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Investigating the *in vitro* Anticancer Potential and Phytochemical Constituents of *Cheilanthes hirta* Swartz Plant Extracts

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Cancer, a complex group of diseases characterized by uncontrolled cell growth, remains a leading cause of death worldwide, accounting for approximately 10 million deaths in 2020. Despite advancements in chemotherapy and targeted therapies, survival rates have not significantly improved, necessitating the exploration of novel anticancer agents. This study investigates the *in vitro* anticancer potential and phytochemical constituents of *Cheilanthes hirta* Swartz, a fern known for its medicinal properties. The plant was collected from KwaZulu Natal, South Africa and extracts were prepared using water, ethanol and methanol. The phytochemical and FTIR screening were carried out using standard procedure and anticancer activities of the extracts were assessed against prostate (PC-3 and DU-145), human T-lymphocytes (SKU-T) and gastric (AGS) cancer cell lines using the MTT assay. The phytochemical screening revealed the presence of tannins, terpenoids, saponins, flavonoids, cardiac glycosides, anthraquinones, phlobatannins, alkaloids and steroids. FTIR spectroscopy identified the functional groups such as hydroxyl, carboxylic acid, terminal alkynes, ketones, phenols and phosphate ions. The cytotoxicity results showed that the ethanol extract exhibited the most potent antiproliferative effects on prostate cancer cell lines, while the aqueous extract had the strongest effect on the gastric cancer cells. This study highlights the potential of *C. hirta* as a source of bioactive compounds for anticancer drug development, however, further investigation into its mechanisms of action and therapeutic efficacy is needed.

Keywords: Anticancer, *Cheilanthes hirta*, Phytochemicals, MTT assay.

INTRODUCTION

Cancer stands as a multifaceted array of disorders characterized by unregulated cellular growth and proliferation within the body, often leading to the invasion and destruction of healthy tissues and organs. According to the WHO [1], cancer ranks as a prominent global cause of mortality, accounting for nearly 10 million deaths in 2020. The year also recorded approximately 18.1 million new cases worldwide [2]. Among these reported cases, breast cancer (2.26 million), lung cancer (2.21 million), colon and rectal cancer (1.93 million), prostate cancer (1.41 million), non-melanoma skin cancer (1.20 million) and stomach cancer (1.09 million) emerged as the most prevalent incidences [1]. The genesis of cancer is believed to arise from a combination of genetic factors and three primary classes of external agents. These agents encompass physical carcinogens, including ultraviolet and ionizing radiation; chemical carcinogens like asbestos, components within tobacco smoke, alcohol, aflatoxin and arsenic; and biological carcinogens such as infections stemming from specific viruses, bacteria or parasites [1]. Additionally, a lack of adequate intake of dietary fibre, fruits and vegetables, coupled with factors like obesity and insufficient physical activity, significantly contributes to an elevated risk of cancer [3].

In South Africa, cancer is a significant contributor to mortality, representing 10% of national deaths. Statistics reveal that 51.3% of diagnosed cancers occur in females, whereas males account for 48.6% of these cases within the country [3]. Targeted

therapies like radiation and chemotherapy are commonly employed in the treatment of cancer. Despite substantial investments and advancements in cancer chemotherapy, the overall survival rates for patients have not markedly improved in numerous countries [4]. Moreover, the long-term side effects of chemotherapy raise significant concerns [5]. These side effects encompass a range of issues from short-term symptoms like nausea and vomiting to long-term complications such as ulceration, anorexia, malabsorption, anaemia and sepsis [6]. Presently, there exists no anticancer treatment that guarantees 100% efficacy without any side effects [7-10]. Hence, there exists an imperative need for the development of novel drugs that can serve as potent anticancer agents while addressing the challenges associated with chemotherapy, including drug resistance and toxicity.

More than 60% of the approved anticancer drugs in existence either originate from natural products or their derivatives, highlighting the pivotal role of medicinal herbs as a noteworthy reservoir of anticancer agents [11-13]. Extensive screening of more than 3000 plant species has led to the discovery of crucial anticancer drugs like vincristine, vinblastine, taxol, indicine-N-oxide, etoposides and camptothecin. These drugs have demonstrated the capacity to impede cancer cell growth by regulating apoptosis and autophagic pathways [14,15]. Medicinal plants globally serve as a bountiful source of medicine, renowned for their minimal side effects and cultural acceptance [16].

