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Original Article

Antiproliferative effects of pinostrobin and 5,6-dehydrokavain isolated from leaves of *Alpinia zerumbet*

Walter A. Roman Junior[a,b,*], Denise B. Gomes[a], Barbara Zanchet[a], Amanda P. Schönell[b], Kriptsan A.P. Diel[b], Thais P. Banzato[c], Ana L.T.G. Ruiz[c], João E. Carvalho[c,d], Angelita Neppel[e], Andersson Barison[e], Cid Aimbiré M. Santos[f]

[a] *Programa de Pós-graduação em Ciências da Saúde, Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brazil*
[b] *Grupo de Pesquisa em Fitoquímica e Farmacologia de Produtos Naturais, Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brazil*
[c] *Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, Divisão de Farmacologia e Toxicologia, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[d] *Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[e] *Departamento de Química, Universidade Federal do Paraná, Curitiba, PR, Brazil*
[f] *Laboratório de Farmacognosia, Departamento de Farmácia, Universidade Federal do Paraná, Curitiba, PR, Brazil*

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ABSTRACT

Natural products are a major source of drugs for the treatment of cancer. The species *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm, Zingiberaceae, is widely distributed in Brazil where it is known as "colônia". The leaves are commonly used in the treatment of hypertension and dyspepsia, however, the effects of *A. zerumbet* extracts and isolated substances on human cancer cells remain to be elucidated. This study was designed to identify the chemical constituents of hydroalcoholic and dichloromethane extracts from *A. zerumbet* leaves and to investigate their *in vitro* antiproliferative activity. The isolated phytochemicals included kaempferol, dihydro-5,6-dehydrokavain, 5,6-dehydrokavain, and pinostrobin. The hydroalcoholic extract inhibited cellular proliferation only at high concentrations, while the dichloromethane extract showed a moderate antiproliferative effect against leukemia and lung tumor cell lines. 5,6-Dehydrokavain showed potent cytostatic activity against glioblastoma cells and a moderate effect on all other tumor cell lines. Pinostrobin showed potent activity against leukemia and breast tumor cell lines and moderate cytostatic effect against ovarian cell. Furthermore, this is the first report on the isolation of kaempferol and pinostrobin from *A. zerumbet* leaves. Moreover, the purification process described in this study was effective. These results suggest that *A. zerumbet* leaves are a promising source of anticancer compounds.

Introduction

Cancer is a serious public health problem representing the second largest cause of death (Siegel et al., 2016). Over the next two decades, approximately 22 million cases per year are expected worldwide (McGuire, 2016). Cancer is the name given to a set of more than 100 diseases that have in common the uncontrolled growth of (malignant) cells that invade tissues and organs and can spread (metastasize) to other parts of the body. Dividing rapidly, these cells tend to be very aggressive and uncontrollable, causing the formation of tumors or malignant neoplasms (Almeida et al., 2005; Elkady et al., 2016).

The treatment of cancer is based mainly on surgical resection of the tumor mass and/or administration of radiotherapy, immunotherapy, and/or chemotherapy. However, many cancers still exhibit only modest clinical responses to protocols developed for either primary tumors or metastases (Costa-Lotufo et al., 2010). Moreover, many anticancer agents have high rates of adverse reactions and toxicity as well as a low selectivity for tumor cells (Prakash et al., 2013; Topcul and Cetin, 2014). Therefore, with the objective of finding more effective and safe treatments, pharmacological studies with substances isolated from plants, as well as synthetic derivatives based on these natural compounds, have intensified (Harvey et al., 2015; Newman and Cragg, 2016).

* Corresponding author.
E-mail: romanwa@unochapeco.edu.br (W.A. Junior).

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Natural products have been reported to act directly or indirectly *via* multiple cell signaling pathways. Thus, the combination of traditional chemotherapeutic drugs with extracts and/or isolated substances could provide an effective alternative for cancer treatment or overcome chemoresistance (Apaya et al., 2016). Among natural products, phenolic compounds, including flavonoids, have been shown to have an array of pharmacological activities (Kristo et al., 2016) such as anti-inflammatory, cancer chemopreventive, and chemotherapeutic (García-Lafuente et al., 2009; George et al., 2017).

