Antileukemic Activity of *Tillandsia recurvata* and Some of its Cycloartanes

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Abstract. *Background: Approximately 250,000 deaths were caused by leukemia globally in 2012 and about 40%-50% of all leukemia diagnoses end-up in death. Medicinal plants are a rich source for the discovery of new drugs against leukemia and other types of cancers. To this end, we subjected the Jamaican ball moss (Tillandsia recurvata) and its cycloartanes, as well as some analogs, to in vitro screening against a number of leukemia cell lines. The WST-1 anti-proliferation assay was used to determine the anticancer activity of ball moss and two cycloartanes isolated from ball moss and four of their analogs against four leukemia cell lines (HL-60, K562, MOLM-14, monoMac6). Ball moss crude methanolic extract showed activity with a 50% inhibition concentration (IC_{50}) value of 3.028 μg/ml against the Molm-14 cell line but was ineffective against HL-60 cells. The six cycloartanes tested demonstrated varying activity against the four leukemia cancer cell lines with IC_{50} values ranging from 1.83 μM to 18.3 μM. Five out of the six cycloartanes demonstrated activity, while one was inactive against all four cell lines. The preliminary activity demonstrated by the Jamaican ball moss and its cycloartanes against selected leukemia cell lines continues to throw light on the broad anticancer activity of ball moss. Further studies to evaluate the efficacy of these molecules in other leukemia cell lines are required in order to validate the activity of these molecules, as well as to determine their mechanisms of action and ascertain the activity in vivo in order to establish efficacy and safety profiles.*

Leukemia is a common type of cancer that affects the blood or bone marrow with a majority of diagnoses made in adults, even though the disease accounts for one-third of all cancer in children under the age of 15 years and 25% of cancer occurring before the age of 20 years (1). Children suffer mostly from acute forms of leukemia, while chronic myeloid leukemia is occurs in adults and rarely seen in children. Chronic lymphocytic leukemia accounts for about one-third of all leukemia cases, while acute myeloid leukemia accounts for more than one-third of all leukemia deaths (2). According to recent global cancer statistics, leukemia is the seventh and ninth leading cause of death due to cancer in males and females, respectively, accounting for about 250,000 deaths annually (3). In the United States alone, it is estimated that 48,610 new cases and 23, 720 deaths will be reported in 2013 (2).

Unlike other forms of cancer, chemotherapy is the most frequently used form of treatment for leukemia. The chemotherapeutic agents are used either in combination or as single agents, and transfusion of blood components are usually applied as supportive treatment. In some circumstances, bone marrow transplantation is also used in treating leukemia.

Plant-derived natural products have contributed significantly to the treatment of leukemia, including agents such as vinblastine and vincristine derived from the vinca-alkaloids from the periwinkle plant, *Catharanthus roseus*, which was collected from Jamaica for investigation in Canada for its anti-diabetic properties but ended-up yielding a treatment for leukemia (4, 5). Even though these treatments may have greatly improved the prognosis for individuals with leukemia, chemotherapy-associated toxicities remain a major problem in the application of these therapies. As a result, there is a great need for the continuous search for effective and less toxic drugs. To this end, the objective of this study was to evaluate the antileukemia activity of the Jamaican ball moss (*Tillandsia recurvata*) and its cycloartanes, which have shown promise against other cancer cell lines (6, 7).

Materials and Methods

Plant material and chemical samples. The Jamaican ball moss (*T. recurvata*) was collected at Kingston, Jamaica and processed as previously reported (7, 8). A voucher specimen of the plant was

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Table I. *Results of the antiproliferative activity of cycloartanes against HL-60, K652, Molm-14 and MonoMac6 cells.*

Compound (Figure 1 structure)	IC_{50} (μM)			
	HL-60	K562	MOLM-14	MonoMac6
Cycloart-23-ene-3,25-diol (**1**)	16.10±2.14	4.06±0.53	11.62±1.53	18.30±5.04
Cycloartane-3,24,25-triol (**2**)	7.07±30	4.27±2.12	3.15±0.04	3.70±0.09
Cycloart-25-ene-3,24-diol (**3**)	NI	NI	NI	NI
3,23-Dioxo-9,19-cyclolanost-24-en-26-oic acid (**4**)	2.53±0.28	1.83±0.61	NI	8.72±2.14
24,25-Dihydroxycycloartan-3-one (**5**)	NI	NI	4.58±0.89	2.89±0.71
Hydroxycycloart-23-en-3-one,25, (**6**)	NI	5.13±1.02	NI	2.89±0.25