Cheilanthes hirta Swartz (Fig. 1), a fern species within the Pteridaceae family, is commonly referred to as the hairy lipfern (in komankoma) in the Eastern Cape Province of South Africa. It typically inhabits rocky terrains, cliffs, crevices, boulder bases and leaf-litter areas along forest margins and in low scrub, often preferring exposed locations or light shade [17]. Its habitat extends across Swaziland, eastern and southern tropical Africa, southern Africa and the western Indian Ocean region [17]. According to Foden & Potter [18], this fern is found across various South African regions, including the Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Mpumalanga, Northern Cape, Limpopo, Northwest and Western Cape. *C. hirta* reproduces vegetatively through sporadically branched rhizomes, forming small clonal stands. Its growth cycle follows a distinct seasonal pattern, primarily occurring during the wetter summer months. However, in exposed environments or prolonged drought conditions, the plants may become dormant [17].

Fig. 1. *Cheilanthes hirta*

The fronds of *C. hirta*, described as more herbaceous to fairly coriaceous, exhibit a narrow lance-like shape with fine divisions [19,20].

Ferns are known to synthesize a diverse range of secondary metabolites such as lycopodium alkaloids, fawcettimine alkaloids, lycodine alkaloids, triterpenoids, flavones and phenolic acids, many of which exhibit valuable biological activities. These phytochemicals could hold promise for the development of new treatments for various diseases [21]. Specifically, ferns from the genus Pteris (Pteridaceae) are known to produce a diverse array of secondary metabolites. This includes a variety of chemical compounds such as *ent*-kaurane diterpenoids and pterosin-sesquiterpenes, flavonoids, benzenoids and benzenoid derivatives. The Pteridaceae family is particularly characterized by the presence of pterosins and *ent*-kaurane diterpenoids as signature phytochemicals [21].

Several studies have shown that the genus Cheilanthes has a range of phytochemicals such as flavonoid, glycosides and proanthocyanidin content and possesses antioxidant and anti-inflammatory activities [22,23]. *C. hirta* has been reported as one of the South African medicinal plants, recognized for its efficacy in treating various types of wounds, including septic wounds, sores, burns, ulcers, boils and cuts, *etc*. [23-25]. The decoction derived from the branches of this plant has been documented as an effective remedy for ailments such as colds, herpes, sore throats, asthma, headaches and herpes [17,22,26]. Additionally, the powdered rhizome of *C. hirta* serves as an anthelmintic agent, specifically employed for the treatment of tapeworm infestations [17,22].

According to a study conducted by Twilley *et al*. [27], the rhizomes of *Cheilanthes contracta* (Kunze) Mett. Ex Kuhn, formerly known as *Cheilanthes hirta* Sw. has been traditionally used as an ingredient in remedies for cancer treatment. The study further reported that the 50% aqueous ethanol extract of fresh rhizomes of the plant exhibited potent anticancer activity against murine metastatic sarcoma (sarcoma 180) cells. However, the same extract was found to be inactive against murine lymphoid leukemia (L-1210) and murine lung carcinoma (Lewis) cell lines. In addition, plant species such as *Cheilanthes dalhousiae* Hook, which contains flavone-5-O-glycosides have been reported to possess anticancer activity [28]. Also, *Cheilanthes farinosa* has been reported to possess antiproliferative and apoptotic activity in human liver cancer cells and is not deleterious towards non-cancerous macrophage cell lines. Also, research has shown that the fern species *C. farinosa* exhibits antiproliferative and apoptotic effects in human liver cancer cell lines. Significantly, the extract was not found to be deleterious towards non-cancerous macrophage cell lines, suggesting a selective cytotoxicity towards the cancer cells [29].

The anticancer efficacy of plants is attributed to a variety of constituent secondary metabolites, including catechins, polyphenols and flavonoids [30-32]. As a result, the purpose of this study is to examine the phytochemical composition of *C. hirta*, identify the functional groups of the phytocompounds and assess the *in vitro* anticancer potential of aqueous, ethanol and methanolic extracts of plant against prostate (PC-3, DU-145 and SKU-T) and gastric (AGS) cancer cell lines.

EXPERIMENTAL

Plant collection and identification: The plant samples were collected in consultation with the traditional and indigenous people of northern KwaZulu Natal, with additional samples purchased at the Muthi market in Durban, South Africa. Subsequent identification of the plant species occurred at Department of Plant Sciences Laboratory, University of Fort Hare, Alice Campus, South Africa, where a voucher specimen was deposited for documentation purposes.

