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In Vitro Cytotoxicity Activity of Acteoside From Leucas Indica Flowers

KEYWORDS: Acteoside, Leucas indica, Cytotoxicity Activity, MTT Assay, MCF-7 cell line, Vero Cell Line

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ABSTRACT *The study was aimed to evaluate the in vitro cytotoxicity activity of acteoside isolated from the crude methanolic extract of Leucas indica flowers on the MCF-7 cell line. The acteoside was tested for its inhibitory effect on MCF-7 cell line. The in vitro cytotoxicity of acteoside on MCF-7 cell was evaluated by the MTT assay. Acteoside has significant cytotoxicity effect on MCF-7 cell line in concentration range between 1.0 µg/ml to 250 µg/ml by using MTT assay. IC50 value and R2 value of acteoside on MCF-7 cell and Vero cell were 7.7 and 0.9968, 249.7 and 0.9545 respectively by MTT assay. From the performed assay, acteoside shows greater activity on MCF-7 cell line and little activity on Vero cell line and these values indicates that acteoside from Leucas indica flowers extract shows significant cytotoxicy activity.*

INTRODUCTION

Plants have long history used in the treatment of cancer. Active constitutes of Catharanthus roseus, Angelica Gigas, Podophyllum peltatum, Taxus brevifolia, Podophyllum emodii, Ocrosia elliptica, and Campototheca acuminata have been used in the treatment of advanced stages of various malignancies (1). Leucas indica, Family - (Lamiacae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The flowers are given with honey to treat cough and cold in children. Traditionally, the whole plant is taken orally for analgesic, antipyretic, antirheumatic, anti-inflammatory and antibacterial treatment and its paste is applied topically to inflamed areas (2). The plant contains various phytochemical constituents mainly triterpenoids, Ursolic acid and β-sitosterol, Nicotine, Sterols, glucoside, diterpenes, oleanolic acid, ursolic acid, phenolic compounds [4-(24-hydroxy-1-oxo-5-n-propyltetracosantyl)-phenol] (3-9). The antioxidant activity of Leucas indica might be due to their phenolic compounds (10) and also Leucas indica having antioxidant, cytotoxity, and antinociceptive activity (11).

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation - 7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers. The Michigan Cancer Foundation is now known as the Barbara Ann Karmanos Cancer Institute.

Cancer is one of the most life-threatening diseases and causes serious health problems in both developed and developing countries. It is a group of diseases characterized by the deregulated proliferation of abnormal cells that invade and disrupt surrounding tissues (12). Therefore, investigations for finding new anticancer compounds are imperative and interesting. After taking into consideration the immense side effects of synthetic anticancer drugs, many researchers are making concerted efforts to find new and natural anticancer compounds. The screening of plant extracts has been of great interest to scientists in the search for new drugs for effective treatment of several diseases (13).

The aim of the present work was to evaluate the in vitro cytotoxic activity of acteoside isolated from Leucas indica flowers methanolic extract to support the pharmacological effects. Although numerous studies have shown the medicinal values of this plant, there still remains ample scope for further in depth research. Accordingly, we disclose herein the in vitro cytotoxic effects of acteoside from the flowers parts of Leucas indica to further establish the scientific basis of the traditional uses of this plant.

MATERIALS AND METHODS

MATERIALS

Cell line and culture

Human breast cancer MCF-7 cell line (GDC055) Human Adenocarcinoma cell lines were obtained from National centre for cell sciences Pune (NCCS). African green monkey kidney Normal cell line (Vero). The cells were maintained in RPMI-1640 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.

Reagents

RPMI-1640 was purchased from GIBCO/BRL Invitrogen (Caithershurg, MD). Fetal bovine serum (FBS) was purchased from Gibco laboratories Trypsin, methylthiazolyl diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

METHODS

Preparation of plant extracts

Accurately weighed 10 gms of Leucas indica flower powder was extracted with 250 ml methanol by stirring at 50°C for 3 hr. The extracts were then filtered through whatmann filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure.

Isolation of acteoside

Isolation of acteoside from crude methanolic extract of leucas indica flowers using preparative HPLC method.

Thumba (Leucas indica) as identified and authenticated by a Dr. P.N Sudha, Department of chemistry, Thiruvallur University and was collected in Nov 2013.

Cytotoxicity Assay

Micro culture tetrazolium (MTT) assay

Principle

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 (14-15).

Procedure

The in-vitro cytotoxicity of Leucas indica flower extracts on MCF-7 cell line was determined by the MTT assay (Mosmann et al., 1983). Cells (1 × 10^5/well) were plated in 100 µl of medium/well

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in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence then, cells were incubated in the presence of various concentrations of the extracts in 0.1% DMSO for 48 h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/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 was added. Viable cells were determined by the absorbance at 570 nm with reference at 655nm. Measurements were performed in 3 times, and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a micro plate reader using wells without sample containing cells as blanks. All experiments were performed in triplicate.