Alpinia zerumbet (Pers.) B.L. Burtt & R.M. Sm, Zingiberaceae, is native to China and Japan and cultivated in Brazil where is popularly known as falso-cardamomo, pacová, gengibre-concha, and colônia (Lorenzi and Matos, 2002; Lorenzi and Souza, 2008). This plant is herbaceous, tropical perennial, rhizomatoza, with stem short that can reach up to 3 m tall. It bears funnel-shaped flowers and the leaves are lanceolate, aromatic, and of coriaceous consistency (Correa et al., 2010; Saboo et al., 2014). The *A. zerumbet* leaves are traditionally used in dyspepsia treatment and as ananthelmintic, in addition to their antimicrobial, anti-inflammatory, and antihypertensive properties (Almeida, 1993; Correa et al., 2010). In addition to kava pyrones, dihydro-5,6-dehydrokavain (DDK), and 5,6-dehydrokavain (DK), rutin, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucuronide, (+)-catechin, and (−)-epicatechin, have been reported (Mpalatinos et al., 1998; Elzaawely et al., 2007; Xuan and Teschke, 2015; Kumagai et al., 2016). The pharmacological properties attributed to extracts of *A. zerumbet* leaves, flowers, seeds, and rhizome include diuretic and hypotensive effects (Laranja et al., 1991; Mendonça et al., 1991; Lahlou et al., 2003), antioxidant (Elzaawely et al., 2007), vasodilatory (Pinto et al., 2009; Victório et al., 2009), hypolipidemic (Lin et al., 2008), and antidepressant-like effect (Bevilaqua et al., 2016). Further, antineoplastic effects of *Alpinia officinarum* Hence (Ghil, 2013) and *Alpinia galanga* (L.) Willd. (Samarghandian et al., 2014) rhizome extracts have been described. However, there are no reports regarding the antiproliferative activity of *A. zerumbet* extracts and isolated substances.

In this context, the present study aimed to investigate the chemical composition and the *in vitro* antiproliferative effects of *A. zerumbet* leaf extracts and their major constituents.

Material and methods

Standards and chemicals

All solvents and reagents were of analytical grade and water was distilled and deionized. The solvents used were ethyl acetate, methylene chloride, ethanol, and hexane (Vetec®, Rio de Janeiro, Brazil). Nuclear magnetic resonance (NMR) experiments (^{1}H and ^{13}C) were performed on a Bruker Avance 400 spectrometer (400 and 100 MHz, respectively) using $CDCl_3$ as solvent. Chemical shifts of ^{1}H and ^{13}C NMR were expressed in ppm (δ) using TMS at 0.00 ppm as an internal standard, and coupling constants (J) in Hz. Column chromatography was performed on silica gel (Merck, Darmstadt, Germany; 230–400 mesh ASTM), and analytical thin layer chromatography (TLC) was performed using silica gel plates (Kieselgel 60 F_{254}, Merck). The spots were visualized using ultraviolet light (366 nm) or by spraying with 10% H_2SO_4 in methanol followed by heating at 110 °C (10 min).

Plant material

The leaves of *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm, Zingiberaceae, were collected in Chapecó (SC), Brazil (26°58′36.06″S and 52°44′27.18″W). The plant material was identified by Osmar dos Santos Ribas, the herbarium curator of the Municipal Botanical Museum of Curitiba (PR), where a voucher specimen was deposited (MBM #306196).

Preparation of extracts of A. zerumbet *and chemical isolation*

The leaves of *A. zerumbet* were dried at room temperature (25 ± 5 °C), pounded in a knife mill (Ciemlab®, CE430), passed through sieve (425 μm; 35 Tyler/Mesh), identified, and stored protected from light. The extracts were produced by maceration (5 days) at room temperature using dry-milled leaves of *A. zerumbet* (100 g) in solvent (1:20, w/v), first, with dichloromethane and, subsequently, 70% ethanol. After filtration through Büchner funnel, the dichloromethane (DEA) and hydroalcoholic (HEA) extracts were concentrated by evaporation under reduced pressure, lyophilized, weighed, and stored in a freezer at −20 °C.

A sample of DEA extract (4 g) was dissolved in hexane and submitted to column chromatography using a stationary phase silica gel (Merck, Darmstadt, Germany) and eluted with hexane:ethyl acetate (EtOAc) (90:10, v/v) increasing in polarity to 90% EtOAc (v/v) to yield four subfractions. The subfractions were analyzed by TLC using hexane:EtOAc (80:20, v/v) as the mobile phase, visualized at 366 nm, and revealed with H_2SO_4 (10% in methanol) followed by heating at 110 °C (10 min). Subfraction 2 (0.032 g) was observed as a spot by TLC analysis and was identified as compound **1**. Subfraction 3 (0.316 g) was further separated using flash column chromatography with dichloromethane as an eluent, producing three additional subfractions. Subfraction 3.1 (0.042 g) was identified as compound **2**.

The HEA extract (50 g) was diluted in distilled and deionized water (500 ml) and partitioned with EtOAc (five times; 500 ml). The fraction EtOAc (5.2 g) was fractionated using column chromatography (Sephadex LH-20) with MeOH as an eluent. TLC analysis using DCM: MeOH (90:10, v/v) as the mobile phase was used to identify five subfractions. Subfraction 4 (0.053 g) was identified as compound **3**.

