The Anti-cancer Activity of *Vernonia divaricata* Sw against Leukaemia, Breast and Prostate Cancers *In Vitro*

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ABSTRACT

Background: Vernonia divaricata is one of five endemic Vernonia species of Jamaica. The ethnomedicinal uses of other species have been established, however, scientific validation of this species has not yet been done and as such this paper is aimed at identifying the anti-cancer activity of V divaricata against leukaemia, breast and prostate cancer cell lines.

Methods: Leaves and stems of V divaricata were dried and milled into powder. The crude hexane and methanol extracts of the leaves and stems were obtained and bio-assayed using WST-1 cell proliferation assay against leukaemia, breast and prostate cancer cell lines.

Results: The crude hexane and methanol extracts of V divaricata were able to significantly retard the growth of the MCF-7 (breast), HL-60 (leukaemia) and the PC-3 (prostate) cancer cell lines. The crude methanol extract of the stem was the strongest, exhibiting anti-proliferation activity with IC_{50} values of 10.14, 12.63 and 9.894 µg/ml for the HL-60, MCF-7 and PC-3 cancer cell lines, respectively, with the most potent toward prostate cancer.

Conclusion: The medicinal use of V divaricata as an anti-cancer agent was corroborated as the crude hexane and methanol extracts demonstrated potent anti-proliferation activity and as such hold potential for further research and development into a drug to prevent or treat various cancers.

Keywords: Anti-cancerous, breast cancer, Jamaican, leukaemia, medicinal plant, prostate cancer, Vernonia divaricata

La Actividad Anticancerosa de la *Vernonia divaricata* Sw contra la Leucemia, y el Cáncer de Mama y de Próstata *In Vitro*

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RESUMEN

Antecedentes: La Vernonia divaricata es una de las cinco especies endémicas de Vernonia de Jamaica. Aunque se han establecido los usos etno-medicinales de otras especies, aún no se ha realizado una validación científica de esta especie. Por tal razón, este trabajo está dirigido a identificar la actividad anticancerosa de V divaricata contra las líneas celulares del cáncer de mama, el cáncer de próstata, y la leucemia.

Métodos: Hojas y tallos de V divaricata fueron deshidratadas, molidas, y convertidas en polvo. Los extractos crudos de hexano y metanol de las hojas y tallos fueron obtenidos y analizados mediante ensayo de proliferación celular WST-1 contra las líneas celulares del cáncer de mama, el cáncer de próstata, y la leucemia.

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Resultados: Los extractos crudos de hexano y metanol de V divaricata lograron retrasar significativamente el crecimiento de las líneas celulares de cáncer MCF-7 (mamas), HL-60 (leucemia) y PC-3 (próstata). El extracto de metanol crudo del tallo fue el más fuerte, exhibiendo una actividad antiproliferativa con valores IC_{50} de 10.14, 12.63 y 9.894 µg/ml para las líneas celulares de cáncer HL-60, MCF-7 y PC-3, respectivamente, con una mayor potencia hacia el cáncer de próstata.

Conclusión: El uso medicinal de V divaricata como agente contra el cáncer fue corroborado por cuanto el extracto de hexano y el extracto de metanol crudos mostraron una potente actividad antiproliferativa, y por ende un buen potencial para la ulterior investigación y desarrollo de un medicamento para prevenir o tratar varios tipos de cáncer.

Palabras claves: Anticancerígeno, cáncer de mamas, Jamaica, leucemia, planta medicinal, cáncer de próstata, Vernonia divaricata

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INTRODUCTION

Ethno-medicinal practices have formed the basis for many drug discoveries. Jamaica hosts a wide array of medicinal plants, 199 of which have been identified to contain anticancer properties and a large number is still left to be validated (1). Cancer is a physiological disease that affects approximately 12.7 million persons worldwide annually and resulted in 7.6 million deaths in 2008 (2). Breast and prostate cancers are among the top five cancers diagnosed worldwide, affecting 10.9% and 7% of the total population, as estimated by Ferlay *et al* (2). Leukaemia diagnosis is also significant, with 3% of the total population affected (2). As such, research and use of medicinal plants as potential anti-cancer agents will aid in reducing the cancer incidence in today's society.

The Vernonia divaricata Sw (Asteraceae) is an endemic Jamaican herb that grows erect and is known for its folkloric uses, which are similar to those identified within the genus having medicinal properties (3–5). The most commonly used Vernonia species is the V amygdalina, which is used traditionally to control or treat cancer, malaria, diabetes, to fight diseases caused by gastrointestinal parasites and to boost the immune system (5–7). In our continuous search for plants with anti-cancer properties, we report herein the anti-proliferation activity of V divaricata against selected cancer cell lines.

