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Cytotoxic activities of *Hypoxis hemerocallidea*, *Helichrysum caespititium* and *Dicoma anomala* extracts on DU145 prostate cancer cell line

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Background: Cancer mortality rate is still increasing every year despite advanced treatment regimes. Medicinal plants are one of the most important sources of anticancer agents.

Aim: This study was conducted to evaluate the cytotoxic effects of the aqueous and methanolic extracts of *Hypoxis hemerocallidea*, *Helichrysum caespititium* and *Dicoma anomala* on prostate cancer cell line (DU145) *in vitro*.

Setting: This is an in vitro study conducted under controlled laboratory settings at the University of Johannesburg, Department of Biomedical Sciences, South Africa.

Methods: Corms of the plants were collected and extracted with aqueous and methanolic solvents using the direct maceration method. DU145 cells were treated, respectively, with the aqueous and methanolic extracts of *H. hemerocallidea*, *H. caespititium* and *D. anomala* at various concentrations. Cell viability was quantified using the bisBenzimide H33342 trihydrochloride (Hoechst 33342) and propidium iodide (PI) dual-staining method assay after 48 h.

Results: The aqueous extracts of *H. hemerocallidea* and *H. caespititium* did not result in significant inhibition of DU145 cell line. However, an antiproliferative effect on the DU145 cell line was seen with *D. anomala* aqueous extracts at the concentrations of 15 µg/mL and 62.5 µg/mL, respectively. Additionally, methanolic extracts from *H. caespititium* and *D. anomala* arrested DU145 cell line at 15 µg/mL and 31 µg/mL concentrations in comparison to the untreated cell line and melphalan treatment control. However, not much termination was noticeable from the methanolic extracts of *H. hemerocallidea* on the cell line at 48 h.

Conclusion: These findings indicate that the methanolic extracts of *D. anomala* can act as a potential anticancer agent, with further analysis recommended to isolate the active compound and to understand its mechanism of action.

Contribution: The potential for *D. anomala* to be an alternative is supported by these findings, provided that active anticancer constituents are successfully characterised.

Keywords: cancer; cell line-DU145 prostate; cytotoxicity; cell proliferation; medicinal plants.

Introduction

Background

Cancer has led to approximately 10 million deaths in the year 2020, with prostate cancer rated as the second most common solid cancer in men and the fifth cause of cancer mortality worldwide (Chhikara & Parang 2022). According to the age-specific rate, in the African continent, new prostate cancer diagnoses were 93 171, of which 47 249 resulted in deaths (Gandaglia et al. 2021). Access to surgery and hormonal therapy treatments has improved; however, patient management was delayed and shifted towards the coronavirus disease 2019 (COVID-19) pandemic. Diagnostic and treatment deficiency gap in developing countries such as South Africa are still persisting (Berenguer et al. 2023).

Medicinal plants contribute to healthcare in many countries, both developed and developing, including South Africa. Thirty thousand plant species are categorised as higher plants, with 3000 used medicinally (Van Wyk & Prinsloo 2018). Plants have been reported to contain effective bioactive secondary metabolites such as phenols, flavonoids and alkaloids, which possess numerous disease-preventing or curing properties (Mangisa et al. 2021).

Recent cases highlight the dualism in health-seeking behaviours, which are often driven by barriers in conventional healthcare such as overcrowded facilities and the severe side effects associated with prolonged use of conventional medicine (Mothibe & Sibanda 2019). As a result, the *Hypoxis hemerocallidea*, *Helichrysum caespititium* and *Dicoma anomala* plants were chosen for this study based on anecdotal evidence. These plant extracts are commonly used when benign prostatic hyperplasia or prostate cancer is suspected, offering alternative treatment options rooted in traditional practices. However, a significant research gap exists between these traditional practices and scientific validation; hence, the initial cytotoxicity determination that this study focuses on. It has been claimed that *H. hemerocallidea* can be used as an immune-modulating phytotherapy in the management of immune-related diseases such as the common cold, flu, rheumatic arthritis, cancer, and human immunodeficiency virus or acquired immunodeficiency syndrome (HIV and/or AIDS) (Elbagory, Hussein & Meyer 2019). Additionally, a concoction of *H. caespititium* has been taken for the treatment of gonorrhoea and other prostate-related ailments (Mamabolo et al. 2018). Also, it has been alluded that pharmacological evaluations previously conducted on the *H. caespititium* plant found antibacterial and antifungal activities. The anticancer mechanisms of the extracts, however, have not been explored (Maroyi 2019). According to the literature, anticancer activity has been performed on *Dicoma capensis*, which belongs to the same family as *D. anomala*. The study by Chota, George and Abrahamse (2020) further indicated that this plant species possesses novel chemicals that act as anticancer agents against three different breast cancer cell lines, namely, MCF-7, MDA-MB-231 and MCF-12A (Chota, Abrahamse & George 2024).

