Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 13 (2024), Article ID 236294, 05 pages
DOI: 10.4303/JDAR/236294

Research Article

Anticancer Effect against Pancreatic Cell Line and Leukemia Cell Line Study of *Scoparia dulcis*

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Received: 01 April 2024; Manuscript No: JDAR-24-132214; **Editor assigned:** 03 April 2024; PreQC No: JDAR-24-132214 (PQ); **Reviewed:** 17 April 2024; QC No: JDAR-24-132214; **Revised:** 22 April 2024; Manuscript No: JDAR-24-132214 (R); **Published:** 29 April 2024; **DOI:** 10.4303/JDAR/236294

Abstract

Cancer is a pathological state characterised by the uncontrolled proliferation and dissemination of abnormal cells originating from the body's own tissues. Evaluating *in-vitro* cytotoxicity has become a widely used screening method for determining whether natural sources have anticancer potential. Utilising 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, the MTT test was utilised to assess the anti-tumour efficaciousness of a hydroalcoholic extract obtained from *Salvia dulcis L.* Preliminary phytochemical analysis was also performed. To evaluate cell viability and inhibition, the MTT Assay was utilised. Cell viability decreased and growth inhibition increased in a concentration-dependent manner in the hydroalcoholic extract of *Scoparia dulcis L.* Additionally, it induced changes in cell morphology. *S. dulcis* IC50 values on Pancreatic and leukaemia cell lines were 104.56 g/ml and 73.51 g/ml, respectively, during a 24 hour incubation period, concentration range of 50 g to 150 g by MTT test. Doxorubicin was utilised as a standard control in the study. According to the findings, the hydroalcoholic extract of *S. dulcis* displays substantial anti-cancer effect on Pancreatic and leukaemia cell lines.

Keywords: *Scoparia dulcis*; *In-vitro* cytotoxicity; Soxhlet extraction; Cytotoxicity

Introduction

The most prevalent and fatal illness nowadays is cancer. The majority of currently used cancer medicines have side effects, and not all cancers respond to treatment in the same way. As a result, novel approaches or chemicals are required. The use of plant-based medications as the main source of chemotherapy treatments has good potential. Additionally, the advantage of third world nations still use herbal items as their main source of health treatment. Accordingly, examining for antitumor chemicals can be done by using plants as herbal remedies Nowadays, you may find *Scoparia dulcis L.*, a small plant of the *Plantaginaceae* family, as a scattered herb or subshrub in most tropical regions of the world. *S. dulcis* is employed in traditional medicine as well as a therapeutic agent for the management of gastrointestinal ailments, diabetes, hypertension, and other related conditions. Pharmacological research has shown that the hydroalcoholic extracts of this plant have several therapeutic properties, such as being effective against diabetes, inflammation, fever, microbes, tumours, liver damage, and pain [1-8].

Materials and Methods

Maintenance of cell lines

Acquired from NCCS in Pune, India, the MIA PA CA cell line is a human pancreatic adenocarcinoma. High-glucose DMEM medium was utilized to cultivate the cells, in which 10% FBS and 1% antibiotic-antimycotic solution were added as supplements. At a temperature of 37°C, the culture was maintained in a CO_2 incubator with an atmosphere containing 5% CO_2 and 18%-20% O_2. Every 2 days, the cells were sub cultured. The current investigation made use of Passage 38.

Acquired from NCCS in Pune, India, the THP-1 cell line is a human acute leukemia cell line. High-glucose DMEM medium was utilized to cultivate the cells, in which 10% FBS and 1% antibiotic-antimycotic solution were added as supplements. At a temperature of 37°C, the culture was maintained in a CO_2 incubator with an atmosphere containing 5% CO_2 and 18%-20% O_2. Every 2 days, the cells were sub cultured. The current investigation made use of Passage 38.

Plant material

S. dulcis (*Plantaginaceae*) was acquired from Sri Venkateswara University in Tirupati, Andhra Pradesh, following verification. These plants are maintained in the Guru Nanak Institutions Technical Campus herbarium, which is part of the School of Pharmacy (Figure 1).

