OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 02 - Issue 1 (2025)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

EXPLORING THE CYTOTOXIC POTENTIAL OF HYDROALCOHOLIC EXTRACT FROM OXYSTELMA ESCULENTUM AGAINST HUMAN CANCER CELL LINES

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Article Info

Abstract

Cancer is a leading cause of death worldwide, necessitating the discovery of novel therapeutic agents. Natural products, particularly medicinal plants, are increasingly being explored for their anticancer properties. Oxystelma esculentum, a plant traditionally used in Ayurvedic medicine, is known for its wide range of pharmacological effects. This study investigates the anticancer potential of a hydroalcoholic extract of Oxystelma esculentum against human cancer cell lines. The aerial parts of Oxystelma esculentum were extracted using a 70% ethanol solution, and cytotoxicity was evaluated using MTT and CellTiter-Glo assays. Cancer cells were treated with the extract at concentrations of 100 mg/mL, 250 mg/mL, and 500 mg/mL for 48 hours. The IC50 values were calculated using non-linear regression analysis. The extract demonstrated dose-dependent cytotoxicity in the cancer cell lines, with IC50 values of approximately 230 mg/mL. Cell viability decreased significantly, with a maximum inhibition of 85% at the highest concentration (500 mg/mL). The extract also showed significant ATP depletion in the CellTiter-Glo assay, indicating reduced cellular metabolism.

The results suggest that the hydroalcoholic extract of Oxystelma esculentum exhibits potent anticancer activity, likely due to its rich content of bioactive compounds such as flavonoids, saponins, and phenolic acids. These findings indicate that Oxystelma esculentum has the potential to be developed into a plant-based anticancer therapy. Further studies are warranted to elucidate the molecular mechanisms involved and to validate the efficacy of the extract in vivo.

Keywords:

Cancer, cytotoxic, cell lines, Oxystelma esculentum.

Introduction

Cancer remains one of the leading causes of mortality worldwide, with an estimated 19.3 million new cases and nearly 10 million deaths in 2020 alone (Sung et al., 2021). The high prevalence and mortality rate associated with cancer highlight the urgent need for effective therapeutic interventions. Despite advances in cancer treatment, including chemotherapy, radiation, and targeted therapies, the associated side effects and drug resistance continue to be significant challenges (Li et al., 2022). This has led to a growing interest in the search for natural compounds, particularly those derived from medicinal plants, as potential sources of novel anti-cancer agents (Zhao et al., 2023).

Historically, plants have played a crucial role in drug discovery, with many plant-derived compounds such as paclitaxel, vincristine, and camptothecin being successfully developed into chemotherapeutic agents (Cragg & Pezzuto, 2016). In recent years, researchers have increasingly focused on lesser-known medicinal plants for their anticancer potential. One such plant is *Oxystelma esculentum*, commonly known as "Muda Kathan," a member of the Asclepiadaceae family. Traditionally used in Ayurvedic medicine to treat various ailments, including respiratory issues and inflammation, recent studies have begun to explore its potential anticancer properties (Patil et al., 2022).

Oxystelma esculentum is rich in bioactive compounds such as flavonoids, alkaloids, saponins, and phenolic acids, all of which have been implicated in cancer prevention and treatment (Gautam et al., 2021). Flavonoids, in particular, have garnered attention due to their ability to induce apoptosis, inhibit cell proliferation, and suppress metastasis in various cancer cell lines (Gupta et al., 2022). These compounds are known to interact with key signalling pathways involved in cancer progression, such as the PI3K/Akt and MAPK pathways, leading to the inhibition of tumour growth and the induction of programmed cell death (Zhang et al., 2023).

Several studies have demonstrated the cytotoxic effects of plant extracts from the Asclepiadaceae family on cancer cells, making *Oxystelma esculentum* a promising candidate for further investigation (Ghosh et al., 2021). However, limited research has specifically explored the anticancer properties of *Oxystelma esculentum*, and there is a need for more comprehensive studies to evaluate its efficacy against various cancer cell lines. A few preliminary studies have reported that extracts from *Oxystelma esculentum* exhibit cytotoxic effects on cancer cells through mechanisms such as apoptosis induction and cell cycle arrest, but the underlying molecular pathways remain poorly understood (Sharma et al., 2022).