Hypoxidaceae are delineated from the African family branch of the Asparagales. The genus name 'Hypoxis' is derived from the Greek word 'hypo', meaning 'below', and 'oxy', meaning 'sharp' (Bassey, Combrinck & Chen 2023). *Hypoxis hemerocallidea* is mostly found in the wild areas of the Eastern Cape, KwaZulu-Natal, Gauteng and Limpopo provinces. Its medicinal purposes include treating human immunodeficiency virus (HIV) infections and inflammatory conditions. The plant is now used by traditional healers to treat diverse types of cancers and tumours, such as urinary diseases, testicular tumours, prostatitis, prostate hypertrophy and benign prostate hyperplasia (Matyanga et al. 2020). A study conducted by Albrecht (as cited in Matyanga et al. 2020) demonstrated that rooperol, a compound identified in *H. hemerocallidea*, possesses significant therapeutic potential relevant to the management of several conditions, including prostate cancer, inflammation and HIV.

Helichrysum caespititium belongs to the Asteraceae or Compositae family. Caespititium is derived from the Latin word 'caespitose', which means very much tufted and matted, referring to the cushion-forming or mat-forming growth habit seen with the species (Akinyede et al. 2022). The geographical distribution of *H. caespititium* ranges in Lesotho, South Africa, Swaziland and Zimbabwe (Maroyi 2019).

The medicinal purposes of *H. caespititium* include sexually transmitted infections, nausea, aphrodisiac, headache, wounds and ulceration (Seleteng-Kose, Moteetee & Van Vuuren 2019).

Dicoma species are from the Asteraceae family of flowering plants known as asters, daisies or sunflowers. They are among the chemically most diverse groups of flowering plants. The name '*Dicoma*' is derived from two Greek words: 'di', meaning 'two', and 'kome', meaning 'tuft of hair', referring to the double row of pappus bristles (Mangisa et al. 2021). The plant is native to sub-Saharan Africa and is widely distributed across the country. It is predominantly found in the following provinces: Limpopo, Gauteng, North West, Mpumalanga, KwaZulu-Natal, Free State and Northern Cape (Chota et al. 2020). *D. capensis*, which belongs to the same family as *D. anomala*, has demonstrated anticancer activities; hence, it is further used traditionally for the treatment of cancer, malaria, fever, diabetes, ulcers, colds and coughs (Mangisa et al. 2021). There appear to be increasing recommendations to explore traditional herbal alternatives, as they have numerous phytochemicals with pharmacological activities. Therefore, this study investigated the cytotoxic effects of *H. hemerocallidea*, *H. caespititium* and *D. anomala* extracts on the DU145 prostate cancer cell line.

Materials and methods

Hypoxis hemerocallidea herbarium specimen number Toona 01, *H. caespititium* Toona 02 and *D. anomala* 03 corms were collected from Bojanala District in Rustenburg Municipality, Mamerotse (25°25′03.0″S, 27°20′28.0″E) and Mogajane (25.4870°S, 27.3618°E) villages, Bafokeng area, on April 2024. The plant habitat was along the plain roadside, fully exposed to the sun, and the plants were moderately to abundantly distributed. In addition, the DU145 (ATCC® HBT-81™) prostate cancer cell line used was purchased from Highveld Biological, Cellonex, South Africa.