Figure 1: *Scoparia dulcis L.*

Preparation of extract

The drug substance is placed on a filter paper shaped like a thimble, and then stored in a glass thimble for preservation. The thimble is equipped with both a syphon tube and an inlet tube. At the top, the cylinder encloses the condensed water. The complete assembly is placed into the flask's neck, which has a circular bottom and a hydroalcoholic solution (70:30) inside of it. The flask should be heated using either a water bath or a heating mantle. The vapours from the solvent travel up the inlet tube and condense on the thimble as they pass into the condenser. The condensed solvent comes into contact with the drug placed in the thimble and dissolves. The solution then reaches the top end of the syphon tube. This method ensures a consistent flow of solvent vapours is maintained in the thimble, allowing the dissolved drug to return to the flask. Finally, the heating process is halted, and the solution in the flask is carefully filtered in order to collect the desired extract (Figure 2).

Figure 2: Soxhlet extraction

Preliminary phytochemical screening

In order to identify multiple active ingredients, a qualitative chemical screening was conducted on the hydroalcoholic extract of *S. dulcis*. This involved performing various chemical tests [9-12].

MTT assay: To assess the cytotoxicity of a substance on 2 different cell lines. The sample details as shown in Table 1.

Table 1: Specifics of the study samples received

S. No.	Sample Name/ Code	Concentrations (µg/ ml)	Cell line
1	SD	5 (12.5,25,50,100 and 200)	MIA PA CA 2 and THP-1

The MTT test is a widely used colorimetric technique for evaluating cell growth and cytotoxicity. The function of this is to monitor the formation of formazan crystals through the reduction of MTT, which is a water-soluble tetrazolium dye with a yellow Colour. Liver cells generate mitochondrial lactate dehydrogenase, which converts MTT into insoluble formazan crystals. These crystals become purple when dissolved in an appropriate solvent. The number of living cells is directly proportional to the intensity of this color, which may be measured using a spectrophotometer at 570 nm.

Materials:

1. Cell lines: MIA PA CA 2–Human pancreatic adenocarcinoma cell line (From NCCS, Pune)
2. Cell lines: THP-1–Human acute leukemia cell line (From NCCS, Pune)
3. Cell culture medium: RPMI-1640 medium (Cat No: A10491, Gibco, Invitrogen)
4. Cell culture medium: DMEM high glucose medium-(#12430, Gibco, Invitrogen)
5. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
6. Fetal Bovine Serum (#RM10432, Himedia)
7. Antibiotic-Antimycotic solution (100x) (Cat No: 15240096, Gibco, Invitrogen)
8. MTT Reagent (5 mg/ml) (#4060 Himedia)
9. DMSO (#PHR1309, Sigma)
10. Doxorubicin (#D1515, Sigma)
11. DMSO (#TL1006, Himedia)
12. 96-well plate for culturing the cells (From Corning, USA)
13. T25 flask (#12556009, Biolite-Thermo)
14. 50 ml centrifuge tubes (#546043 TORSON)
15. 1.5 ml centrifuge tubes (TORSON)
16. 10 ml serological pipettes (TORSON)
17. 10 ul to 1000 ul tips (TORSON)

Equipment:

1. Centrifuge (Remi: R-8C).

2. Pipettes: 2 μl-10 μl, 10 μl-100 μl, and 100 μl-1000 μl.
3. Inverted biological microscope (CKX415F, Olympus, Japan)
4. 37°C incubator with 5% CO_2 humidified atmosphere (Healforce, China)
5. Microplate reader with 96 wells (ELX-800, BioTek, USA)

Assay controls:

i. Medium control refers to a control group in an experiment that does not include any cells.

ii. A negative control is a medium that contains cells but does not contain the chemical or substance being tested.

iii. The positive control comprises a cell-containing medium supplemented with a 1 uM/ml concentration of doxorubicin.

