



Short Communication

Anti-angiogenic properties of the Jamaican ball moss, (*Tillandsia recurvata* L.)

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Abstract

The island of Jamaica is known for its rich biodiversity and its abundance of medicinal plants being used as ethno medicines. Given the high occurrence of cancer worldwide and the major source of the discovery of new lead molecules being medicinal plants, this research undertook to investigate the anti-angiogenic properties of a chloroform extract from the Jamaican ball moss previously shown to exhibit anti-cancer properties in our lab and its dicinnamate isolates, 1,3-di-O-Cinnamoyl-glycerol (D1) and (E)-(2-(3-(3-(3,4-dimethoxyphenyl)acryloyloxy)-2-hydroxypropoxy)-2-oxoethyl)triphenylphosphonium bromide (D2) along with known chemotherapeutic drug, paclitaxel. This was investigated using a modified version of the ex vivo aortic ring sprouting assay. Results show that angiogenesis was reduced in the presence of the chloroform crude extract at 20 µg/ml and 30 µg/ml and also in the presence of D1 at 10 µg/ml and 20 µg/ml when compared to the control. The results substantiate the promising anti-cancer properties of the Jamaican ball moss and may prove useful in anti-cancer agents' drug discovery.

Keywords: Jamaican ball moss, *Tillandsia recurvata* L., anti-angiogenesis, cancer.

Introduction

Cancer is a broad group of diseases all involving de-regulated cell proliferation and is the leading cause of death in Jamaica¹ and the third leading cause of death worldwide^{2,3,4} with an annual projection of 26 million new cases by 2030 of which 17 million deaths will occur⁵. It is a well-established theory that the growth of malignant tumours parallels the incremental increase in vascular growth. Tumours starved of angiogenesis will remain benign for an indefinite period while those exposed experience a rapid exponential growth. The tumour angiogenic switch seems to be stimulated when the equilibrium of angiogenic inhibitors to stimulators is shifted toward a pro-angiogenic locale^{6,7}. It is therefore prudent to investigate molecular entities that are not just able to reduce cancer cell viability but also angiogenesis as well.

The island of Jamaica is known for its rich biodiversity and its abundance of medicinal plants used as ethno medicines. Bioactive screens originated here in Jamaica on the plant *Catharanthus roseus*, commonly known as periwinkle from which the world now benefits from the widely used chemotherapeutic drugs, vincristine and vinblastine. The indigenous plant, *Petiveria alliacea* commonly known as guinea hen weed has also been found to exhibit promising anti-cancer activity^{8,9,10} and is presently being used in the nutraceutical industry for the treatment of prostate cancer. Other bioactive plants both local and abroad include; *Cannabis sativa*¹¹, *Carica*

papaya^{11,12}, *Stevia rebaudiana*¹³, *Chromolaena odorata*¹⁴, *Vitex trifolia var*¹⁵, *Punica gratum*¹⁶, *Prosopis juliflora*¹⁷ amongst many others¹¹. Medicinal plants remain a major source for the discovery of new lead molecules for development into novel therapies¹⁸, such as; paclitaxel, vincristine, and camptothecin¹⁹.

This research undertook mechanistic anti-cancer screens on the Jamaican Ball Moss; *Tillandsia recurvata* L. (Bromeliaceae) previously reported to exhibit promising anticancer activity²⁰. The plant is found throughout the island and is an epiphytic weed that grows abundantly on Mango trees, Oak trees and on power lines. The plant is characterized by the presence of a rudimentary root system and shoots having 5-8 linear leaves forming a rosette, covered with peltate, absorptive trichomes. Older plants develop multiple ramets which form a spherical tussock range in size of a golf ball up to the size of a basketball, hence the common name "ball moss"²¹. In Brazil, the plant is used against rheumatism, ulcers and haemorrhoids²². In our lab, preliminary in vivo studies revealed that apart from the inhibition of tumour growth by the crude extract of ball moss by way of apoptosis, tumours in the treatment group had no blood compared to tumours in the control group. On the basis of this observation, we hypothesized that inhibition of angiogenesis was one of the possible mechanisms of action of the Jamaican ball moss and undertook to investigate the possible anti-angiogenic action of this plant.

Material and Methods

Chemicals: BD Matrigel Matrix was purchased from BD bioscience and dimethyl sulfoxide (DMSO) was purchased from American Bioanalytical.

Anti-angiogenic assay: This assay is a modified method of the ex vivo aortic ring sprouting assay of Grant et al.²³. The sprouts represent all phases of angiogenesis (invasion, proliferation, migration and tube formation). The thoracic aorta was dissected from 5-6 weeks old Sprague-Dawley rats (Harlan, Frederick, Maryland) weighing 170 g each and the peri-aortic fibro adipose tissue was removed and transverse sections (1-2 mm) made. The aortic rings were rinsed extensively in 5 consecutive washes of EBM (endothelial cell basal media). Twenty four well culture plates were used to embed the rings in 200 µl of Matrigel ensuring that the lumen was parallel to the base of the plate. Following the addition of 1 ml of EBM without ECGS (endothelial growth supplement) to each well, the rings were incubated for 24 h at 37°C and 5% CO₂ in humidified air. The media was subsequently replaced with 1 ml of EBM containing ECGS and varying concentrations (0-30 µg/ml) of LM extracts and compounds for 4-5 days after which sprout formation was evaluated. The minimum drug concentration that inhibited capillary sprout formation compared to control were captured with a Nikon FDX-35 camera mounted onto a Nikon Eclipse TE300 microscope linked to a computer for visualization and processing of digital images. All animals were housed in the Institute of Human Virology Animal Facility at the University of Maryland, School of Medicine in accordance with the institutional guidelines. The experiment was repeated 3 times.

