs [12-13]. These references provide the traditional backbone to our preliminary objectives to screen the cytotoxic potential of bark of *S. racemosa*.

Dulbecco's Modified Eagle medium (DMEM), Fetal bovine serum (FBS), RNase A, ethidium bromide, penicillin and streptomycin solution were purchased from Himedia laboratories, Mumbai, India. Trypsin and MTT were obtained from Sigma Aldrich, Bangalore (India). Dimethyl sulphoxide, other chemicals and solvents used in this study were of analytical grade and obtained from Merck Limited, Mumbai (India).

The dried bark material was purchased from the local market of district Neemuch (Madhya Pradesh, India) authenticated by Pharmanza Herbal Pvt. Ltd. Anand, India (PHPL/HB/060). The dried bark material was powdered (250g) and extracted with hexane, chloroform, ethyl acetate and n-butanol for 72 h using soxhlet apparatus and water extract was prepared using hot extraction method. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator whereas water extract has been evaporated and dried under the hot air oven and used for the further study.

BRL-3A (normal rat liver cell) and Hep3B (human hepatoma cell) cell lines have been obtained from the National Centre for Cell Science (NCCS) Pune. The cells were maintained in DMEM supplemented with 10 % FBS, penicillin (100 IU/ml), streptomycin (100µg/ml) and amphotericin-B (5µg/ml) in a humidified atmosphere of 5 % CO_2 at 37 °C.

Both normal and cancer cells were pre incubated at a concentration of 2×10^6 cells/ml in culture medium for 3 h at 37 °C and 6.5 % CO_2 and 75 % relative humidity. Cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and various concentrations of different extracts (1000 µg/ml-0.05 µg/ml) and standard were added into micro plates (96 flat bottomed tissue culture grade wells). Cell cultures were further incubated for 24 h at 37 °C and 6.5 % CO_2. The supernatants were removed and cell layers were washed with phosphate buffer saline and 10 µl of MTT labeling mixture, was added to that and incubated further for 4 h in a humidified atmosphere at 37 °C. 100 µl of DMSO was added to each well and then incubated for the period of overnight to dissolve the formazan crystals formed. Absorbance of the samples was measured using a microplate enzyme linked immunosorbent assay (ELISA) reader at wavelength 570 nm. Each extract and control was assayed in triplicate in three independent experiments. Cells were observed at different time intervals during incubation in the presence of the test item. The effect of different extracts and fractions of *S. racemosa* on the viability of normal liver and hepatoma cells were presented as the % of viability using following formula. Concentration that inhibits 50 % of cell growth was used as a parameter for cytotoxicity. IC_{50} values have been determined from plot of the dose response curve between log of compound concentration and percentage growth inhibition. IC_{50} value has been derived using curve fitting methods with Graph Pad Prism as statistical software (Ver. 5.02).

% viability = (A_{570} of treated cells–A_{570} of blank cells)/(A_{570} of controlled cells–A_{570} of blank cells) × 100.

Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula %cytotoxicity = 100–% cell viability [14].

In the present study, the cytotoxic effect of different extracts of *S. racemosa* bark was evaluated on human hepatoma cell (Hep3B) and normal liver cell (BRL-3A) compared to standard drug doxorubicin using MTT assay method after 24 h of treatment. The results revealed that amongst all extracts tested, ethyl acetate extract and chloroform extract showed potential effectiveness against Hep3B cell lines with IC_{50} value (µg/ml) of 63.45 and 75.55 respectively and not affected normal cells BRL-3A (>1000 µg/ml). Moderate effectiveness of n-butanol extract against Hep3B cell lines was also observed with IC_{50} 111.3µg/ml. Fig. 1 and 2 showed the graphical representation of concentration in µg/ml versus % cell inhibition of standard doxorubicin and five different extracts of *S. racemosa* against Hep3B and BRL-3A cell lines respectively using MTT assay. Table 1 represents the IC_{50} value of doxorubicin and five different extracts of *S. racemosa* bark against Hep3B cell line.

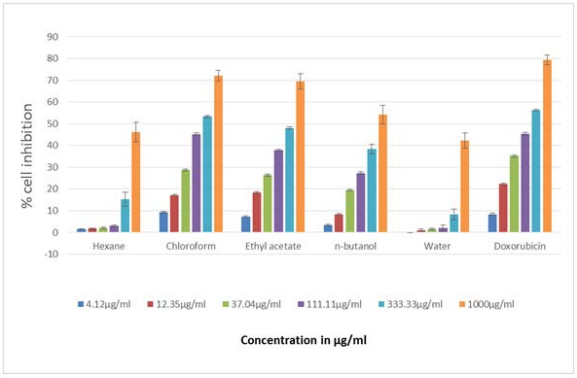
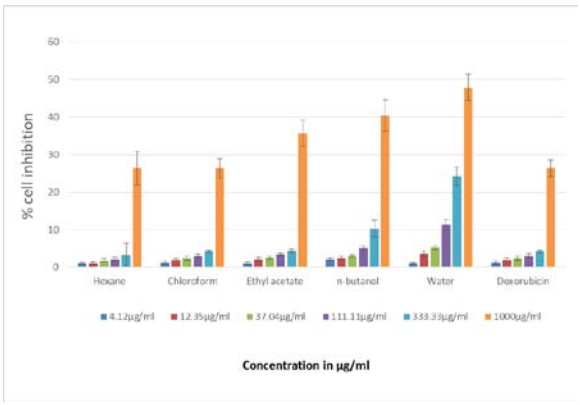


Fig. 1: It shows Graphical representation of concentration versus %cell inhibition of standard doxorubicin and five different extracts of *Symplocos racemosa* against Hep3B cell line

The results are shown as mean±SEM from three independent experiments



ig. 2: It shows Graphical representation of concentration versus % cell inhibition of standard doxorubicin and five different extracts of *Symplocos racemosa* against BRL-3A cell line

The results are shown as mean±SEM from three independent experiments

Different extracts of the bark exhibited diverse activity on cell lines and the observed selectivity is a result of sensitivity of the particular cell line to the active compounds present in the extract. Among the different extracts of *S. racemosa* bark, ethyl acetate and chloroform extract showed cytotoxic potential against human hepatocellular carcinoma cells, without affecting adversely to the normal cells. It may be due to the presence of phytochemicals such as phenolic glycosides, steroids, triterpenoids and flavonoids reportedly found present in the bark. In continuation to this research work, further investigations are ongoing for fractionation of bioactive extracts of the bark and identification of the possible phyto constituents responsible for the anticancer activity. This preliminary evaluation showing anticancer potential of this drug has provided a platform to establish more scientific claims to the traditional report of its use in cancer. The phytochemicals with prominent activity may serve as a novel therapeutic alternative in the treatment or prevention of hepatocellular carcinoma and deserved to be investigated further using more specific *in vivo* models of liver cancer.

Table 1: It shows IC_{50} value of doxorubicin and five different extracts of *S. racemosa* against Hep3B cell line

Name of extract/standard drug	IC_{50} value (µg/ml)
Hexane	1000
Chloroform	75.55
Ethyl acetate	63.45
n-butanol	111.3
Water	1000
Doxorubicin	55.63

IC_{50}: Concentration that reduces the mitochondrial activity by 50%. The results are shown as mean±SEM from three independent experiments

CONFLICT OF INTERESTS

Declared None

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