Fresh leaves of *A. zerumbet* (2 kg) were extracted by decoction in distilled water (10 l) for 15 min. After filtration, the aqueous extract (AEA) was reduced to 1000 ml by evaporation under reduced pressure followed by liquid partition with chloroform (1000 ml). The chloroform fraction (CFA) was concentrated by evaporation under reduced pressure and weighed (0.785 g). For recrystallization, to CFA (0.785 g), 100 ml of distilled water was added at 100 °C and the solution filtered through a glass filtration funnel. The filtrate was immediately refrigerated at −8 °C for 48 h, after which, the crystals were filtered using a Büchner funnel and stored in the hood with anhydrous Na_2SO_4. The crystals obtained (0.045 g) were analyzed by TLC using $CHCl_3$:EtOH (9:1, v/v) as an eluent and visualized at 366 nm or by spraying with 10% H_2SO_4 in methanol followed by heating at 110 °C (10 min) (Itokawa et al., 1981). The crystals isolated using this method were identified as compound **4**.

Antiproliferative assay

The antiproliferative effect of the DEA and HEA extracts, and of compounds **2** and **4** was investigated using the protocol described by Monks et al. (1991). A panel of nine human cancer cell lines [U251 (glioma, CNS), MCF-7 (breast), NCI-ADR/RES (ovarian cell expressing a multiple drugs resistance phenotype), 786-0 (kidney), NCI-H460 (lung, non-small cell), PC-3 (prostate), OVCAR-03 (ovarian), HT-29 (colon adenocarcinoma), and K-562 (chronic myeloid leukemia)], kindly provided by Frederick Cancer Research & Development Center, National Cancer Institute, Frederick, MA, USA and one immortalized human cell line (HaCat, keratinocyte) provided by Dr. Ricardo Della Coletta (University of Campinas) were used. Stock and experimental cultures were grown in 5 ml of

RPMI-1640 supplemented with 5% fetal bovine serum (RPMI/FBS 5%) with penicillin:streptomycin mixture 1000 U/ml:1000 μg/ml (1 ml/l; RPMI-1640).

Stock solutions of the samples (5 mg) were prepared in DMSO (50 μl) followed by successive dilutions in RPMI/FBS 5% to give final concentrations of 0.25, 2.5, 25, and 250 μg/ml. Doxorubicin was used as a positive control at final concentrations of 0.25, 2.5, 25, and 250 μg/ml.

Cells in 96-well plates (100 μl cells/well, cell densities: 3–7 × 10^4 cells/ml) were incubated with each of the four concentrations of sample solution or doxorubicin (100 μl/well) in triplicate (n = 3), for 48 h at 37 °C and 5% of CO_2. Before (T0 plate) and after (T1 plates) sample addition, cells were fixed with 50% trichloroacetic acid (50 μl well) and stained with sulforhodamine B to quantitate cell proliferation using the reading at 540 nm. The GI_{50} (concentration that produces 50% cell growth or cytostatic effect) and the TGI (concentration that resulted in total cellular growth inhibition) values were determined through non-linear regression applied to a sigmoidal curve using Origin 8.0 software (OriginLab Corporation).

Table 1
Values of GI_{50} and TGI (μg/ml) for *Alpinia zerumbet* leaves hydroalcoholic (HEA) and dichloromethane (DEA) extracts against different cell lines.

Cell lines	GI_{50} (μg/ml)		TGI (μg/ml)	
	HEA	DEA	HEA	DEA
U-251	32.73	22.89	222.00	81.82
MCF-7	27.58	9.44	213.60	69.43
NCI/ADR-RES	[a]	7.79	[a]	[a]
786-0	202.53	24.51	[a]	52.80
NCI-H460	12.17	5.85	228.88	62.35
PC-3	226.33	23.05	[a]	78.34
OVCAR-3	18.39	2.05	[a]	69.17
HT-29	45.13	23.68	[a]	60.51
K-562	66.98	6.12	[a]	55.69
HaCat	0.93	4.44	133.31	74.34

Note: Human tumor cell lines: glioblastoma (U-251), breast (MCF-7), ovarian expressing the resistance phenotype (NCI/ADR-RES), 786-O (kidney), non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-3), colon (HT-29), leukemia (K-562); human immortalized keratinocyte (HaCat). GI_{50} = 50% growth inhibition; TGI, total inhibition of growth.

[a] Effective concentration higher than the highest tested concentration (250 μg/ml).

