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INVITRO CYTOTOXIC ACTIVITY OF LEAVES OF HIBISCUS SABDARIFFA L. AGAINST HEPG$_2$ CELL LINE

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ABSTRACT

The aim of the present study was to evaluate *in vitro* cytotoxic activity of ethanolic extract of leaves of *Hibiscus sabdariffa* on HepG$_2$ cell lines. Qualitative phytochemical screening tests were performed to detect phytochemicals in the extract. The cytotoxic activity of the extracts of *Hibiscus sabdariffa* on HepG$_2$ cells was investigated *in vitro* through MTT assay. The results showed decreased cell viability and increased cell growth inhibition in a dose dependent manner. From the results it can be concluded that *Hibiscus sabdariffa* possess potential of bioactive components which are responsible for biological activities that is useful for liver cancer treatment.

KEYWORDS: *Hibiscus sabdariffa*, MTTassay, HepG$_2$, dose dependent.

INTRODUCTION

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by external factors, such as tobacco, infectious organisms and an unhealthy diet and internal factors, such as inherited genetic mutations, hormones and immune conditions. These factors may act together or in sequence to cause cancer. Ten or more years often pass between exposure to external factors and detectable cancer. Treatments include surgery, radiation, chemotherapy, hormone therapy, immune therapy and targeted therapy (drugs that specifically interfere with cancer cell growth).[1] Cancer is one of the most common leading causes of mortality worldwide. Cancer is an uncontrolled growth of cells resulting in lack of differentiation and ability to invade local tissues and metastasis which are proliferate

individually throughout the body. During metastasis, cancer cells enter the blood stream and are carried to distant parts of the body where they form other similar growths. Synthetic drugs are available for the treatment of cancer but they are not free from adverse effects. Chemotherapy and radiation therapy are major clinical treatment used for the control of early stages of tumor but these methods has serious side effects. Nature has provides human a variety of useful sources mainly plants for discovery and development of drugs against dreadful diseases. Traditional herb as an effective system of treatment of cancer and many diseases. Drugs from medicinal plants are found to be comparatively less toxic and side effects.[2]

Progress in medicinal plant research has undergone a phenomenal growth during last decade. Worldwide trend towards the use of natural plant remedies has created an enormous need for information about the properties and uses of medicinal plant as antitumor, antianalgesic, insecticides. Besides medicines, plants provides thousands of novel compounds, such as fragrance, flavorings, dyes, fibers, foods, beverages, etc. Roselle (*Hibiscus sabdariffa* L.) is known for delicacy and also for medicinal properties. It is a plant which is widely grown in Central and West Africa and South East Asia.[3] The approach of *H. sabdariffa*is equally significant in alternative system of medicine as well as in conventional system of medicine. *H. sabdariffa*is an aromatic, astringent, cooling herb that is currently used Tropical areas. It is known to have diuretic effects, to help lower fevers and is an antiscorbutic. The leaves are antiscorbutic, emollient, diuretic, refrigerant, and sedative. The plant is also reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, purgative and resolvent. It is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, and neurosis.[4-5] The present study was carried out to evaluate the phytochemical screening and cytotoxic activity of *Hibiscus sabdariffa* against $HepG_2$ cell line.

MATERIALS AND METHODS

Collection and Authentification of plant material

The leaves of *Hibiscus Sabdariffa* were collected from Orathanadu, Thanjavur District, Tamilnadu. The plant material was washed thoroughly 2-3 times with running tap water and once sterile with distilled water. Then the leaves were shade dried and easily powdered separately and stored in well closed bottles for further analysis. The plant was authenticated at the Rapinet Herbarium, St. Joseph's college, Thiruchirapalli. Tamil Nadu.

Preparation of Extract

For preparing extract, 10gm of the powdered sample was mixed with 100ml of ethanol and kept for 48hrs at room temperature to ensure maximum metabolite extraction. The extract obtained was filtered and concentrated. The extraction method is based on the solubility of the constituents of the *Hibiscus sabdariffa* sample in ethanol. The filtrate placed into the thimble of the soxhlet extraction apparatus chamber. The sample was extraction for 12 hours at 4 cycles per hour. After extraction, the solvent was removed by the means of a rotary evaporator, yielding the extracted compound.

Qualitative Phytochemical Screening

Preliminary Qualitative screenings were performed using standard procedure.[6-7]

***Invitro* Cytotoxicity Assay**

Cell lines

Cell lines Hepatic carcinoma ($HepG_2$ cell line) were used in this study and procured from National Centre for Cell Sciences, Pune, India.

Culture medium

The liver cancer cell line $HepG_2$ preserved in RPMI-1640 medium tissue culture flasks at $37^{\circ}C$ under a humidified 5% CO2 and 95% air.

Preparation of test solution

The ethanolic extract were separately dissolved in distilled DMSO and the volume was made up with medium supplemented with 2% inactivated FBS to obtain a solution of 1mg/ml concentration and sterilized by filtration. From this stock solution, five different lower dilutions (100, 200, 400, 800, 1000µg/ml) were prepared.