NI: No inhibition.

identified at the Institute of Jamaica Herbarium where it is deposited under Accession Number: IJ 3411. A methanolic extract was used for the study. Cycloart-23-ene-3,25-diol (**1**), and cycloart-25-ene-3,24-diol (**3**) were identified and isolated from samples of *T. recurvata* as previously reported (9-11). Cycloartane-3,24,25-triol (**2**), 3,23-dioxo-9,19-cyclolanost-24-en-26-oic acid (**4**), 24,25-dihydroxycycloartan-3-one (**5**), and hydroxycycloart-23-en-3-one,25 (**6**) which are close analogs of the cycloartanes identified in *T. recurvata*, were sourced commercially from ChemFaces Biochemical Co Ltd., Wuhan, China. The compounds were all at least 98% pure as confirmed by High performance liquid chromatography (HPLC). The structures of the six cycloartanes are presented in Figure 1.

Cell lines and culture medium. Two leukemia cell lines HL-60 and K562 were obtained from the American Type Culture Collection (Manassas, VA, USA), while MOLM14 and MonoMac6 leukemia cells were obtained from Dr. Rena Lapidus of the University of Maryland School of Medicine. The cells were maintained in minimum essential medium supplemented with 10% fetal calf serum, 1% L-glutamine, 2% penicillin–streptomycin, and 0.2% gentamicin.

Antiproliferation assay. The 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate (WST-1) (Roche, Indianapolis, IN, USA) colorimetric assay was used (12). Briefly, on the day the experiment was initiated, cells were plated into 96-well plates in 50 μl of medium and incubated overnight. The next day, approximately 18 h after plating, 50 μl of medium containing the required drug concentration was added per well. The compounds and extracts were solubilized in DMSO. Cells were plated at a density so that 72 h after drug addition, the cells were in log phase (500-2,000 cells/well). The cells are allowed to proliferate for 72 h 37˚C in humidified atmosphere of 5% CO_2. The experiment was terminated using WST-1 (Roche) 10 μl per well and absorbance was read at 450 nm/690 nm. The effect of drugs on growth was assessed as the percentage of cell viability. The 50% inhibition concentration (IC_{50}) values were determined from the extract dose *versus* control growth curves using GraphPad Prism software (La Jolla, CA, USA). All experiments were carried out in duplicate and the mean results with standard deviations determined.

Results

The antiproliferative activity of the Jamaican ball moss and two cycloartanes isolated from ball moss and four of their analogs against four leukemia cancer cell lines, HL-60 (acute promyelocytic leukemia); K562 (acute lymphoblastic leukemia); MOLM-14 and monoMac6 (acute monocytic leukemia) were determined in this study. The results of the antiproliferative activity of the Jamaican ball moss against two leukemia cell lines are presented in Figure 2. The six cycloartanes were tested for antiproliferative activity against all four leukemia cell lines and the results are presented in Table I.

The ball moss extract in this study showed selectivity in inhibiting the MOLM14 cell line but failed to inhibit the HL-60 cell line. The cycloartanes exhibited a varying activity with compounds **1** and **2** exhibiting activity against all four leukemia cell lines, compound **4** active against three cell lines, and compounds **5** and **6** active against two cell lines each. Compound **3** was not active against any of the cell lines.

Discussion

The bioactivity demonstrated by the Jamaican ball moss against the leukemia cell lines, as well as the activity of its cycloartanes, demonstrate their broad anticancer activity, considering previous findings showing that the Jamaican ball moss has anticancer activity against other cell lines including prostate and breast cancers and melanoma (7, 8). Ball moss has also exhibited activity in other assays, helping to explain its possible mechanism of anticancer action. Notable amongst these is its antiangiogenic activity (13), inhibition of FMS-like tyrosine kinase 3 (FLT3)(9), as well as induction of cell death *via* apoptosis (7). The inhibition of FLT3 kinase validates the activity of ball moss against the Molm-14 leukemia cell line in this study, as