Results

Chemical constituents of Alpinia zerumbet

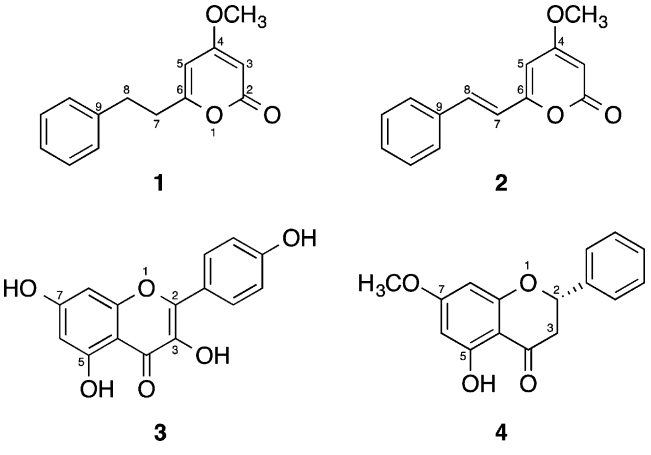
The purification techniques employed allowed the isolation of four compounds from the *A. zerumbet* leaf extracts (HEA, DEA, and AEA); these compounds were identified by comparison of their experimental spectra (IR, NMR ^{1}H, and ^{13}C) with those previously described: DDK (**1**), DK (**2**) (Itokawa et al., 1981; Mpalatinos et al., 1998), 3,4′,5,7-tetrahydroxyflavone (kaempferol; **3**) (Markham et al., 1978), and 5-hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one (pinostrobin, PTB; **4**) (Smolarz et al., 2006; Ramirez et al., 2013).

Diidro-5,6-dehydrokavain (DDK; **1**): It was obtained as colorless needles, melting point 96–98 °C; $C_{14}H_{14}O_3$, IR n_{max} (cm^{-1}; KBr disk) 1725 (pyrone carbonyl), 1650, 1530 (C=C), 1238, 1129 (C—O—C), 1600, 725, 600 (benzene ring) cm^{-1}; ESI-MS: 231.1 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) ppm (δ): 2.79 (2H, t; J = 8 Hz, 7-H), 2.96 (2H, t; J = 8 Hz, 8-H), 3.81 (3H, s, MeO-), 5.51 (1H, d; J = 2.2 Hz, 3-H), 5.92 (1H, d; J = 2.2 Hz, 5-H), 7.19–7.34 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) ppm (δ): 33.72 (C-8), 36.13 (C-7), 56.82 (MeO-), 88.21 (C-3), 101.96 (C-5), 128.53 (C-12), 129.44 (C-10), 129.49 (C-14), 129.52 (C-11), 129.55 (C-13), 141.23 (C-9), 166.26 (C-6), 167.81 (C-2), 173.76 (C-4).

5,6-Dehydrokavain (DK; **2**): It was obtained as yellow needles, melting point 136–138 °C; $C_{14}H_{12}O_3$, IR n_{max} (cm^{-1}; KBr disk) 1710 (pyrone carbonyl), 1620, 1525 (C=C), 1230, 1125 (C—O—C), 1650, 700, 620 (benzene ring) cm^{-1}; ESI-MS: 229.1 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) ppm (δ): 3.87 (3H, s, MeO-), 5.62 (1H, d; J = 2.2 Hz, 3-H), 6.24 (1H, d; J = 2.2 Hz, 5-H), 6.86 (1H, d; J = 16 Hz, 7-H), 7.43 (1H, d; J = 16 Hz, 8-H), 7.36–7.59 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) ppm (δ): 56.61 (MeO-), 89.50 (C-3), 102.73 (C-5), 120,12 (C-7), 128.10 (C-14), 128,44 (C-10), 129,41 (C-13), 129.52 (C-11), 130.23 (C-12), 136.31 (C-9), 136.74 (C-8), 160.55 (C-2), 166.63 (C-6), 173.34 (C-4).

3,4′,5,7-Tetrahydroxyflavone (kaempferol; **3**): It was obtained as yellow crystalline powder, melting point 276–278 °C; $C_{15}H_{10}O_6$, IR n_{max} (cm^{-1}; KBr disk) 3440 (OH), 1681 (C=O), 1600 (C=C), 1375 (C=C), 1335, 1303, 1267, 1165 (C—O) 746, 748, 705. ESI-MS: 286.1 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) ppm (δ): 6.17 (1H, d, J = 2.1 Hz, 6-H), 6.37 (1H, d, J = 2.1 Hz, 8-H), 6.89 (2H, d, J = 9.0 Hz, 3′,5′-H), 8.07 (2H, d, J = 9.0 Hz, 2′,6′-H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm (δ): 99.30 (C-6), 94.56 (C-8), 104.67 (C-10), 116.35 (C-3′), 116.38 (C-5′), 123.70 (C-1′), 130.76 (C-6′), 130.78 (C-2′), 137.01 (C-3), 148.09 (C-2), 158.35 (C-9), 160.67 (C-4′), 162.54 (C-5), 165.64 (C-7), 177.41 (C-4).