MATERIALS AND METHOD

The leaves and stem samples (250 g) of the *V* divaricata plant were collected from St Andrew, Jamaica, and verified by the Herbarium Department at the Institute of Jamaica (accession number: IJ35262). The plant material was then dried and pulverized into powder prior to solvent extraction with one litre of hexane and subsequently with one litre methanol. The crude extracts were dried *in vacuo* to obtain dark green residues (hexane extracts: stem 0.7 g, leaves 3.1 g and methanol extracts: stem 0.5 g, leaves 2.4 g). The residues were dissolved in dimethyl sulfoxide (DMSO) and stored at -20 C until bioassay.

Three human tumour cell lines (HL-60, MCF-7 and PC-3) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were maintained in minimum essential media supplemented with 10% fetal calf serum, 1% L-glutamine, 2% penicillin-streptomycin and 0.2% gentamicin.

The WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzene disulfonate) (Roche) colorimetric assay was used (8). The cells were trypsinized and plated into 96-well plates in 50 µl of media and incubated overnight. Approximately 18 hours after plating, 50 µl of media containing the required drug concentration was added per well. Cells were plated at a density to initiate a 72-hour post-drug addition, and thus the cells will be in log phase (500-2000 cells/well). The compounds and extracts were solubilized in DMSO. The cells were then allowed to proliferate for 72 hours at 37 °C in humidified atmosphere of 5% carbon dioxide (C Q). The experiment was terminated using WST-1 (Roche) 10 µl per well and absorbance read at 450 nm/690 nm. The effect of drugs on growth was assessed as per cent of cell viability compared to the control. The IC_{50} values were determined from the extract dose versus control growth curves using Graphpad Prism software. All experiments were carried out in duplicate and the mean results determined.

RESULTS

The crude methanol and hexane extracts were tested for their bioactivity against three cancer cell lines: MCF-7 (breast), HL-60 (leukaemia) and the PC-3 (prostate). The IC₅₀ values obtained showed significant anti-proliferation when the samples were tested. The crude hexane extract of the leaves caused growth retardation of 24.20 μ g/mL, 22.06 μ g/mL and 26.27 μ g/mL with the leukaemia, breast and prostate cancer cell lines, respectively (Fig. 1). The crude hexane extract of the stem (Fig. 2) had IC₅₀ values of 13.84 μ g/mL (leukaemia), 22.15 μ g/mL (breast) and 21.94 μ g/mL (prostate). However, the crude methanol leaves extract produced the strongest anti-proliferation (Fig. 3) as the IC₅₀ values were



Fig. 1: The anti-proliferation activity of leaves of the crude hexane extracts of *Vernonia divaricata* in the WST-1 cell proliferation assay after 72 hours of incubation.



Fig. 2: The anti-proliferative activity of the crude hexane extract of *Vernonia divaricata* stems in the WST-1 cell proliferation assay after 72 hours of incubation.



Fig. 3: The anti-proliferation activity of the crude methanol extract of *Vernonia divaricata* leaves in the WST-1 cell proliferation assay after 72 hours of incubation.

10.14 μ g/mL (leukaemia), 12.63 μ g/mL (breast) and 9.894 μ g/mL (prostate) for the respective cancer cell lines.

DISCUSSION

The medicinal use of *V* divaricata as an anti-cancer agent was significant in retarding the growth of cells from the three different cancer cell lines (HL-60, MCF-7 and PC-3) as depicted in this research. Other *Vernonia* species have been known to contain anti-cancer activity, such as *V* cinerea, *V* acuminata and *V* amygdalina, which may be due to the presence of sesquiterpene lactones within these plants (9–13).

The stems produced a greater anti-proliferation activity, as seen with the crude hexane extract of *V divaricata*

against leukaemia, when compared with the crude hexane extract of the leaves. There was no significant difference in the IC₅₀ values against the breast cancer MCF-7 cell line (22.15 *vs* 22.06 μ g/mL for the stem and leaves, respectively) and the prostate cancer cell line (21.94 *vs* 26.27 μ g/mL for the stem and leaves, respectively).

The crude methanol extract of the leaves of V *divaricata* was the most potent overall in preventing the growth of the different cancer cells. There was strong antiproliferation activity against prostate cancer (IC₅₀: 9.894 µg/mL), which was significantly stronger in retarding growth compared to 10.14 µg/mL and 12.63 µg/mL with the leukaemia and breast cancer cell lines, respectively.

From the literature, extract of *V cinerea* revealed the presence of 12-oleanen-3-ol-3 β -acetate, lupeol, β -sitosterol and stigmasterol. The anti-cancer effect of *V divaricata* may be due to the steroids, esters, triterpinoids and glycosides present within the methanolic extract of the stem and bark, similar to those found in the *V cinerea* plant (14, 15).

CONCLUSION

The crude hexane and methanol extracts of *V* divaricata exhibited anti-proliferation activity against breast, prostate and leukaemic cancer cell lines. The methanol leaves extract was the most potent of the three extracts. *Vernonia divaricata* has been identified as a potential anti-cancer agent based on the findings of this study. Further studies will be required to isolate the active compounds in *V* divaricata.

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