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PHYTOCHEMICAL ANALYSIS & IN VITRO ANTICANCER ACTIVITY OF METHANOL EXTRACT OF DECALEPIS HAMILTONII ROOT AGAINST HEPATIC CANCER CELL LINES (HepG2)

Devi Manickam* and Preetha D.

Department of Biochemistry, Muthayammal College of Arts & Science, Rasipuram – 637408, Namakkal Dist, Tamil Nadu, India.

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***Correspondence for Author**
Dr. Devi Manickam
Department of Biochemistry, Muthayammal College of Arts & Science, Rasipuram – 637408, Namakkal Dist, Tamil Nadu, India.

ABSTRACT

Hepatocellular carcinoma is the third leading cancer causing death Worldwide. Currently research focuses on the use of medicinal plants for the treatment of cancer, due to its resistance to chemotherapeutic agents. The present study was designed to evaluate the preliminary phytochemical analysis and in vitro anticancer activity of methanol extract of Decalepis hamiltonii root against hepatic cancer cells (HepG2). Various concentrations (125, 250, 500, 750 & 1000µg/ml) of methanol extract of Decalepis hamiltonii was taken for cytotoxicity assay using MTT assay and calculated the percentage of cell viability. The phytochemical analysis revealed the presence of flavonoids, saponins, tannins, steroids, cardiac glycosides, phenols, resins and thiols. The methanol extract of Decalepis hamiltonii showed therapeutic values against HepG2 cell line with CTC_{50} values 476.67±1.3 respectively. 1000µg/ml of Decalepis hamiltonii has maximum (76.86±1.4) cytotoxicity effect against HepG2. The result of our study revealed that the methanol extract of root of Decalepis hamiltonii has a cytotoxic effect on HepG2 cell line in a concentration dependent manner. The isolation of pure compounds and determination of the bioactivity of individual compounds will be further performed.

KEYWORDS: Decalepis hamiltonii, Phytochemical analysis, In vitro anticancer activity, Hepatic cancer cells (HepG2).

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality in India and fifth commonest cancer Worldwide.[1] Hepatocellular carcinoma (HCC) is the lethal and common malignancies in the human population, with approximately 550 000 new cases and many deaths occur due to HCC per year.[2] The incidence of hepatocellular carcinoma differs depending on aspects such as geographic location, sex, age, race and ethnicity, environmental exposure to certain agents, as well as presence of other risk factors.[3] Hepatocellular carcinoma most commonly occurs in the presence of cirrhosis as a result of long standing chronic liver disease.[4] HCC is particularly attributed to these exposures due to the extensive oxidative stress and release of inflammatory cytokines induced by viral infection in the setting of liver inflammation. Diabetes, obesity, smoking and alcohol abuse have also been associated with the development of HCC, but with reduced frequency.[5] There has been urgent need for the treatment of HCC.

According to World health Organization, 80% of people living in rural areas depend on medicinal herbs as primary health care system. Herbal medicines have a vital role in the prevention, treatment of cancer and medicinal herbs are commonly available.[6] Use of plants for medicinal remedies is an integral part of the Indian cultural life. Many traditional healers and herbalists in India have been treating cancer patients for many years using various medicinal plant species.[7] Hence, an attempt has been made to screen some medicinal plants used for the prevention and treatment of cancer in India. It is generally known that ethnomedical data provide substantially increased chance of finding active plants relative to random approach.[8] Herbal compounds are known to play a major impact in all the stages of HCC. Therefore, there has been an increase in the research for the use of plant derived compounds as potential anticancer agents against HCC for a novel drug development.[9,10]

This study aimed to evaluate the preliminary phytochemical analysis and in vitro anticancer activity of methanol extract of Decalepis hamiltonii root against hepatic cancer cells (HepG2).

MATERIALS AND METHODS

Specimen collection

The Decalepis hamiltonii root for the present study was collected from local market, Chennai, Tamil Nadu, India. Identification and authentication of roots were done at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India.

Preparation of extract

The root of Decalepis hamiltonii were air dried under shade and powdered to 40 meshes coarse powder. The powder was extracted with petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol, ethanol and distilled water by using soxhlet apparatus. The residue was filtered and the solvent were evaporated under reduced pressure and stored for further studies.

Phytochemical screening for secondary metabolites

The qualitative phytochemical analysis of various extracts of Decalepis hamiltonii root were carried out for the presence of alkaloids, flavonoids, saponins, steroids, glycosides, phenols, thiols and resins by using method adopted in similar surveys.[11]

In vitro cytotoxicity of methanol extract of Decalepis hamiltonii against HepG2.

Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT assay

Principle

The ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.[12]

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the

partial monolayer in microtitre plates. The plates were then incubated at 37º C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37º C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm.

The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of Individual group}}{\text{Mean OD of Control group}} \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of phytochemical screening of the roots of Decalepis hamiltonii were presented in Table 1. Phytochemical analysis showed the presence of tannins, saponins, steroids, flavonoids, phenols, resins, thiols, cardiac glycosides and carbohydrates.