Preparation of extract: The plant materials were washed thoroughly under running tap water to get rid of all the debris and soil. They were then dried at room temperature for 2 weeks, after which the air-dried plant material was finely ground and packed in self-seal airtight polythene bags for further use. The plant extracts were prepared by standard methods. Approximately 30 g of each of dried powdered materials were soaked in 250 mL of water, ethanol and methanol, respectively, with shaking on a Labcon platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h. Each mixture was stirred for 12 h using a sterile glass rod. Plant extracts were filtered using Whatman No. 1 filter paper. The filtrates from ethanol and methanol were concentrated under reduced pressure and at 45 ºC using a rotary (Cole Parmer SB 1100, Shangai, China) evaporator. The filtrate from the water extract was freeze-dried using a freeze-dryer. The crude extracts were all wrapped in foil and stored at -20 ºC until use. Plant extract stock solutions for anticancer assays were made by dissolving 0.04 g crude extracts of ethanol and methanol in 2 mL solution (containing 0.1% DMSO) and the water crude extract in double distilled water. All the extracts were vortexed and sequentially passed through 0.45 µm and 0.22 µm sterile filters. The prepared aliquots of extracts were wrapped in foil and stored at -20 ºC until use.

Ethical studies: Ethical approval to conduct the study was obtained from the Inter-Faculty Research Ethics Committee of the Faculty of Science and Agriculture, University of Fort Hare with reference number REC-270710-028-RA.

Qualitative phytochemical screening: The phytochemical constituents of crude ethanolic, aqueous extracts and powdered plant material of *C. hirta* were tested for alkaloids, tannins, steroids, phlobatannins, saponins, flavonoids, cardiac glycosides (keller-kiliani test) and terpenoids (Salkowski's test) following the established protocols [33-36]. The presence of phytochemicals was determined by observing a colour change or the formation of a precipitate after introducing the specified reagent(s).

Test for alkaloids (Mayer's and Dragendroff 's test): Briefly, 500 mg of powdered plant material was mixed in 5 mL of 1% aqueous HCl, then heated in a water bath for 5 min and filtered. Subsequently, 1 mL of resulting filtrate was treated with a few drops of Mayer's reagent and the appearance of a cream-coloured precipitate indicated the presence of alkaloids. Another portion of the filtrate was treated with Dragendroff's reagent, leading to the formation of an orange or orange-red precipitate, indicating the presence of alkaloids

Test for tannins: A quantity of 500 mg of dried powdered material was boiled in 20 mL of water in a test tube and filtered. Following filtration, a few drops of 0.1% $FeCl_3$ were added to 1 mL of filtrate, the resulting solution was observed for the appearance of a brownish-green or blue-black colouration, indicating the presence of tannins.

Test for phlobatannins: The aqueous extract was boiled with 1% aqueous HCl and observed for the deposition of red precipitate, indicating the presence of phlobatannins.

Test for saponins: Approximately 2 g of crude powdered material was boiled in 20 mL of distilled water using a water bath and subsequently filtered. Following this, 10 mL of resulting filtrate was mixed with 5 mL of distilled water, vigorously shaken and observed for a stable persistent froth. The froth was then mixed with 3 drops of olive oil, vigorously shaken once more and observed for the formation of an emulsion, indicating the presence of saponin.

Test for flavonoids: A portion of powdered material was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The resulting mixture was filtered and 4 mL of filtrate was shaken with 1 mL of dilute NH_3 solution. The appearance of a yellow coloration served as an indication of the presence of flavonoids.

Test for steroids: In testing for steroids, 2 mL of acetic anhydride was added to 0.5 g of ethanolic extract along with 2 mL conc. H_2SO_4. The colour changes from violet to blue or green indicating the presence of steroids.

Test for terpenoids (Salkowski's test): Briefly, 5 mL of extract was mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of H_2SO_4 to form a layered structure. The presence of a reddish-brown colouration at the interface indicated the presence of terpenoids.

Test for cardiac glycosides (Keller-Kiliani test): In this procedure, 5 mL of extract was treated with 2 mL of glacial acetic acid containing one drop of $FeCl_3$ solution. This mixture was then underplayed with 1 mL of conc. H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Cytotoxicity assay

Cell culture: Human prostate carcinoma cells (PC-3 and DU-145), human T-lymphocytes (SKU-T) and gastric adenocarcinoma (AGS) cell lines were obtained from the International Centre for Genetic Engineering and Biotechnology, Department of Integrative Biomedical Sciences, South Africa. The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin. The cultures were then maintained at 37 °C in a humidified atmosphere of 5% CO_2 in 95% air.