5-Hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one (pinostrobin (PTB); **4**): It was obtained as yellow needles, melting point 96–98 °C; $C_{16}H_{14}O_4$, IR n_{max} (cm^{-1}; KBr disk) 3432 (OH), 1635 (C=O), 1620 (C=C), 1390 (C=C), 1350, 1311, 1301, 1245, 1178 (C—O) 759, 749, 700. ESI-MS: 271.0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) ppm (δ): 2.72 (1H, dd, J = 16.6, 3.2 Hz, 3-H), 2.98 (1H, dd, J = 16.6, 12.7 Hz, 3-H), 3.82 (3H, s, 7-OMe), 5.24 (1H, dd, 12.7, 3.2 Hz, 2-H), 6.05 (1H, d; J = 2.2 Hz, 6-H), 6.10 (1H, d; J = 2.2 Hz, 8-H), 7.35–7.42 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) ppm (δ): 46.52 (C-3), 56.31 (C-7-OMe), 80.20 (C-2), 94.41 (C-8), 97.28 (C-6), 106.20 (C-10), 127.33 (C-5′), 127.39 (C-6′), 129.51 (C-3′), 129.78 (C-4′), 129,79 (C-2′), 141.13 (C-1′), 164.48 (C-7), 166.67 (C-5), 167.28 (C-9), 191.88 (C-4).



Antiproliferative effects

The HEA extract inhibited 50% of the growth of various human tumor cell lines (Fig. 1): immortalized keratinocytes (HaCat; GI_{50} = 0.93 μg/ml), lung (NCI-H460; GI_{50} = 12.17 μg/ml), ovarian (OVCAR-3; GI_{50} = 18.39 μg/ml), and breast (MCF-7; GI_{50} = 27.58 μg/ml); however, the HEA extract could not induce total growth inhibition (TGI > 200.00 μg/ml) (Table 1). In contrast, the DEA extract showed a potent cytostatic effect against human