Steps followed: For MIA PA CA 2 cells

1. In the absence of the test agent, add 200 μl of a cell suspension containing 20,000 cells per well to each well of a 96-well plate. Allow an estimated 24 hours for the proliferation of the cells.
2. Add the test agent's specified concentrations as shown in the Excel sheet's findings section.
3. Place the plate in a controlled environment with a temperature of 37°C and a 5% concentration of carbon dioxide for a duration of 24 hours.
4. Remove the plates from the incubator after incubation. Discard the wasted medium and proceed to add MTT reagent to the entire volume. To achieve 0.5 mg/mL, adjust the concentration.
5. To prevent the plate from being exposed to light, it is advisable to wrap it with aluminium foil.
6. After 3 hours, return the dishes to the incubator and allow them to rest. (Note: Different cell lines require varying times for incubation. When doing comparisons within a single experiment, the incubation period should remain consistent).
7. Subsequent to eliminating the MTT reagent, introduce 100 μl of DMSO solubilization solution.
8. Gyratory shaking with gentle motion will promote dissolution. Occasionally, it may be necessary to perform pipetting movements in both upward and downward directions in order to fully dissolve the MTT formazan crystals in thick cultures.
9. At reference wavelengths of 570 nm and 630 nm, the absorbance should be measured with a spectrophotometer or an ELISA reader.
10. The formula is employed to determine the percentage of cell viability

$$\% \text{ cell viability} = [\text{OD of treated cells}/\text{OD of Untreated cells}] * 100$$

11. Y=Mx+C, a linear regression equation, was used to calculate the IC50 value. In this instance, Y had a value of 50, and the viability graph provided the values for M and C.

Steps followed: For THP-1 cells:

1. In the absence of the test agent, add 200 μl of a cell suspension containing 20,000 cells per well to each well of a 96-well plate. Allow an estimated 24 hours for the proliferation of the cells.
2. Add the test agent's specified concentrations as shown in the Excel sheet's findings section.
3. Place the plate in a controlled environment with a temperature of 37°C and a 5% concentration of carbon dioxide for a duration of 24 hours.
4. Remove the plates from the incubator after incubation. Discard the wasted medium and proceed to add MTT reagent to the entire volume. To achieve 0.5 mg/mL, adjust the concentration.
5. To prevent the plate from being exposed to light, it is advisable to wrap it with aluminium foil.
6. After 3 hours, return the dishes to the incubator and allow them to rest. (Note: Different cell lines require varying times for incubation. When doing comparisons within a single experiment, the incubation period should remain consistent).
7. Subsequent to eliminating the MTT reagent, introduce 100 μl of DMSO solubilization solution.
8. Gyratory shaking with gentle motion will promote dissolution. Occasionally, it may be necessary to perform pipetting movements in both upward and downward directions in order to fully dissolve the MTT formazan crystals in thick cultures.
9. At reference wavelengths of 570 nm and 630 nm, the absorbance should be measured with a spectrophotometer or an ELISA reader.
10. The formula is employed to determine the percentage of cell viability

$$\% \text{ cell viability} = [\text{OD of treated cells}/\text{OD of Untreated cells}] * 100$$

11. Y=Mx+C, a linear regression equation, was used to calculate the IC50 value. In this instance, Y had a value of 50, and the viability graph provided the values for M and C.

The concentrations utilized for the study: In this investigation, we evaluated the test chemical's cytotoxic effects on the MIA PA CA 2 and THP-1 cell lines. The chemical concentrations employed for cell therapy were as shown in Table 2.

Table 2: Specifics of the drug treatments administered to the cell lines utilized in the study

S. No.	Test	Cell Line	Concentration treated to cells
1	Untreated	MIA PA CA 2 and THP-1	No treatment
2	Std. Control	MIA PA CA 2 and THP-1	1 μM/ml
3	Blank	-	Only media without cells
4	SD2	MIA PA CA 2 and THP-1	12.5 μg/ml, 25 μg/ml, 50 μg/ml,100 μg/ml and 200 µg/ml

Results

Scoparia dulcis

After a 24-hour treatment period, the Table 3 displayed the % cell viability values and IC50 values of the supplied test drug against the Human pancreatic cancer (MIA PA CA 2) cell line and Human acute leukemia cell line (THP-1) and was depicted in bar graphs as shown below Figure 3.