Determination of tumour cells viability and counts: The viability of DU-145, PC-3, SKU-T and AGS cells was assessed using the trypan blue staining method [37]. The cell concentration per millilitre was determined using a haemocytometer and the desired cell numbers were calculated using the following formula:

$$\text{Cells/mL} = 10^4 \times (\text{Average count per square}) \times (\text{Dilution factor})$$

Study design: The plate was divided into three groups; complete media, untreated cell solution as a control and treatment with *C. hirta* at different concentrations. The treatment was carried out in triplicate.

***In vitro* cell viability assay:** The antiproliferation of the plant extracts was evaluated *in vitro* on DU-145, PC-3, SKU-T and AGS cell lines using a modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay described by Mosmann [38]. Briefly, 6×10^4 cells/well in 100 µL complete media were seeded into a 96-well cell culture plate and allowed to attach for 24 h. After 24 h, the spent media was aspirated and the cells were treated with a plant extract diluted to 200 µg/mL of complete media (10 µg/mL). In the 96-well culture plate, 100 µg/mL of diluted plant extract was added and serial dilution was performed, with untreated cell media solution serving as a control. The 96-well cell culture plates were then incubated for 72 h at 37 ºC in a humidified atmosphere of 5% CO_2 and 95% air. Following incubation, 10 µL MTT (2.5 mg/mL) was added to each well and incubated for another 3-4 h before stopping the experiment by adding a sodium dodecyl sulphate 10% in 0.1 N HCl to solubilize the formed formazan and leaving overnight. After 72 h, the optical density in the wells was measured using a microplate reader (Thermo Multiskan Go, USA) at a wavelength of 595 nm. The absorbance values obtained from the control wells were averaged and this value was considered to be 100% cell viability. The cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis: Statistical analysis was performed on MINITAB version 12 for Windows (Minitab Inc., USA). One-way analysis of variance was used to test for whether there are any statistically significant differences between the means of samples in comparison to controls. The P values less than 0.05 were considered significantly different.

RESULTS AND DISCUSSION

Previous research indicates a scarcity of data regarding the phytochemical composition of ferns and their potential pharmacological uses [21]. This study is however the first to report the phytochemical screening, FTIR analysis and anticancer activity of *C. hirta*. The qualitative phytochemical screening of the crude powdered materials and extracts of *C. hirta* revealed the presence of flavonoids, tannins, terpenoids, cardiac glycosides, saponins, anthraquinone, phlobatannins, alkaloids and steroids as shown in Table-1.

The phytochemical constituents found in similar ferns include alkaloids [39], flavonoids [40,41], polyphenols [41,42], terpenoids [43] and steroids [44]. In particular, research into the genus Pteris (Pteridaceae) has identified several secondary metabolites such as *ent*-kaurane diterpenoids, pterosin-sesquiterpenes, flavonoids, benzenoids and their derivatives. In addition, alkaloids, glycosides, tannins and flavonoids have been identified in *Cheilanthes tenuifolia* (Burm.f.) Swartz, which belongs to the same family as *C. hirta* [45].

TABLE-1
QUALITATIVE PHYTOCHEMICAL SCREENING OF *Cheilanthes hirta*

Phytochemicals	Status
Tannins	Detected
Terpenoids	Detected
Saponins	Detected
Flavonoids	Detected
Cardiac glycosides	Detected
Alkaloids	Detected
Steroids	Detected
Phlobatannins	Detected

FTIR spectral studies: Perkin-Elmer Universal ATR 100 Fourier transform infrared spectrophotometer (FTIR) was used to determine the functional groups in the aqueous extract in the range of 400-4000 cm^{-1}. The FTIR spectrum revealed that the aqueous extract of *C. hirta* possessed phytochemicals with different functional groups at 1035.76, 1357.36, 1608.64, 2157.46, 2906 and 3252.32 cm^{-1} (Fig. 2). The peaks at 2157.46 cm^{-1}, 1608.64 cm^{-1} and 1035.76 cm^{-1} were strong, while the others varied from weak to medium. Various compounds were detected including hydroxyl groups, carboxylic acid, terminal alkynes, ketones, phenols and phosphate ions. The FTIR analysis identified a strong presence of C≡C (terminal alkynes) at 2157.46 cm^{-1}, C=O (ketone) at 1608.68 cm^{-1} and PO_3 stretch at 1035.76 cm^{-1}. There was also an indication of the presence of phenols at 1357.36 cm^{-1} as shown by the O–H stretch, carboxylic acid at 2906 cm^{-1} and hydroxy compound (H-bonded) at 3252.32 cm^{-1} (Table-2).