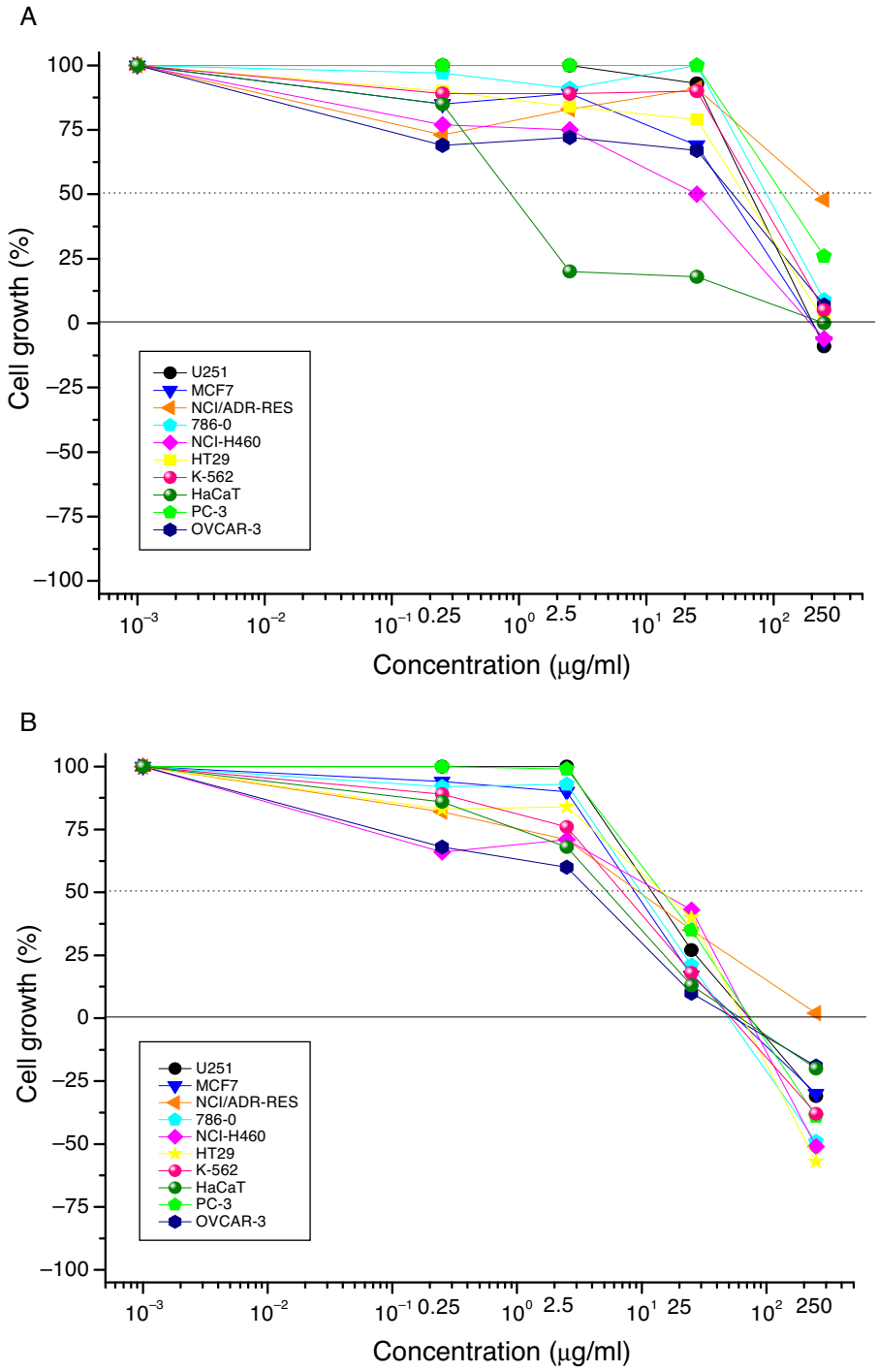


Fig. 1. *In vitro* antiproliferative effects of *Alpinia zerumbet* leaves extracts. (A) Hydroalcoholic extract (HEA); (B) dichloromethane extract (DEA); concentration range: 0.25–250 μg/ml; exposition time: 48 h; human tumor cell lines: glioblastoma (U-251), breast (MCF-7), ovarian expressing the resistance phenotype (NCI/ADR-RES), 786-O (kidney), non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-3), colon (HT-29), and leukemia (K-562); human immortalized cell line: keratinocytes (HaCat).

ovarian (OVCAR-3; GI_{50} = 2.05 μg/ml) and lung tumor cells (NCI-H460; GI_{50} = 5.85 μg/ml) in addition to total growth inhibition of all cell lines at concentrations from 52.80 to 81.82 μg/ml (Fig. 1 and Table 1).

Among the isolated compounds, DK (**2**) and PTB (**4**) were evaluated in the antiproliferative assay (Fig. 2). DK (**2**) showed a more potent antiproliferative effect than did PTB (**4**) and showed cytostatic effects against almost all the cell lines tested with a GI_{50} from 0.25 to 5.03 μg/ml (Table 2). Interestingly, PTB (**4**) was more active against the breast cancer cell line (MCF-7) with GI_{50} < 0.25 μg/ml and the leukemia cell line (K562; GI_{50} = 0.91 μg/ml) (Table 2).

Discussion

The family Zingiberaceae is a rich source of substances having a therapeutic value, such as flavonoids, which have been detected in several species (Iwashina, 2000) and are considered phytochemical markers of the order Zingiberales (Pugialli et al., 1993). Previous studies on *Alpinia* rhizome and seed have reported the isolation of alpinetin from *Alpinia speciosa* (Krishna and Chaganty, 1973; Itokawa et al., 1981); pinocembrin and PTB from *Alpinia rafflesiana* (Mohamad et al., 2004); and galangin-3-methyl ether, galangin, and pinobanksin from *Alpinia calcarata* (Hema and Nair, 2009). Rutin,