The direct maceration extraction method was used, as adapted from Fonmboh et al. (2020). Five grams of dried *H. hemerocallidea*, *H. caespititium* and *D. anomala*, respectively, were mixed with 50 mL of methanol (MeOH) and 50 mL of aqueous (deionised water [ddH2O]) as solvents. The mixtures were placed on a constant mechanical shaker for 40 h at room temperature (20°C – 24°C); extracts were then filtered, and the supernatant was concentrated under reduced pressure using a vacuum rotary evaporator. The concentrated extracts were stored in small glass bottles at 4°C until further use (Ghomari et al. 2019). The residue was used for experiments.

Prostate cancer cell line DU145 was cultured in RPMI 1640, low-glucose cell culture medium, and foetal bovine serum (FBS) was purchased from GE Healthcare Life Sciences (Logan, UT, United States). Cultures of cells were seeded into 96-well microtitre plates at a density of 5000 cells/well using a volume of 100 µL aliquots in each well. The microtitre plates with cells were incubated at 37°C, with 5% CO2 for 24

h prior to the addition of the extracts compound to allow for the attachment of cells. Extracts were solubilised in dimethyl sulfoxide and diluted to the desired concentrations of 15, 31, 62.5, 125, 250 and 500 µg/mL in the medium. They were added to wells with exponentially growing cells, and all experiments were performed in triplicate.

The treatment medium was removed after 48 h and replaced with 100 µL DPBS-Dulbecco's Phosphate Buffered Saline with Ca^{2+} and Mg^{2+} containing Hoechst 33342 at a final concentration of 5 µg/mL and incubated for 30 min at room temperature (20°C – 24°C). For cell death detailing effects, propidium iodide (PI) was added at a final concentration of 100 µg/mL using 20 µL per well of a 1 mL stock just before image acquisition, which was carried out using an ImageXpress Micro XLS fluorescent microscope and evaluated with MetaXpress software. Thus, the concentration to inhibit 50% of the cell population was determined.

Statistical analysis

Experiments were conducted in triplicate, and data represented the mean ± standard deviation (s.d.). The statistical significance was measured by one-way ANOVA, and *p*-values < 0.05 were considered significant.

Ethical considerations

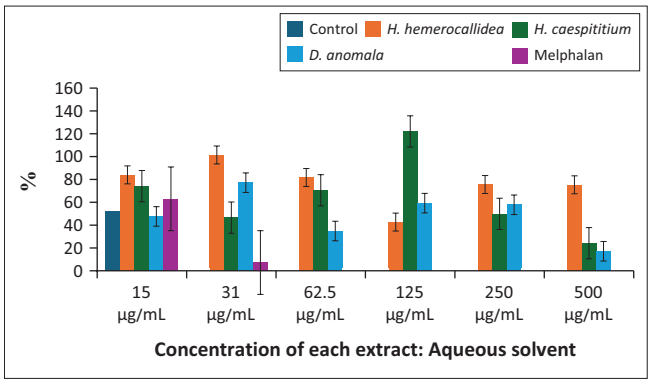
Ethical clearance to conduct this study was obtained from the Faculty Research and Innovative Committee (FRIC) of the Faculty of Health and Environmental Sciences, Central University of Technology on 25 September 2025.

Results

In comparison to the untreated cells and melphalan control, a reduction in viability of the DU145 cell line was seen in *D. anomala* aqueous extracts at concentrations of 15 µg/mL and 62.5 µg/mL. Conversely, the *H. hemerocallidea* and *H. caespititium* aqueous extracts did not result in significant DU145 cell growth inhibition. Therefore, *H. hemerocallidea* and *H. caespititium* aqueous extracts did not exhibit much cytotoxicity even at the maximum extract concentration of 500 µg/mL, although at this concentration, *D. anomala* aqueous extracts still proved to be potent (Figure 1).

Treatment of DU145 prostate cancer cells with methanolic extracts from *H. caespititium* and *D. anomala* resulted in a concentration-dependent inhibition of cell proliferation. Specifically, concentrations of 15 µL/mg and 31 µL/mg of these extracts significantly arrested the DU145 cell line, an effect comparable to the positive control, melphalan, and a notable reduction compared with the untreated control (Figure 2). In contrast, the methanolic extract of *H. hemerocallidea* showed minimal inhibition of cell growth.