Table 1: Phytochemical screening for various extracts of root of Decalepis hamiltonii

Secondary metabolites	PE	B	C	E	A	M	Et	Aq
Alkaloids	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	+	++	+++	++	-
Glycosides	+	-	-	+	+	+	+	-
Steroids	+	+	+	+	+	++	+	-
Saponins	+	-	-	-	+	+	-	-
Tannins	+	-	-	+	+++	+++	++	-
Phenols	+	-	-	+	+	+	-	-
Resins	-	-	-	+	+	+	-	-
Thiols	-	-	-	+	-	+	-	-
Carbohydrates	++	-	+	+	+	++	++	-

(PE – Petroleum ether, B – Benzene, C – Chloroform, E – Ethyl acetate, A – Acetone, M – Methanol, E – Ethanol, Aq – Aqueous)

The screening of methanol extract were found to possess all secondary metabolites except alkaloids such as flavonoids, steroids, glycosides, saponins, tannins, phenols, resins, thiols and carbohydrates. The aqueous extract does not contain any secondary metabolites. The

petroleum ether extract contain glycosides, steroids, tannins, phenols and carbohydrates. Steroids alone present in the benzene extract. Chloroform extract contains only steroids and carbohydrates. The acetone extract contains all metabolites except alkaloids and thiols. Saponins and alkaloids were absent in ethyl acetate extract. Ethanol extract found to contain flavonoids, steroids, glycosides, tannins and carbohydrates. Alkaloids were found to be absent in all the extracts.

Cytotoxic properties of Decalepis hamiltonii against HepG2 cell line

The result of our study revealed that methanol extract of root of Decalepis hamiltonii has a cytotoxic effect on HepG2 cell lines in a concentration dependent manner. The extract showed moderate therapeutic values against Hep G2 cell line with CTC_{50} values 476.67±1.3 respectively. Morphological studies also confirmed that the methanol extract of Decalepis hamiltonii root has got potential cytotoxic effect (Table 2 & Figure 1).

Table 2: Cytotoxic properties of Decalepis hamiltonii against HepG2 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	1000	76.86±1.4	476.67±1.3
	750	54.81±1.4	
	500	52.48±0.6	
	250	26.14±2.2	
	125	16.48±1.2	

The medicinal value of the plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds.[13]

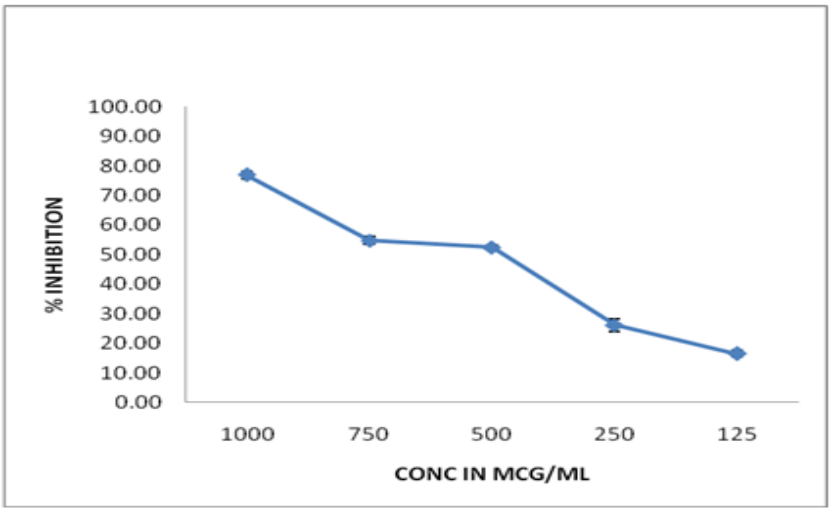

Figure 1: Cytotoxic properties of Decalepis hamiltonii against HepG2 cell line

Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells, whereas the rest may damage the healthy cells and tissues. The use of medicinal plants for cancer treatment has been increasing due to its availability, affordability and relatively lesser side effects when compared to the commercially available chemotherapeutic agents.[14,15] Plants contain several phytochemicals, which possess strong antioxidant activities. The antioxidants may prevent and cure cancer and other diseases by protecting the cells from damage caused by 'free radicals' – the highly reactive oxygen compounds.[16] Many plant-derived products have been reported to exhibit potent antitumour activity against human cancer cell lines.[17] Plant derived natural products such as flavonoids, terpenes, alkaloids etc. have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects.[18]

CONCLUSION

The present study was formulated to understand the phytochemical analysis and *in vitro* anticancer properties of Decalepis hamiltonii roots. In this study the phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, steroids, phenols, tannins, and fixed oil and fats. The study of cytotoxicity assay indicated the potential of methanol extract of Decalepis hamiltonii root which could be a source of anticancer therapeutic agent against HepG2 cell line. Thus, our results show that the methanol extract of Decalepis hamiltonii root possess good potential for use as cancer chemotherapeutic agent.

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