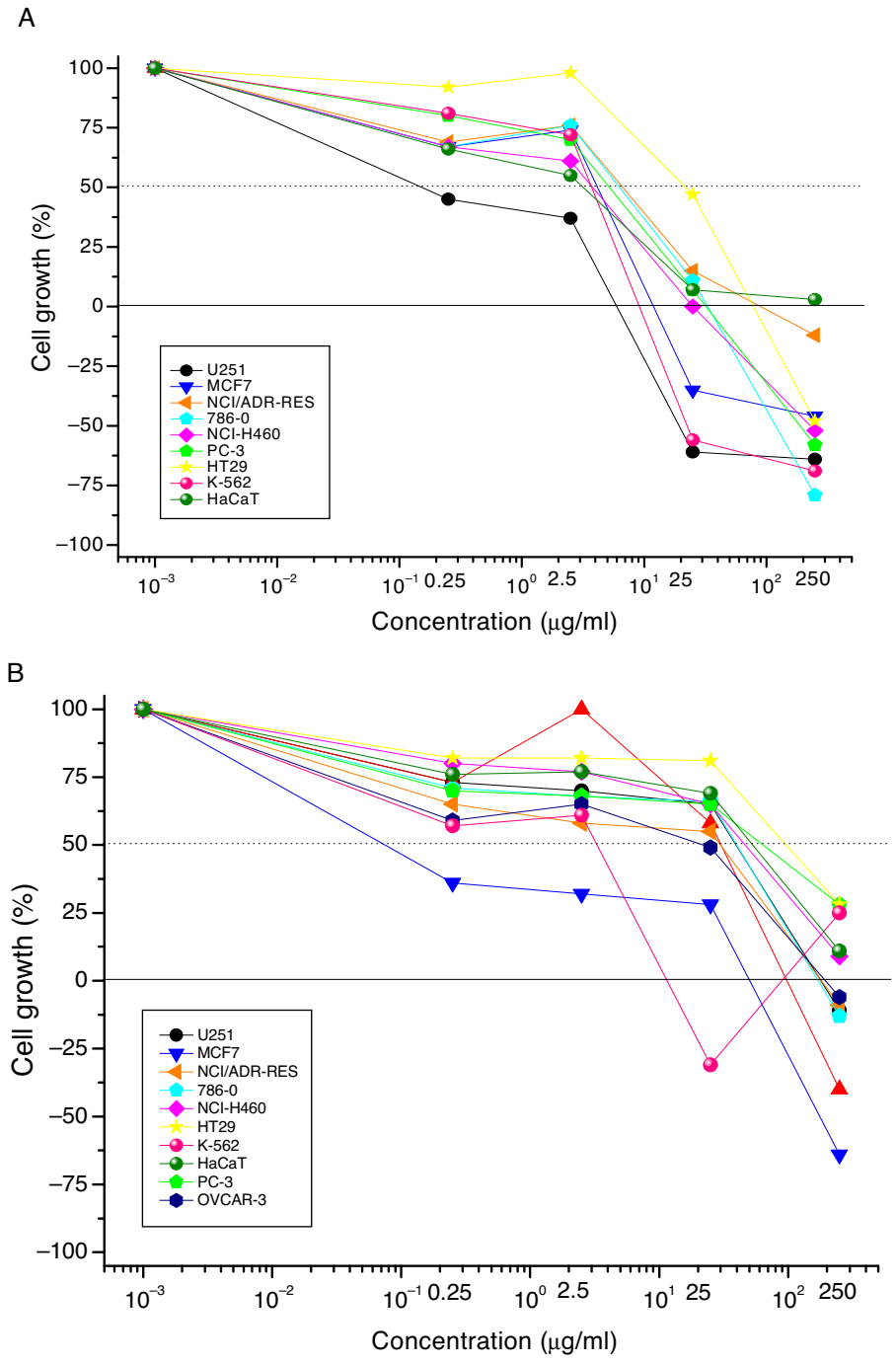


Fig. 2. *In vitro* antiproliferative effects of 5,6-dehydrokavain (A) and pinostrobin (B) isolated from *Alpinia zerumbet* leaves. A: 5,6-dehydrokavain; B: pinostrobin; concentration range: 0.25–250 μg/ml; exposition time: 48 h; human tumor cell lines: glioblastoma (U-251), breast (MCF-7), ovarian expressing the resistance phenotype (NCI/ADR-RES), 786-O (kidney), non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-3), colon (HT-29), and leukemia (K-562); human immortalized cell line: keratinocytes (HaCat).

kaempferol-3-*O*-glucuronide, and kaempferol-3-*O*-rutinoside have been isolated from *A. zerumbet* leaves (Mpalatinos et al., 1998; Victório et al., 2009).

In this study, four compounds were isolated from *A. zerumbet* leaves, namely DDK (**1**), DK (**2**), kaempferol (**3**), and PTB (**4**). DDK (**1**), and DK (**2**), which are common constituents of the genus *Alpinia* (Pugialli et al., 1993; Iwashina, 2000), have been identified in the roots and leaves of *A. zerumbet* (Mpalatinos et al., 1998; Kuster et al., 1999; Chompoo et al., 2011). However, this is the first report of kaempferol (**3**) and PTB (**4**) isolation from *A. zerumbet* leaves and, thus, contributes to the chemotaxonomic information related to *Alpinia* species.

According to the literature, alcoholic extracts from other *Alpinia* species have shown antiproliferative effects against MCF-7 cells. A methanolic extract of *A. officinarum* rhizomes (85 μg/ml) inhibited MCF-7 cell proliferation by inducing programmed cell death and arresting the cell cycle at the S phase (Ghil, 2013). Moreover, a 48 h treatment with an ethanolic extract of *A. galanga* (L.) Willd. rhizomes at 250 μg/ml reduced MCF-7 cell viability ($GI_{50} = 170.0 \pm 5.9$ μg/ml) as evidenced by

Table 2
Values of GI_{50} and TGI (μg/ml) for 5,6-dehydrokavain (DK) and pinostrobin (PTB) isolated from *Alpinia zerumbet* leaves against different cell lines.

Cell lines	GI_{50} (μg/ml)		TGI (μg/ml)	
	DK	PTB	DK	PTB
U-251	0.25	27.33	4.43	228.40
MCF-7	2.85	<0.25	17.28	17.67
NCI/ADR-RES	4.95	4.35	106.64	[a]
786-0	5.03	12.11	28.08	221.63
NCI-H460	2.04	27.41	25.96	[a]
PC-3	4.19	34.81	31.26	[a]
OVCAR-3	[a]	3.97		[a]
HT-29	24.77	89.70	79.03	[a]
K-562	2.81	0.91	9.79	235.62
HaCat	1.53	30.76	150.60	[a]

Note: Human tumor cell lines: glioblastoma (U-251), breast (MCF-7), ovarian expressing the resistance phenotype (NCI/ADR-RES), 786-O (kidney), non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-3), colon (HT-29), leukemia (K-562); human immortalized keratinocyte (HaCat). GI_{50}, 50% growth inhibition; TGI, total inhibition of growth.

[a] Effective concentration higher than the highest tested concentration (250 μg/ml).

phosphatidylserine residue exposure and plasma membrane permeation (Samarghandian et al., 2014).

