PHYTOPHARMACOLOGICAL INVESTIGATIONS OF BIOACTIVES FROM *Symplocos racemosa* BARK WITH SPECIFIC REFERENCE TO ANTICANCER ACTIVITY

ABSTRACT

Hepatocellular carcinoma (HCC) is the one of the world's commonest cancer, ranking third with very high morbidity and mortality rates with poor prognosis. With the increasing incidence of HCC globally, especially in China, Asia, Africa; therapeutic approaches for HCC are very limited currently. Therefore, there is an urgent quest for improvement of therapeutic activity and selectivity of anticancer agents or drug combinations from natural source with no or limited toxicity for developing cancer therapeutics. Considering the continuing need for effective anticancer agents, medicinal plants play inexhaustible source of anticancer drugs in term of both variety and mechanism of action. Over 50% of anticancer drugs approved by United states Food and Drug Administration, originated from natural resources, especially from terrestrial plants. *Symplocos racemosa* Roxb. belongs to a unigeneric family Symplocaceae; is a small evergreen tree; 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, steroids and flavonoids with various pharmacological effects. The ethnobotanical literature indicates the utility of the bark in liver complaints, bowel complaints such as diarrhoea, dysentery and dropsy, skin diseases, ear/eye disease, uterine complaints, vaginal and menstrual disorders, tumors, fever, ulcers and scorpion-string bite. In Ayurveda pittaja arbuda and medoja arbuda tumors are reported to be treated with bark of *S. racemosa* in combination with other drugs. These references provide the traditional backbone to our objectives to screen the chemoprevention and cytotoxic potential of bark of *S. racemosa*. So the aim of the study is to evaluate the anticancer potential of bark of *S. racemosa* against the hepatocellular carcinoma using *in vitro* and *in vivo* method.

Symplocos racemosa barks have been procured and evaluated according to Ayurvedic Pharmacopoeia of India. The fourteen different extracts of *S. racemosa* bark have been tested for *in vitro* cytotoxicity assay (MTT assay) on human hepatocellular carcinoma (Hep3B) cells. The result revealed that ethyl acetate soluble fraction of methanol extract (ESME) showed the highest cytotoxic effect (IC_{50} value 32.55 µg/ml) as compared to standard doxorubicin (IC_{50} value 55.43 µg/ml). Bioactivity guided isolation has been performed to investigate the responsible phytoconstituents for the cytotoxic effect. ESME loaded on the glass column and eluted with

hexane and different proportion of ethyl acetate. Various fractions of different volumes were collected and checked for the presence of phytoconstituents using the chromatography. Various eluted fractions have been evaluated using *in vitro* cytotoxicity assay (MTT assay) on human hepatocellular carcinoma (Hep3B) cells and rat liver cells (BRL-3A). The fractions eluted with hexane and ethyl acetate (85:15, 80:20, 70:30) showed the potent cytotoxic effect against Hep3B cells whereas found to be noncytotoxic to normal rat liver cells. The results revealed that the ESME showed the presence of β-sitosterol, stigmasterol, hexadecanoic acid and ursolic acid. The ESME, stigmasterol and ursolic acid have been evaluated using rat hepatoma cells (H4TG) induced experimental HCC *in vivo* rat model after obtaining Institutional Animal Ethical committee approval (Registration number: ARL/PT/019/2014). The results revealed that all the bioactives showed the significant chemo preventive and cytotoxic effects as compared with standard drug 5-FU. The methanol extract, ESME, stigmasterol and ursolic acid have been tested for the antioxidant activity using DPPH free radical and H_2O_2 scavenging assay methods and for the apoptosis effect using DNA fragmentation assay using gel electrophoresis. The results revealed that the samples showed the significant antioxidant activity, defined DNA fragmentation pattern and exhibited the apoptosis effect. Taken together, the potential anticancer activity of bark of *S. racemosa* against hepatic cancer *in vitro* and *in vivo* and its partial molecular mechanisms of activities were investigated in this experimental study first time. The results demonstrated that the bark of *S. racemosa* has a powerful chemo preventive and anticancer activity against human hepatocellular carcinoma and inhibit the hepatoma growth *in vivo* through the apoptotic and antioxidant effects without significant effect on normal cells. H4TG induced hepatocellular carcinoma in the rat model has been first time developed and evaluated; it may be applied for the evaluation of chemotherapeutics agents further. These bioactives may be investigated further for the pharmacokinetic and pharmacodynamic parameters, may act as lead compounds for the treatment of hepatocellular carcinoma, and deserve to be investigated further to come in to clinical practice after proper safety assessment.

Keywords: S*ymplocos racemosa*, Hepatocellular carcinoma, Cytotoxicity, Apoptosis