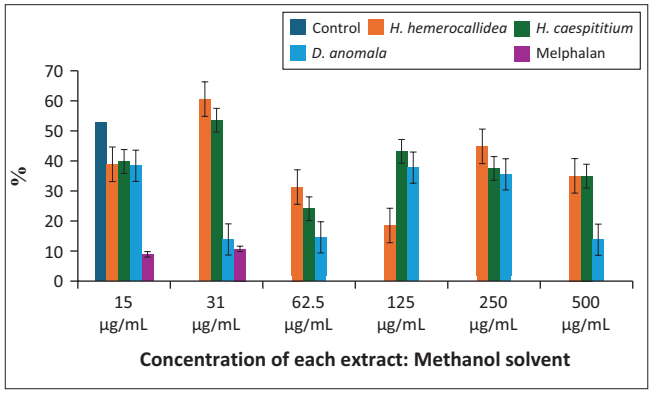
Further analysis revealed that methanolic extracts were more effective than their aqueous counterparts at the same concentrations (15 µL/mg and 31 µL/mg) in reducing the



Control, untreated cell line; solvent, aqueous (ddH_2O); melphalan, cell line treated with melphalan; *H. hemerocallidea, Hypoxis hemerocallidea; H. caespititium, Helichrysum caespititium; D. anomala, Dicoma anomala*.

Note: s.d. is represented as error bars. The bar graph represents the average of one individual experiment performed in quadruplicate.

FIGURE 1: Cytotoxicity activity of Aqueous extracts from *Hypoxis hemerocallidea, Helichrysum caespititium* and *Dicoma anomala* on DU145 prostate cancer cell line.



Control, untreated cell line; solvent, MeOH; melphalan, cell line treated with melphalan; *H. hemerocallidea, Hypoxis hemerocallidea; H. caespititium, Helichrysum caespititium; D. anomala, Dicoma anomala*.

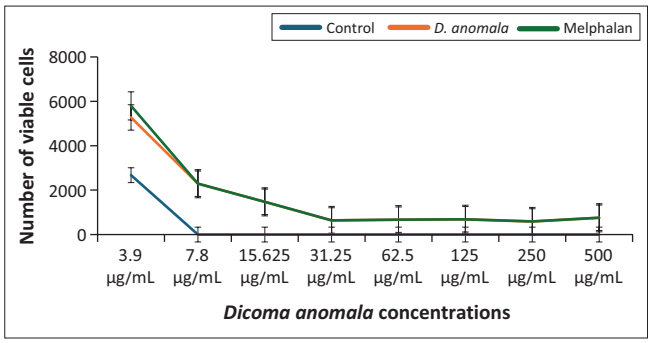
Note: s.d. is represented as error bars. The bar graph represents the average of one individual experiment performed in quadruplicate.

FIGURE 2: Cytotoxicity activity of Methanolic extracts from *Hypoxis hemerocallidea, Helichrysum caespititium* and *Dicoma anomala* on DU145 prostate cancer cell line.

viability of DU145 cell line (Figures 1 and 2). The distinct cytotoxic effects were observed with the methanolic extracts of *H. caespititium* and *D. anomala*. DU145 cell proliferation rates were found to be 54% and 14% after treatment with 31 µL/mg of the methanolic extracts of *H. caespititium* and *D. anomala*, respectively.

These findings suggest that the methanolic extract of *D. anomala* is cytotoxic to the DU145 prostate cancer cell line, demonstrating a more potent antiproliferative effect than the other tested extracts (Figures 1 and 2).

In comparison with aqueous extracts of *H. hemerocallidea*, *H. caespititium* and *D. anomala* showed minimal effects on the DU145 cell line, similar to the methanolic extracts of *H. hemerocallidea* and *H. caespititium*. However, DU145 cell line treated with methanolic *D. anomala* showed a more cytotoxic effect. Therefore, the inhibition concentration (IC) of *D. anomala* methanol extracts proved to be cytotoxic against the DU145 cell line, with an IC value of 18.98 µg/mL (R^2 = 94%; Figure 3).