Here, based on the antiproliferative activity, we demonstrated that successive extraction with an increasingly polar solvent resulted in the concentration of bioactive compounds from the DEA extract but not from the HEA extract of *A. zerumbet* leaves. A possible reason is that the more lipophilic chemical constituents present in extracts of lower polarity have greater affinity and greater ease of permeation across cellular membranes (Lee and Houghton, 2005). Under the conditions described by Fouche et al. (2008), the HEA extract showed a weak cytostatic effect against lung (NCI-H460, GI_{50} = 12.17 μg/ml) and ovary tumor cell lines (OVCAR-3, GI_{50} = 18.39 μg/ml). However, in our experiments, the HEA extract actively inhibited the proliferation of immortalized keratinocytes (HaCat, GI_{50} = 0.93 μg/ml), suggesting possible toxic effects on non-tumor tissues. In addition, the DEA extract showed moderate antiproliferative effects against leukemia (K-562), ovary (OVCAR-3 and NCI-ADR/RES), lung (NCI-H460), and breast tumor cell lines (MCF-7) and against immortalized human keratinocytes (HaCat) (Table 1).

Among the isolated compounds, DK and PTB were evaluated against tumor and non-tumor cell lines (Table 2). According to Fouche et al. (2008), compounds with TGI values < 6.25 μg/ml show potent antiproliferative activity. Thus, DK had a potent antiproliferative effect against glioblastoma cells (U-251) with TGI value = 4.43 μg/ml, which was about 33-fold higher than that for the immortalized keratinocyte cell line (HaCat; TGI = 150.60), and with twice the potency of doxorubicin used as a positive control, indicating possible selectivity. The main metabolic pathway of DK in rats and humans is the hydroxylation of the C-12 in the aromatic ring to produce *p*-hydroxy-5,6-dehydrokavain. This metabolite was detected in blood and urine in the first few hours after absorption, producing by desmethylation of the 4-methoxy group of the lactone ring the compound *o*-desmethyl-hydroxy-5,6-dehydrokavain. In rats, approximately 50–75% of administered kavalactones are excreted in the urine, mostly as glucuronide and sulphate conjugates. Approximately 15% is excreted in the bile (Rowe et al., 2011).

With regard to the GI_{50} results, PTB showed a potent cytostatic effect (GI_{50} < 0.25 μg/ml) against breast carcinoma cells (MCF-7) and a 219-fold selectivity when compared to that for immortalized keratinocytes (HaCat, GI_{50} = 30.76 μg/ml). According to the literature, PTB demonstrated a strong antitumor activity in mammary carcinoma cells, which was partially explained by topoisomerase I inhibition (Sukardiman et al., 2000). This enzyme is responsible for DNA strand break repair, allowing the broken strand to rotate on the intact one and reducing the torsional tension of the molecule (Brandão et al., 2010) during the process. The antiproliferative effect of PTB on mammary tumors could also be attributed to an anti-aromatase effect rather than antiestrogenic activity, as demonstrated by Le Bail et al. (2000). Because aromatase is responsible for the conversion of testosterone to estrogen, inhibition of the enzyme could reduce estrogen levels, and therefore the probability of hormone-related cancer (Le Bail et al., 2000).

In addition, PTB presented a potent cytostatic effect on the leukemia cell line (K-562, GI_{50} = 0.91 μg/ml) (Table 2), which is in agreement with the results described by Smolarz et al. (2006). PTB also inhibited endothelial cell proliferation, probably by promoting membrane cell depolarization, suggesting a potential antiangiogenic effect (Siekmann et al., 2013). Due to its lipolyphilicity (Log *P* = 3.1), PTB in rats showed high absorption with a maximum concentration time of about 2 h. A large volume of distribution has been observed, and the metabolism appears to be essentially hepatic. Based on clearance values this compound is mainly excreted *via* non-renal, with serum half-life of approximately 7 h (Sayre et al., 2012, 2015).

Cancer chemotherapy can often prolong life, and provide temporary relief from symptoms and occasionally complete remission. A successful anticancer molecule should kill or incapacitate cancer cells while having reduced toxicity toward normal tissues (Sharma et al., 2016). In this study, DK and PTB showed high selectivity and potent antiproliferative or cytostatic effects against glioblastoma and breast cancer cell lines, implicating a potential role in inhibiting cancer progression. These results add to the literature showing that a number of herbal compounds are cytostatic and induce cell cycle arrest, thereby showing an important mechanism useful for development of new antineoplastic drugs (Sharma et al., 2014).

Conclusions

DK and PTB isolated from the leaves of *A. zerumbet* had antiproliferative effects *in vitro* with high potency and selectivity against glioblastoma (U-251) and breast carcinoma (MCF-7) tumor lines and are potential candidates for development of antineoplasic drugs.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors contribution

WARJ, DBG, BZ, APS, and KAPD contributed in all steps of this study. TPB, ALTGR, and JEC contributed to antiproliferative studies. AN, and AB contributed to chemical analyses. CAMS contributed to design of the study. All theauthors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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