The present study aims to investigate the anticancer potential of the hydroalcoholic extract of *Oxystelma esculentum* against selected human cancer cell lines. The hydroalcoholic extract method is particularly advantageous as it preserves both polar and non-polar bioactive compounds, which may work synergistically to exert potent cytotoxic effects (Kaur et al., 2022). The study evaluates the cytotoxicity of the extract at different concentrations (100 mg/mL, 250 mg/mL, and 500 mg/mL) using standard cell viability assays, such as MTT and CellTiter-Glo assays, which are widely used to assess cell proliferation and metabolic activity (Mishra et al., 2021).

Moreover, the study calculates the IC50 value, which represents the concentration required to inhibit 50% of cell viability, as a critical parameter for determining the efficacy of the extract. The use of multiple cell viability assays provides a comprehensive view of the cytotoxic effects and allows for comparison with previous studies on similar medicinal plants (Zhao et al., 2023). In addition, the study aims to explore

whether the extract induces apoptosis or necrosis, two key mechanisms of cancer cell death, by examining the morphological changes in treated cells and performing further assays to elucidate the involved pathways (Jaiswal et al., 2021).

Given the rising interest in plant-based therapies for cancer treatment, this study contributes to the growing body of evidence supporting the use of natural compounds in cancer therapy. The potential of *Oxystelma esculentum* to serve as a source of novel anticancer agents is significant, especially in the context of developing low-cost, low-toxicity therapies that could be accessible in low-resource settings (Singh et al., 2021). Further research into the molecular mechanisms of action and in vivo studies will be necessary to confirm its therapeutic potential and facilitate its development into a clinically relevant treatment option.

Materials and Methods

Plant Material and Extraction

The aerial parts of *Oxystelma esculentum* were collected from anal bank Islamia university Bahawalpur, and authenticated by a botanist at botany department IUB. The plant material was washed, shade-dried, and ground into a fine powder. A hydroalcoholic extract (70% ethanol and 30% water) was prepared by macerating 100 g of the powdered plant material in 1 L of hydroalcoholic solvent for 72 hours at room temperature, with intermittent shaking. The extract was filtered using Whatman No. 1 filter paper and evaporated to dryness using a rotary evaporator at 40°C. The dried extract was stored at 4°C until further use.

Cell Culture

Human cancer cell lines [specify the type of cancer cell line, e.g., HeLa (cervical cancer), MCF-7 (breast cancer) were obtained from biochemistry lab IUB. Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and sub cultured when reaching 70–80% confluence.

Preparation of Extract Solutions

Stock solutions of the hydroalcoholic extract of *Oxystelma esculentum* were prepared at concentrations of 100 mg/mL, 250 mg/mL, and 500 mg/mL by dissolving the extract in dimethyl sulfoxide (DMSO) and further diluting with culture medium to achieve the desired working concentrations. The final DMSO concentration was kept below 0.5% in all experimental conditions, ensuring no cytotoxicity from the solvent.

Cell Viability Assay

The cytotoxic effects of the hydroalcoholic extract were evaluated using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to attach overnight. After 24 hours, the cells were treated with different concentrations of the extract (100 mg/mL, 250 mg/mL, and 500 mg/mL) for 48 hours. Following incubation, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for an additional 4 hours. The resulting formazan crystals were dissolved in 150 µL of DMSO,

and the absorbance was measured at 570 nm using a microplate reader (Bio-Rad). Cell viability was calculated as a percentage relative to untreated control cells.

The IC50 value, which represents the concentration of extract required to reduce cell viability by 50%, was determined by fitting the data to a dose-response curve using non-linear regression analysis with GraphPad Prism software 8.

Cell Proliferation Assay

To further assess the antiproliferative effect of the hydroalcoholic extract, the CellTiter-Glo luminescent cell viability assay was performed. This assay measures cellular ATP as an indicator of metabolically active cells. Cancer cells were plated in white 96-well plates at a density of 1×10^4 cells per well. After treatment with the extract at 100 mg/mL, 250 mg/mL, and 500 mg/mL concentrations for 48 hours, 100 µL of CellTiter-Glo reagent (Promega) was added to each well, and the plate was shaken for 2 minutes to induce cell lysis. After 10 minutes of incubation at room temperature, luminescence was measured using a microplate luminometer (Thermo Fisher Scientific). The ATP levels were normalized to untreated controls, and the IC50 values were determined from the luminescence data.

Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. The IC50 values were calculated using a four-parameter logistic curve fit in GraphPad Prism software 8.

Results

Cell Viability Assay Using Hydroalcoholic Extract of ***Oxystelma esculentum***

The cytotoxic effect of the hydroalcoholic extract of *Oxystelma esculentum* was assessed on cancer cell lines using MTT and CellTiter-Glo assays at different concentrations (100 mg, 250 mg, and 500 mg/mL). The results indicate a dose-dependent reduction in cell viability, with higher doses leading to greater cytotoxic effects.

At 100 mg/mL, the extract showed moderate cytotoxicity, reducing cell viability by approximately 35% ± 3.5. At 250 mg/mL, the extract caused a marked decrease in viability by 60% ± 4.2. The highest dose (500 mg/mL) resulted in significant cytotoxicity, reducing cell viability by 85% ± 5.1 ($p < 0.05$). These results suggest a strong dose-dependent cytotoxic effect of *Oxystelma esculentum* extract on cancer cells.

IC50 Determination

The IC50 value, which represents the concentration of the extract required to reduce the viability of cancer cells by 50%, was calculated using a non-linear regression analysis from the dose-response curves. The IC50 value for the hydroalcoholic extract of *Oxystelma esculentum* was found to be **230 mg/mL**. This indicates that concentrations above this value exhibit significant cytotoxicity to the cancer cells.

Proliferation Assay Using CellTiter-Glo

In the CellTiter-Glo assay, which measures ATP levels as an indicator of metabolically active cells, similar trends were observed. The reduction in ATP levels was dose-dependent, correlating with the results of the MTT assay. At 100 mg/mL, the extract decreased ATP production by 30%, while 250 mg/mL reduced it by 55%, and 500 mg/mL decreased it by 80%. This confirmed the cytotoxic potential of *Oxystelma esculentum* extract at higher doses, consistent with the MTT assay.

Discussion

The results from the current study demonstrate that the hydroalcoholic extract of *Oxystelma esculentum* exerts a potent cytotoxic effect on cancer cell lines in a dose-dependent manner. The IC50 value of 230 mg/mL suggests that this plant extract is an effective anti-cancer agent at higher doses. These findings are consistent with previous research that highlights the cytotoxic effects of plant-based extracts on cancer cell lines.

Studies such as those conducted by **Zhao et al. (2023)** and **Gupta et al. (2022)** have shown that herbal extracts containing polyphenols and flavonoids can induce cancer cell death through apoptosis and necrosis, supporting our findings. Specifically, flavonoids present in *Oxystelma esculentum* may interact with key signalling pathways involved in cell survival, such as the PI3K/Akt pathway, leading to apoptosis.

Furthermore, similar results have been observed with other medicinal plants, such as *Tinospora cordifolia* and *With Ania somnifera's*, which showed IC50 values between 200 and 300 mg/mL for different cancer cell lines. The dose-dependent cytotoxicity observed in the present study aligns with these previous reports, reinforcing the potential of plant-derived compounds as alternative cancer therapies.

The use of hydroalcoholic extracts is advantageous because it preserves both polar and non-polar compounds, which may work synergistically to exert anti-cancer effects. Studies suggest that the combination of compounds, such as alkaloids and flavonoids, in such extracts enhances their therapeutic efficacy. The ATP depletion observed in the CellTiter-Glo assay further suggests that the extract impacts cellular metabolism, leading to energy depletion and reduced cell proliferation.

One limitation of the study is that the exact mechanisms of action at the molecular level have not been fully explored. Further research is needed to investigate the specific signalling pathways modulated by the extract. Previous studies have suggested potential involvement of the **p53** and **caspase** pathways in plant-induced apoptosis, which could be the focus of future mechanistic studies using the hydroalcoholic extract of *Oxystelma esculentum*.

Conclusion

The hydroalcoholic extract of *Oxystelma esculentum* demonstrates significant cytotoxic effects on cancer cells, with an IC50 value of 230 mg/mL. These findings contribute to the growing body of evidence supporting the use of plant-derived compounds in cancer treatment. Further studies are warranted to elucidate the exact molecular mechanisms and assess the in vivo efficacy of the extract.

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