D. anomala, Dicoma anomala.

Note: Data represent the mean ± s.d. for replicate wells at each of the indicated concentrations.

FIGURE 3: Dose–response curve for the MeOH *Dicoma anomala* extract.

Discussion

This study investigated the cytotoxic effects of extracts from *H. hemerocallidea*, *H. caespititium* and *D. anomala* on the DU145 prostate cancer cell line. Melphalan and untreated cells were used as positive and negative controls, respectively.

Analysis of the aqueous extracts revealed that *H. hemerocallidea* and *H. caespititium* did not inhibit DU145 cell proliferation, even at a high concentration of 500 µg/mL (Figure 1). In contrast, the aqueous extracts of *D. anomala* demonstrated notable cytotoxic activity at this same concentration. This finding aligns with the research of Steenkamp and Gouws (2006), who observed significant cytotoxicity from both *H. hemerocallidea* and *D. capensis* aqueous extracts on the same cell line. However, it contradicts the findings of Mamabolo et al. (2018), who reported low toxicity for *H. caespititium* aqueous extracts in rat hepatocytes, suggesting that the cytotoxicity of these extracts is cell type-dependent.

Furthermore, methanolic extracts of *H. caespititium* and *D. anomala* showed greater cytotoxic activity than the aqueous extracts. *Helichrysum caespititium* and *D. anomala* significantly inhibited DU145 cell proliferation at concentrations of 15 µg/mL and 31 µg/mL, an effect comparable to the positive control, melphalan (Figure 2). However, the methanolic extract of *H. hemerocallidea* showed only a minimal effect. Notably, the methanolic extract of *D. anomala* was the most potent, as it effectively arrested the DU145 cell line at both 15 µg/mL and 30 µg/mL (Figure 3). These results are consistent with the broader anticancer properties of *D. anomala* extracts observed by Chota et al. (2020) in the study performed on other cancers such as breast and lung cancer.

The study's most significant and unexpected finding was that among the three plants traditionally used together, only the methanolic extract of *D. anomala* exhibited strong cytotoxic activity against the DU145 cell line. This outcome suggests that the active anticancer compounds are highly dependent on the extraction method and the specific plant species, which contrasts with the traditional practice of using aqueous preparations of all three plants.

This aligns with previous studies showing that variations in extraction procedures and the natural variability of plants can affect their therapeutic properties (Steenkamp & Gouws 2006). While some research has shown that *H. hemerocallidea* can inhibit the growth of other cancer cell lines, such as MCF-7 (Steenkamp & Gouws 2006), the results of this study suggest that aqueous extracts may have a limited effect on specific carcinoma cell lines (Chota et al. 2020).

Further investigations are needed to fully understand the anticancer mechanism of action of *D. anomala* and *H. caespititium*. Future investigations should focus on demonstrating whether these extracts induce apoptosis (programmed cell death) with minimal necrosis (cell injury), which is a crucial characteristic of a promising anticancer agent. This study encourages in-depth research to characterise the specific compounds responsible for the cytotoxic effects of these plant extracts, particularly *D. anomala*, on DU145 prostate cancer cell proliferation.

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Competing interests

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Authors' contributions

L.M.T. was involved in conceptualisation, methodology, data curation, data analysis and writing. J.M. performed conceptualisation and supervision of the study. P.H.M. was responsible for conceptualisation, methodology, data curation, validation, funding acquisition and review of the writing.

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Data availability

The authors declare that all relevant data are contained within the article and its listed references.

Disclaimer

The study was performed using a purchased cancer cell line and did not involve the use of animals, human tissues, or manipulation of plant materials or organisms. All experimental procedures in this study were conducted in accordance with laboratory standard operating procedures (SOP). The views and opinions expressed in this article are those of the authors and are the product of professional research. They do not necessarily reflect the official policy or position of any affiliated institution, funder, agency or that of the publisher. The authors are responsible for this article's results, findings and content.

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