The percentage cell inhibition was calculated using following formula,

% cell inhibition= 100-{(At-Ab)/ (Ac- Ab)} x100
Where,
At= Absorbance value of test compound
Ab= Absorbance value of blank
Ac=Absorbance value of control

RESULTS

The effect of in-vitro cytotoxicity activity was carried out for acteoside isolated from crude methanolic extract of Leucas indica flowers. Leucas indica extract was screened for its cytotoxicity against MCF-7 and Vero cell lines at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay. Extract response curves constructed between the range of 1.0 µg/ml and 250 µg/ml for acteoside component, express decreasing number of viable cells with increasing concentration of extract. Calculation of IC_{50} and R^2 value was done using graphs generated from Microsoft excel 2003 edition. (Figure 1 and Figure 2). The susceptibility of cells to the extract exposure was characterized by IC_{50} values (Table 1). Results indicate that the antiproliferative effect strengthens with increase in the concentration of extract.

Determination of Cytotoxity by MTT assay

Results are tabulated in table 1 and graphically represented in Figure 1 and Figure 2. The percentage growth inhibition was found to be increasing with increasing concentration of test compounds, as shown in Figure 1. Acteoside effect on MCF-7 cell line and Vero cell line from 1.0 µg/ml to 250 µg/ml (Table 1 and Figure 1, 2) and that IC_{50} value on MCF-7 cell line was 7.7 µg/ml, while acteoside has significant action on Vero cell line, so its IC_{50} found to be 249.7 µg/ml. To be a good drug, the IC_{50} value of such agent should be sufficiently low to avoid any possible unspecific effects. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio guided studies, if it exerts an IC_{50} value $\leq$ 30 µg/ml (16). That means acteoside isolated from flowers extract has little effect on normal healthy body cell. If drug has more effect on Vero cell line that denotes it affects normal healthy body cell and turn out with side effect. While in case of acteoside superior result on MCF-7 cell but little effect on Vero cell. So it shows significant cytotoxicity activity.

Table 1: Determination of cytotoxicity by MTT assay

Component	Conc µg/ml	MCF-7 % Inhibition	IC_{50}	R^2	Vero % Inhibition	IC_{50}	R^2
Acteoside	250.00	76.10	7.7	0.9968	50.05	249.7	0.9545
	125.00	70.55			42.13		
	62.50	64.16			30.85		
	31.25	59.45			22.58		
	15.63	54.87			18.22		
	7.81	50.50			13.46		
	3.91	44.89			9.25		
	1.95	39.25			5.55		
	0.98	32.19			2.20		

Figure 1: ERC (Extract response curve) of Methanolic extract of Leucas indica for MCF-7 cell line by MTT assay

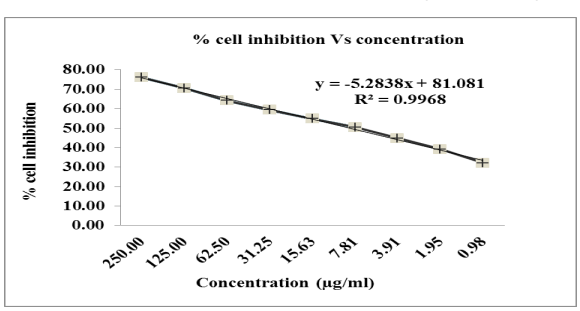
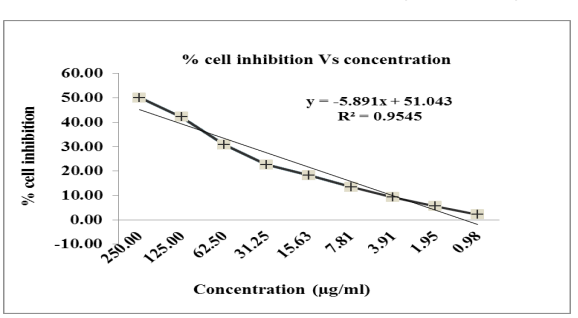


Figure 2: ERC (Extract response curve) of Methanolic extract of Leucas indica for Vero cell line by MTT assay



DISCUSSION

Plant based compounds have been playing an important role in the development of several clinically useful anti-cancer agents such as vinblastine, vincristine, camptothecin derivatives, topotecan, irinotecan, etoposide derived from epipodophyllotoxin and taxol (17). Our study indicates the scope of developing anticancer drugs from Leucas indica flowers. The cytotoxic effect of acteoside is principally contributed by the presence of phenolic group present in this compound.

CONCLUSION

The study demonstrates that acteoside isolated from crude methanolic extract of Leucas indica flowers has prominent cytotoxic effect to be used in many pharmacological as well as biological actions. However, further in-vivo toxicity studies on cell lines are also suggested to confirm this attribution.

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