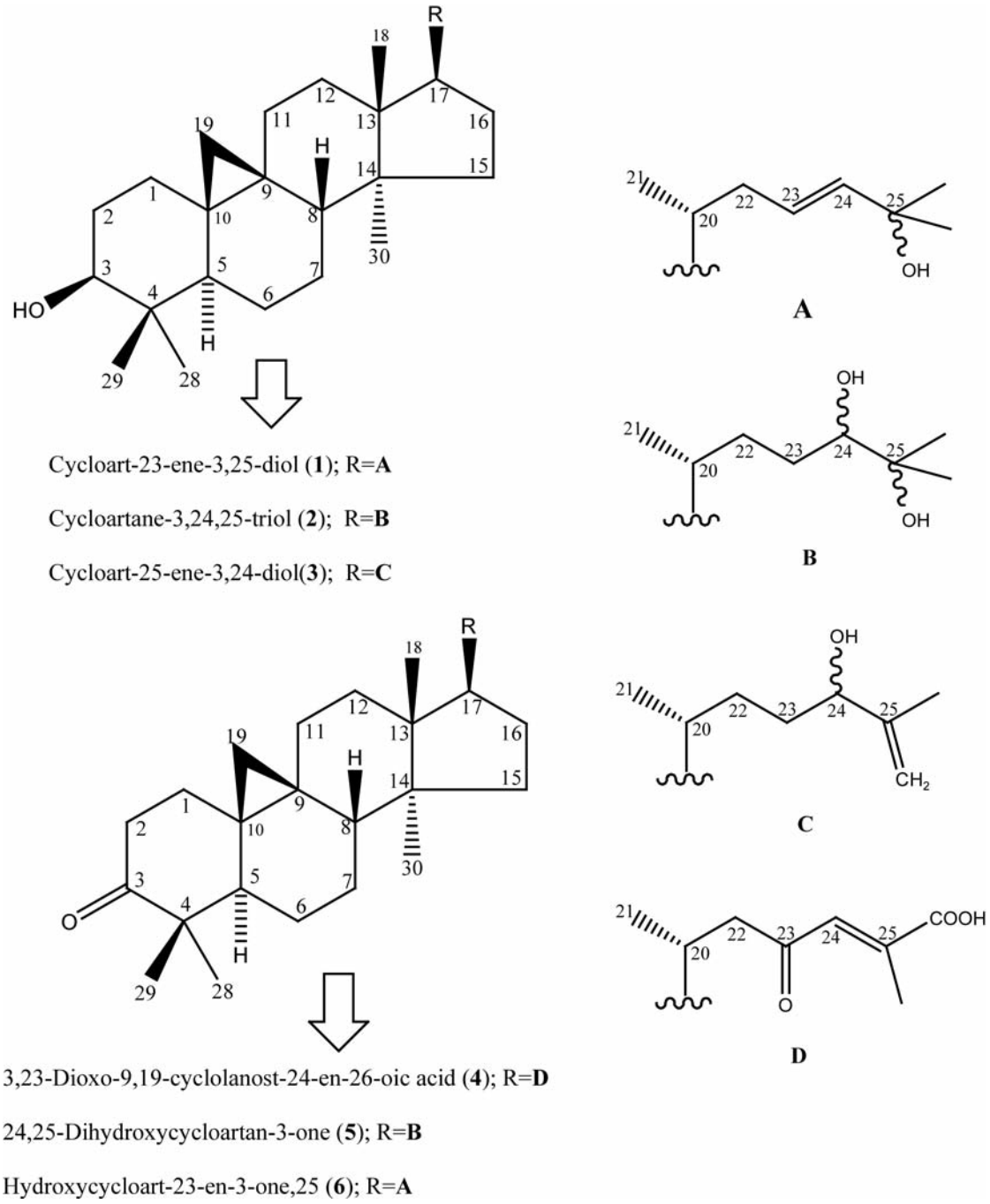

Figure 1. *Structures of the six cycloartanes used in this study.*

FLT3 kinase has been found to be expressed in acute myeloid leukemia cell lines and its mutations are usually associated with poor prognosis (14, 15). The cycloartanes included in this study have also already been shown to possess varying degrees of anticancer activity against other cancer cell lines notably prostate and breast cancer (6). Compounds **1-5** have also demonstrated an inhibitory activity against the myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK), pointing to their possible mechanism of action (6).