Plant material: *Tillandsia recurvata* (extraction and isolation): The whole *T. recurvata* plant was collected from trees and electricity poles at Kingston, Jamaica. A voucher specimen of the plant was identified at the Institute of Jamaica Herbarium where it is deposited with Accession Number: IJ 3411. The collected plant material was air dried, pulverized into powder and exhaustively extracted with chloroform. The resulting residues were subjected to flash chromatography (Varian 971-FP Intelliflash) using normal phase silica gel column (600g). Elution with hexane and ethyl acetate (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 2:8 and 0:10) and re-crystallization yielded pure compounds D1 and D2 (figure-1). The identity of the purity of the compounds were determined using, IR (thin film NaCl) using a Nicolet iS10 FTIR spectrometer and NMR was performed using a Varian Mercury 400 MHz instrument operating at 400.1271 MHz for proton and 100.6120 MHz for ¹³C.

Results and Discussion

Anti-angiogenic investigations showed that the chloroform extract from the Jamaican ball moss and its dicinnamate isolates reduced angiogenesis as shown in table 1. The crude and pure compounds inhibited capillary sprouts at varying concentrations. D1 inhibited sprout formation at 10 µg/ml and

20 µg/ml while the crude extract inhibited sprout formation at 20 µg/ml and 30 µg/ml. D2 showed no activity and this could be due to the two methoxy groups present (figure 1). There is evidence that capillary sprouts are known to be representative of all phases of angiogenesis (i.e. invasion, proliferation, migration and tube formation)²³. The formation of new blood vessels facilitate the supply of nutrients to tumour cells and is an important requirement in tumorigenesis^{24,25}. Evidence not only supports the survival and rapid growth of tumours being analogous to the constant supply of nutrients²⁶ but Algire²⁷ demonstrated that solid tumours themselves induce angiogenesis in vivo while Greenbalt and Shubik²⁸ provided evidence supporting the humoral induction of tumour angiogenesis. It is therefore apparent that several factors play a role in the promotion of angiogenesis which makes its inhibition even more critical. Inhibition of angiogenesis is one of the attributes of some anticancer drugs such as paclitaxel since stopping the development of the vessels that supply nutrients to tumour cells lead to starvation and eventual death of tumour cells²³. It is for this attribute that paclitaxel has been deemed one of the most active anti-cancer drugs²⁹. Results obtained from this current research concretize the anti-tumour activity of the ball moss crude extract and its isolate D1. The ability of the extract to inhibit angiogenesis implicates its promise in being developed as an anti-cancer agent as well as its ability to stop, halt or reverse the onset of certain cancers.

Conclusion

In conclusion, the results obtained from this study validate the anticancer activity of the Jamaican Ball Moss in addition to providing insight into one of its likely mechanisms of action being anti-angiogenesis which can also be deemed chemopreventive. The results may prove useful in anti-cancer drug discovery.

Acknowledgment









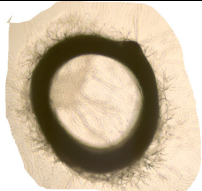
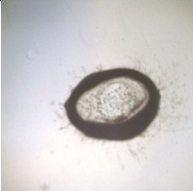
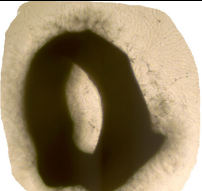

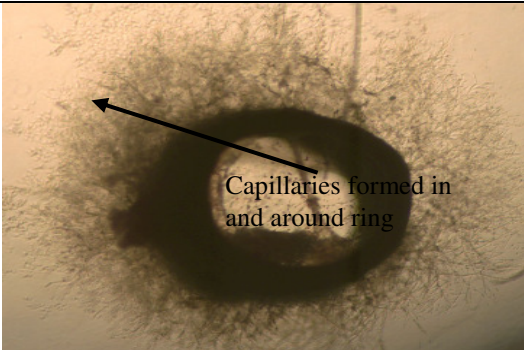
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Table-1
Ex vivo anti-angiogenic activity of Ball Moss and isolated dicinnamates (D1 and D2) compared to paclitaxel, Reduction in angiogenesis as observed by D1 (10 µg/ml) and the ball moss extract (30 µg/ml) compare well with known chemotherapeutic drug paclitaxel (at 10 and 20 µg/ml respectively)

Ball Moss extract	D1	D2	Paclitaxel
			
30 µg/mL	20 µg/ml	20 µg/ml	20 µg/ml
			
20 µg/ml	10 µg/ml	10 µg/ml	10 µg/ml
			
10 µg/ml NA	5 µg/ml (NA)	5 µg/ml	5 µg/ml
Control			
 <p>Capillaries formed in and around ring</p>			

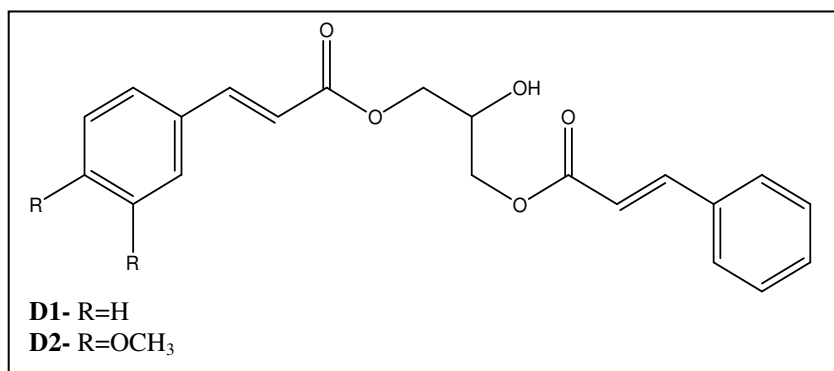


Figure-1
Dicinnamates isolated from Ball Moss