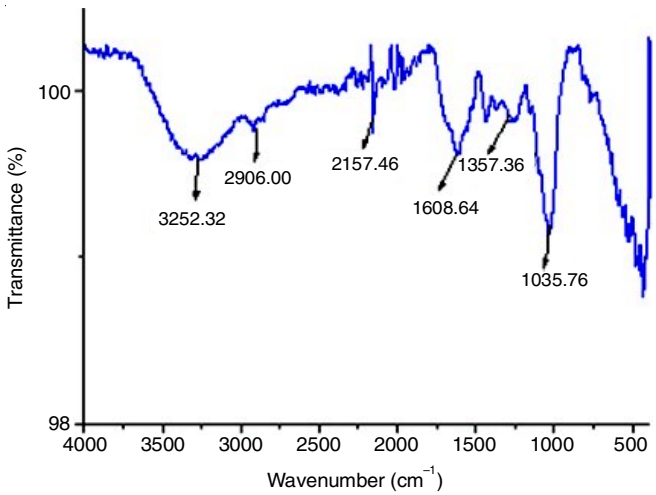

Fig. 2. FTIR spectrum of aqueous extract of *C. hirta*

Anticancer assay: This study explores the *in vitro* antiproliferative effects of methanol, ethanol and aqueous extracts of *C. hirta* on prostate cancer cell lines (PC-3 and DU-145), Human T-Lymphocytes (SKU-T) and gastric cancer cell lines (AGS). The evaluation reveals promising activities and concentration dependent effects across different extracts.

Antiproliferative effect of *C. hirta* on human T-Lymphocytes (SKU-T) cell line: *In vitro* evaluation of the antiproliferative effects of *C. hirta* methanol, ethanol and aqueous extracts on the human T-Lymphocytes (SKU-T) cell line revealed several

TABLE-2
FTIR INTERPRETATION OF FUNCTIONAL GROUPS OF AQUEOUS EXTRACT OF *C. hirta*

Wavenumber (cm^{-1}) (test sample)	Wavenumber (cm^{-1}) [46]	Functional group	Phyto compounds identified	Bioactivity	Ref.
3252.32	3570-3200	H-bonded, O–H stretch	Hydroxy compound, phenols	Antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities and prevention of cardiovascular diseases.	[47,48]
2906.00	3500-2400	Carboxylic acid, O–H stretch	Carboxylic acid	Antimicrobial, antioxidant, anticancer.	[49]
2157.46	2260-2100	Carbon-Carbon triple bond (C≡C)	Terminal alkynes	Antimicrobial	[50]
1608.64	1650-1600	C=O stretch	Ketone compounds	Antimicrobial	[51,52]
1357.36	1410-1310	O–H bond, alcoholic group	Phenol, or tertiary alcohol	Antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities and prevention of cardiovascular diseases, cancers, diabetes, neurological disorders, skin diseases and diseases associated with oxidative stress. It also has anti-analgesic, anti-allergic and anti-Alzheimer's properties	[47,48,53-55]
1035.76	1100-1000	PO_3 stretch	Phosphate ion	Bone and teeth mineralization, energy transfer and storage, regulation of enzymatic activity and buffering agent.	[54,56,57]

promising activities. The ethanol and methanol extracts reduced the percentage proliferation of SKU-T cells from 0.04 to 100 µg/mL in a concentration dependent manner (Fig. 3). Significant reductions in cell proliferation were observed in the lower concentrations of the methanol extract ranging from 11.1 to 100 µg/mL. In contrast, the ethanol extract only had a significant antiproliferative effect at the highest concentration (100 µg/mL). The methanol extract had the overall strongest antiproliferative effects on SKU-T cells, followed by the aqueous extract. Unlike the ethanol and methanol extracts, the antiproliferative effect of the aqueous extract was not concentration dependent. Surprisingly, the antiproliferative effects of the aqueous extract were only significant from concentrations 3.7 to 33.3 µg/mL; lower concentrations (< 3.7 µg/mL) appeared to have proliferation enhancing effects on the cells, with significant increases in proliferation observed at 0.14 and 0.04 µg/mL of aqueous extract treatment.