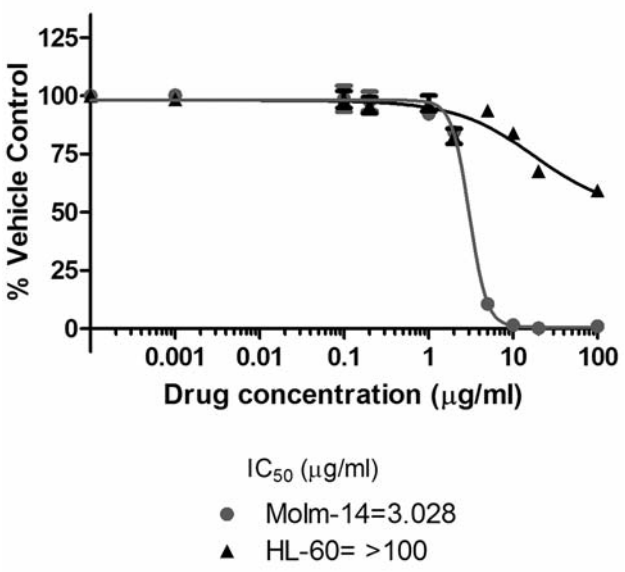

Figure 2. *Antiproliferative effects of the Jamaican ball moss extract on HL-60 and Molm-14 leukemia cells. Cells were plated into 96-well plates and dosed with ball moss methanolic extracts at different concentrations and incubated for 72 h. Cell proliferation was then measured using the WST-1 assay. Results were graphed and the 50% inhibition concentration (IC_{50}) values were calculated using GraphPad Prism software. The ball moss extract showed activity against Molm-14 but not against HL-60. Experiments were performed in duplicate.*

In terms of structure–activity relationship, the six cycloartanes demonstrated varying degrees of activity which could be associated with their structural differences. Compounds **1-3** differ from each other only in the side-chain attached to carbon 17 (Figure 1) but interestingly, only compounds **1** and **2** exhibited activity against all four leukemia a cell lines with compound **3** not having activity against any cell line. Compound **3** was also not active against prostate and breast cancer cell lines in a recent study (6) and this may be attributed partially to its poor solubility. However, it might also be attributed to selectivity, as compound 3 was shown to have antiproliferative activity in the phytohemagglutinin (PHA)- induced T-cell proliferation assay and against Ehrlich ascites tumor cells (16, 17). The most potent inhibition was observed against the K562 acute lymphoblastic leukemia cell line, with an IC_{50} of 1.83 μM by compound **4**. Compound **4** differs from compounds **1** and **2** in that it possesses a 3-oxo group instead of a 3β-hydroxyl group in the number 3 carbon position, as well as a significant difference in the side chain. Compound **5** in this study inhibited MOLM-14 and MonoMac6 leukemia cell lines but not HL-60 and K562. These results are in contrast to those obtained from a previous study that showed compound **5** to be active against HL-60 with IC_{50} of 19.00 μM (18). This difference in activity may be attributed to various factors, including the assay sensitivity, given that the MTT assay was used in a previous study while the WST-1 assay was used in the current study. From the structure–activity relationship, the diverse structures of cycloartanes seem to yield significantly different activity profiles. For example, a cycloartane isolated from the stigma of *Zea mays* L. possessing two hydroxyl groups in the number 2 and 3 carbon positions had no activity against HL-60 compared to compounds **1** and **2** in this study, which have hydroxyl groups in the number 3, 24 and 25 carbon positions (19).

The use of medicinal plants to treat leukemia is a common practice in many developing countries and it is interesting to note that *Astragalus membranaceus*, a plant that is used in Chinese and other folk medicines to treat leukemia and other types of cancer, is also the source of several cycloartanes (20-22). Cycloartanes have also been shown to have anti-inflammatory activity, and cancer-chemopreventive effects, as well multidrug-resistance reversal activity (23, 24). For example, compounds **1** and **2** have been found to enhance drug retention in cells by inhibition of the efflux pump activity promoted by P-glycoprotein, preventing multidrug resistance onset (23).

Conclusion

The activity of Jamaican ball moss extracts and its cycloartanes against leukemia cell lines is another indication that ball moss possesses compounds with broad anticancer activity given our previous reports on its activity against breast, Kaposi sarcoma and prostate cancer (6-8). Even though the antileukemia activity of the Jamaican ball moss extracts is so far not at the same level as the activity of the vinca alkaloids discovered in 1952 by Clark Noble from another Jamaican plant, Cathantheus reseus (5, 25), the preliminary results warrant further studies against other leukemia cell lines. At least 20 cycloartanes have been identified in the organic solvent extracts of Jamaican ball moss and their isolation could yield a cycloartane more potent than the ones already tested. Based on the results obtained from the study of the six cycloartanes here, compound **4** would be a good candidate for structural modification in activity optimization efforts. Further studies are required to validate the activity *in vivo*, as well as for the isolation and screening of other molecules from this plant which could lead to the discovery and development of a new antileukemia agent.

Conflicts of Interest

None declared.

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