Table 3: MTT assay-summary-cell viability values

Drug conc. (μg/ml)	MIA PA CA2	THP-1
Untreated	100	100
Dox-1 μM	39.37	47.14
SD-12.5 μg	89.51	89.89
SD-25 μg	79.13	75.92
SD-50 μg	67.6	62.25
SD-100 μg	51.46	46.62
SD-200 μg	14.26	21.45
IC50 Conc (μg/ml)	104.56	73.51

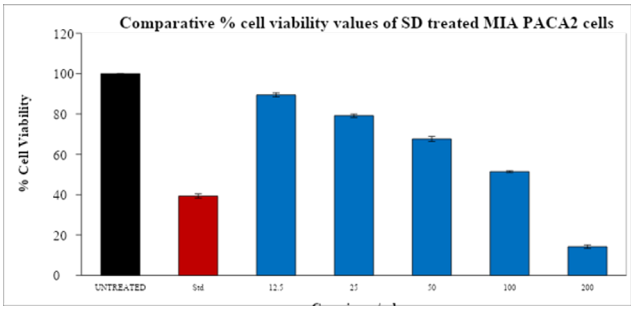

Figure 3: An overlay bar graph depicted the proportion of viable cells of MIA PA CA 2 cells treated with varied doses of SD2 after a 24-hour incubation period

After an incubation period of 24 hours, the superimposed bar graph illustrates the proportion of viable THP-1 cells that were exposed to various concentrations of SD2 (Figure 4).

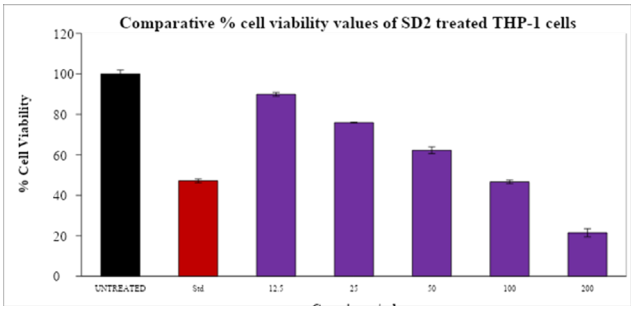

Figure 4: The proportion of viable THP-1 cells that were exposed to various concentrations of SD2

Figure 5 depicted the IC50 values of SD against the MIA PA CA 2 and THP-1.

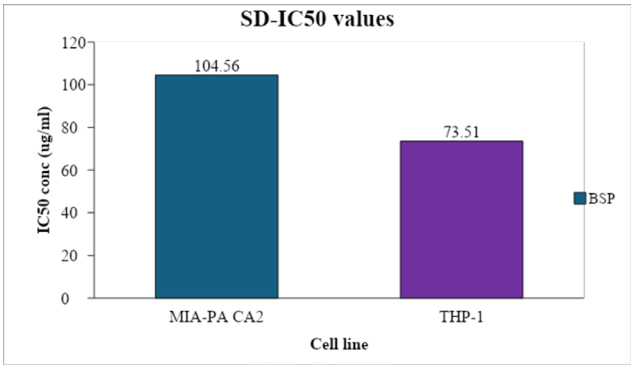

Figure 5: Comparative Bar graph depicted the IC50 values of SD against the MIA PA CA 2 and THP-1 cells after the incubation period of 24 hours

Discussion

Cytotoxicity study of the SD against MIA PA CA 2 and THP-1 cell line

With an IC50 value of 104.56 ug/ml, the test chemical SD2 showed moderate toxicity against MIA PA CA2 and THP-1 cells, according to statistical results from the cytotoxicity research utilising the MTT assay. Furthermore, after a 24-hour incubation period, the test compound showed effective toxicity with an IC50 value of 73.51 ug/ml. The study included doxorubicin as the standard control [13-17].

Microscopic examination of drug-treated pictures of the THP-1 cell line and MIA PA CA 2 obtained at a 20x magnification.

Conclusion

S. dulcis have effective potential capacity against human pancreatic cancer cells with IC50 value 104.56 μg/ml and effective toxicity on human acute leukemia cells with IC50 value of 73.51 μg/ml. *S. dulcis* have more cytotoxic activity against leukemia cell when compared with Pancreatic cancer cells.

Acknowledgement

None.

Conflict of Interest

The authors declared that they have no conflict of interest.

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