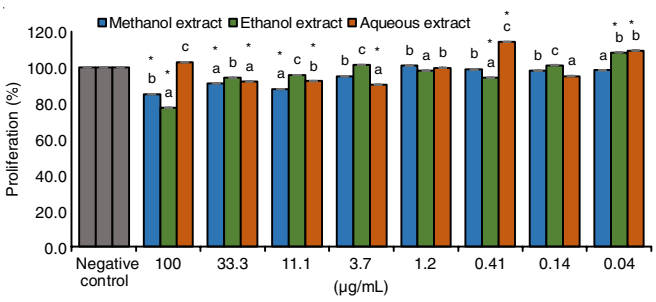

Fig. 3. Antiproliferative effects of the different extracts on the SKU-T cells. Bars with different alphabets are significantly different and bars with "*" are significantly different (higher or lower) from the negative control

Antiproliferative effect of *C. hirta* on prostate cancer cell lines: The *in vitro*, evaluation of the antiproliferative effects of *C. hirta* methanol, ethanol, and aqueous extracts on prostate cancer cell lines PC-3, and DU-145 cells revealed several promising activities. Significant concentration-dependent antiproliferative effects of the aqueous and ethanol extracts on PC-3 cells were observed, with the ethanol extract eliciting the most significant antiproliferative effect, followed by the aqueous extract, while the methanol extract had no significant antiproliferative effect on the PC-3 cells across all concentrations (Fig. 4). Except at 1.2 µg/mL, the ethanol extract elicited significant antiproliferative effects on the cells at concentrations ranging from 0.41 to 100 µg/mL, with the effect at 100 µg/mL being the highest observed across all extracts tested. However, the aqueous extract induced significant antiproliferative effects ranging from 3.7 to 100 µg/mL. Similar to the SKU-T cells, the aqueous and methanol extracts elicited significant proliferation enhancing effects on the PC-3 cells at the lowest concentrations of 0.14 µg/mL and 0.41 µg/mL, respectively.

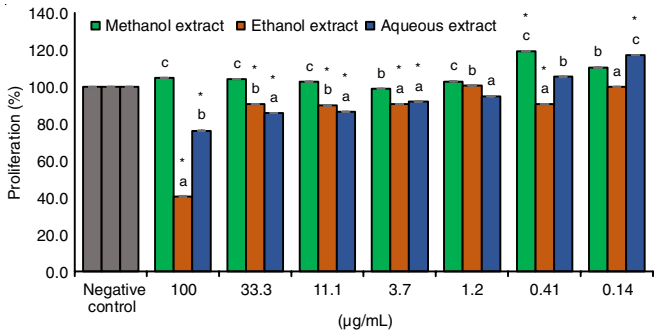

Fig. 4. Antiproliferative effects of the different extracts on the PC-3 cells. Bars with different alphabets are significantly different and bars with "*" are significantly different (higher or lower) from the negative control

The treatments also had significant antiproliferative effects on the DU-145 cells, with only the aqueous extract showing a concentration-dependent trend (Fig. 5). The aqueous extract's activity increased steadily as the percentage proliferation of the cells decreased from 0.04 to 100 µg/mL. The extract's acti-

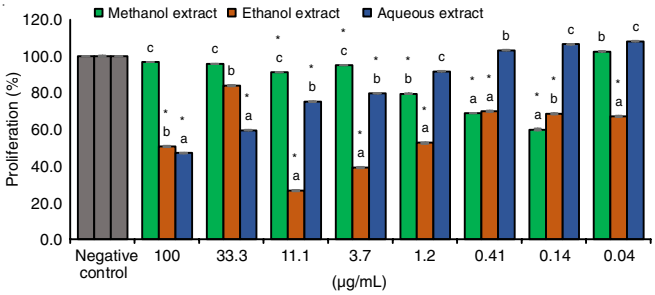

Fig. 5. Antiproliferative effects of the different extracts on the DU-145 cells. Bars with different alphabets are significantly different and bars with "*" are significantly different (higher or lower) from the negative control

vity was significant from 3.7 to 100 µg/mL. Like in the PC-3 cells, the ethanol extract had the highest antiproliferative effects on the DU-145 cells, with significant effects observed at all concentrations except at 33.3 µg/mL. The antiproliferative effects of the ethanol extract were statistically constant from 0.04 to 0.41 µg/mL, after which the effects increased steadily to their maximum at 11.1 µg/mL, where the lowest percentage proliferation of the cells was observed among all the effects elicited by all the extracts at all concentrations tested. The methanol extracts elicited antiproliferative effects that were significant at lower concentrations (0.14 to 11.1 µg/mL). The antiproliferative effect of extract appeared to be inversely proportional to concentration, with increased proliferation of the DU-145 cells observed as the concentration of methanol extract increased.