Cell viability assay[8]

MTT assay is a colorimetric assay that measures the reduction of yellow 3-(4, 5 dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. Cancer $HepG_2$ cells were seeded at the density of 2×10^5 cells/well were plated on into 6 well plated and treated with extract for 24 and 48h. The cells were permitted to adhere for 24 hours, and the growth medium (MEM) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBF. 1ml of medium (without FBF) containing different dilution of drugs was added in

respective wells; 200µl of MTT (5mg/ml in PBS) were added to each well, and the cells incubated for a further 6-7 hrs. in 5% CO2 incubator. After removal of the medium, 1ml of DMSO was added to each well. The effect of extracts on growth inhibition was assessed as present cell viability, where vehicle-treated cells were taken as 100% viable. The cells then exposed to with the medium alone (as positive control). Concentrations of the *H.sabadariffa* leaves extract ranging 50-250µg/ml were used. The supernatant was removed and 50µl of propanol was added and the plates were gently shaken to solubilize the formed formosan. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formosan product.

The cells are then solubilized with an organic solvent (e.g. Isopropanol) and the released, solubilised formosan reagent. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The cells were incubated with the extract for 24h and 48 h and the cell mortality were checked. The plates were placed on a shaker for 15min and the absorbance was read on an enzyme-linked immunosorbent assay (ELISA) reader at 570nm. Each experiment was carried out in triplicate and the half maximal inhibitory concentration (IC_{50}) of each extract as the percentage survival of the cells was calculates according to the formula provided below:

Viability (%) = [(Mean OD/Control OD)-1] x 100

Statistical Analysis

Results are expressed as Mean ± S.D.

RESULTS AND DISCUSSION

Cancer is one of the most dreaded of 20th century and spreading further continuously with increasing in 21th century. Ii is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, growth, local tissue invasion and distant metastases.[9]

Over the past few years, cancer has remained a major cause of the death and number of individual affected with cancer is continuing to expand. Hence a major portion of current pharmacological research is developed to anticancer drug design customized to fit new molecular targets.[10] Due to enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities.[11-12]

Qualitative phytochemical screening

The phytochemical screening of ethanolic extracts of *Hibiscus Sabdariffa* revealed the presences of secondary metabolites such as carbohydrate, saponins, terpeniods, phenol, steroids and coumarins (Table 1).

Table 1: Phytochemical screening of *Hibiscus Sabdariffa* leaves.

S.No	Phytochemicals	Result
1.	Carbohydrate	+
2.	Tannins	-
3.	Saponins	+
4.	Flavonoids	-
5.	Alkaloids	-
6.	Glycosides	-
7.	Terpinoids	+
8.	Phenol	+
9.	Steroids	+
10.	Coumarins	+

(+) indicates presence whereas, (-) indicates absence

Invitro cytotoxic activity of *Hibiscus sabdariffa*

The cytotoxic effects of ethanolic extract of *Hibiscus sabdariffa* on $HepG_2$ cells were performed by the MTT method, which is reliable to detect proliferation of cell. The results of MTT assay showed in Table 2. The results clearly conformed the expresser of ethanolic effects of *Hibiscus sabdariffa* at different concentrations such as 50, 100, 150, 200 and 250µg/ml for 48hrs, resulted in decrease cell viability in a dose dependent manner. The percentage of inhibitory concentration (IC_{50}) of cell proliferation was found to be initiate at the concentration of 168µg/ml of ethanol extract of *Hibiscus Sabdariffa* in $HepG_2$ cell line. Doxorubicin (100µg/ml) was used as a standard.

Table 2: In vitro cytotoxic activity of Hibiscus sabdariffa.

S.No	Treatment	Concentrations of leaves extract (µg/ml)	Absorbance 570 nm	% Cell viability	IC_{50} (µg/ml)
1	HSE treated	50	0.286	87.7±5.3	168
2		100	0.253	77.6±6.1	
3		150	0.226	69.3±4.4	
4		200	0.194	59.5±5.1	
5		250	0.119	36.5±3.5	
6	Doxorubicin	150	0.085	26. 0 ±1.8	

Interest in the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased dramatically over the past twenty years. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells.[13-14] Toxicity on Vero, $HepG_2$ and MCF-7 cells showed, in agreement with our previous studies.[15]

The biological activity of any phytocompounds depends on the type of chemical composition and the concentration of active constituents as well as types and developmental stages of the cancer.[16] The screening of plants for their anticancer properties used cell-based assays and established cell lines, in which the cytotoxic effects of plant extracts could be measured. MTT assay is a nonradioactive, fast and economical assay widely used to quantify cell viability and proliferation. MTT is a yellow water-soluble tetrazolium salt. Metabolically active cells are able to convert the dye to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring.[17] The result of study revealed that ethanol extract of GA and GU has a cytotoxic effect on human liver hepatocellular cells ($HepG_2$) cell line in a concentration-dependent manner.

CONCLUSION

From the results, it can be concluded that the *Hibiscus sabdariffa* leaf extract possess cytoprotective activity against $HepG_2$ cell lines. Further studies will be needed to carry out for the characterization of bioactive compounds responsible for the pharmacological activities.

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