Thus, with the highest activity observed in DU-145 cells, the ethanol extract of *C. hirta* exhibited the best antiproliferative effects on the prostate cancer cell lines. The methanol extract, on the other hand, exhibited the least anti-proliferative effects, with evidence of proliferation enhancing properties in the PC-3 and DU-145 cells.

Antiproliferative effect of *Cheilanthes hirta* Swartz on gastric cancer cell lines: The *in vitro* analysis of antiproliferative effect of *C. hirta* methanol and aqueous extracts on gastric cancer cell lines (AGS) revealed interesting concentration dependent results (Fig. 6). The aqueous extract elicited the greatest antiproliferative effects, which were significant for all tested concentrations (0.04 to 100 µg/mL) except at 0.14 µg/mL. The effects of aqueous extract were significantly

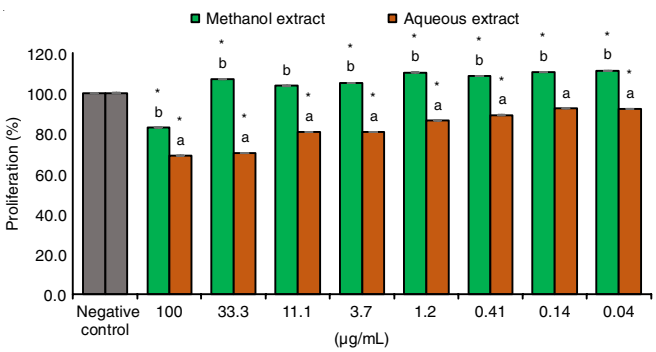

Fig. 6. Antiproliferative effects of the different extracts on the AGS cells. Bars with different alphabets are significantly different and bars with "*" are significantly different (higher or lower) from the negative control

higher than those of the methanol extract across all concentrations tested, with the highest at 100 µg/mL. The methanol extract, on the other hand, only elicited an antiproliferative effect at the highest concentration (100 µg/mL), while at lower concentrations (0.04 to 3.7 µg/mL and 33.3 µg/mL), the extract elicited proliferative effects that increased with decreases in concentration, as the percentage proliferation of the cells was significantly higher than that of the negative control at these concentrations.

Conclusion

This study has provided significant insights into the *in vitro* anticancer potential and phytochemical constituents of *Cheilanthes hirta* Swartz plant extracts. The study found several phytochemicals in *C. hirta* extracts including terpenoids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones, phlobatannins, alkaloids and steroids. The FTIR spectral analysis further confirmed the presence of functional groups associated with these phytochemicals, including hydroxyl groups, carboxylic acids, terminal alkynes, ketones, phenols and phosphate ions. The *in vitro* antiproliferative assays demonstrated that *C. hirta* extracts possess significant anticancer activity against prostate (PC-3 and DU-145), human T-Lymphocytes (SKU-T) and gastric (AGS) cancer cell lines. Among all the extracts, the ethanol extract exhibited the strongest antiproliferative effects on prostate cancer cell lines, particularly the DU-145 cells. The aqueous extract, on the other hand, showed the greatest antiproliferative effects on gastric cancer cell lines. The methanol extract, although less potent, still displayed significant antiproliferative activity, especially at lower concentrations. These findings revealed the potential of *C. hirta* as a source of novel anticancer agents. The presence of diverse bioactive compounds within the plant suggests a multifaceted mechanism of action, potentially involving the induction of apoptosis, inhibition of cell proliferation and modulation of signaling pathways. Further research is warranted to isolate and characterize the specific compounds responsible for these effects and to elucidate their mechanisms of action. Thus, *C. hirta,* with its rich phytochemical profiles and demonstrated anticancer activity, holds promise as a valuable addition to the array of natural anticancer agents. This study contributes to the growing body of evidence supporting the medicinal value of the traditional plants and shows the need for continued exploration of natural resources in fight against cancer.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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