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Research Article

Cytotoxic Activity of Hexane Extracts of *Psidium Guajava L (Myrtaceae)* and *Cassia Alata L (Caesalpineaceae)* in Kasumi-1 and OV2008 Cancer Cell Lines

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Abstract

Purpose: *The cytotoxic effects of hexane extracts of Cassia alata and Psidium guajava leaves were evaluated in OV2008 ovarian and Kasumi-1 leukemia cancer cell lines, respectively.*
Methods: *The cancer cells were exposed to various concentrations of either C. alata (100 – 180 µg/ml) or P. guajava (100 – 500 µg/ml) leaf extract for 24 h. Following treatment, the cells were evaluated using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to determine the cytotoxic effect of the extracts. C. alata extract was also analyzed using high performance liquid chromatography (HPLC).*
Results: *C. alata and P. guajava extracts produced significant ($p < 0.05$) cytotoxicity in OV2008 and Kasumi-1 cell lines, respectively. The IC_{50} values were 160 µg/ml for C. alata and 200 µg/ml for P guajava. Further, the cytotoxicity exhibited by C. alata might be attributable to the flavonoid, kaempferol, which was identified as a constituent of the extract.*
Conclusion: *The results suggest that further chemical analysis and mechanistic investigations should be conducted on P. guajava and C. alata extracts to validate their potential uses for anticancer therapy.*

Keywords: *P. guajava, C. alata,* Cytotoxicity, Kasumi-1, Cancer cell, OV2008

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INTRODUCTION

Psidium Guajava (*P. guajava)* is a plant belonging to the family Myrtaceae and is native to tropical America [1]. In ethno-traditional medicine, extracts of the root, bark and leaves of *P. guajava* have been used to treat a wide range of illnesses such as gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, coughing and hyperglycemia [1]. A limited number of studies have reported cytotoxic activities of *P. guajava* in prostate [2,] and breast [4] cancer cells. There is, however, need for validation of the cytotoxic activity of the plant in other types of cancer cells.

Cassia alata (*C. alata*) is a plant from the family Caesalpiniaceae which has been used traditionally in herbal medicine for the treatment of conditions such as constipation and various skin diseases [5,6] in countries such as Indonesia, Bangladesh, Philippines and Jamaica [7,8]. There is a deficiency of information regarding the traditional use of *C. alata* for cancers, but compounds from *C. alata* have been reported to exhibit anti-angiogenic activity and cytotoxic activity in breast cancer cell lines [9] as well as protective effects against pancreatic cancer [10]. The available scientific evidence to support cytotoxic activities of *C. alata* in cancers such as ovarian cancer is lacking and this has propelled the present study.

Cancer of the ovary is the seventh most frequent cancer in women worldwide [11] and the leading cause of gynecologic cancer-related death in Europe and the USA [12]. In Jamaica, for the period 2003 - 2007, the incidence of ovarian cancer was 4 .6 per 100,000 per year, and is ranked 8th among cancers in women [13]. Leukemia is the leading type of cancer in children aged 0 - 14 years in Jamaica, specifically Kingston and St. Andrew [14] and the 7^{th} and 10^{th} most common cause of cancer death overall in men and women, respectively, in the world [15].

Current pharmacological management of leukemias, such as acute myeloid leukemia (AML), involves the use of cytarabine plus an anthracycline as mainstay therapy [16], while standard primary therapy for ovarian cancers is carboplatin and paclitaxel [17]. The limitations of these current conventional therapies used in the treatment of ovarian cancers and leukemias have in recent years contributed to a significant increase in the targeted screening of plant materials for cytotoxic activities. Such limitations include the severe unwanted effects, drug resistance and relapse that usually accompanies chemotherapy of leukemias[18] and ovarian cancers [17], and the postulation that ethnopharmacologically derived therapies might represent a safer modality of treatment.

For our investigations, we used the OV2008 ovarian and the Kasumi-1 leukemia (AML) cell lines which are commonly used in cytotoxicity screening protocols. Our study is the first to evaluate the cytotoxic effect of hexane extracts of *C.alata* and *P. guajava* leaves in OV2008 and Kasumi-1 cell lines, respectively.

EXPERIMENTAL

Plant material

The *P. guajava* and *C. alata* plants were obtained from the parishes of Kingston and Clarendon in Jamaica, respectively. They were authenticated by Mr. Patrick Lewis at the herbarium of University of the West Indies (UWI) Mona Campus, Jamaica. Voucher specimens (#35349 for *C. alata* and #35449 for *P. guajava)* were also deposited at the herbarium. The leaves of both plants were washed with water. *P. guajava* leaves were dried using a solar drier and *C. alata* leaves were air-dried for approximately 7 days. The dried leaves of *P. guajava* were milled to a fine powder.

For extraction, *C. alata* leaves were dried and homogenized in methanol (100 g in 1000 ml methanol). The extract was filtered and

concentrated *in vacuo* using a rotary evaporator. The methanol (Sigma Aldrich, USA) fraction was then partitioned between hexane (Sigma Aldrich, USA) and water (1:1 v/v) The hexane fraction was then concentrated *in vacuo* and stored at 4 ºC for further studies.

Sixty grams of P*. guajava* leaf powder was placed in a separating funnel and 500 ml of hexane (Sigma Aldrich, USA) was added to it. The mixture was then allowed to stand for 4 days. Subsequently, the hexane extract was concentrated *in vacuo* and the remaining residue was collected and stored at 4 ºC.

Cell culture

OV2008 cancer cells were kindly donated by Stupack Lab from the Moore's Cancer Center, UCSD, California, USA. Kasumi-1 cells were purchased from American type Cell Culture Collection (ATCC). OV2008 cells were propagated in 90 % Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin. Kasumi-1 cells were cultured in RPMI-1640 medium supplemented with 20 % FBS and 1 % penicillin/streptomycin. All the cells were cultured at 37 °C in a humidified atmosphere of 5 % CO_2. All culturing reagents were purchased from Thermo Scientific, USA.

Trypan blue exclusion assay for cell viability

The trypan blue exclusion assay was used to determine the viability of each cell line before the treatment of the cells with extracts. Briefly, 200 µl of each cell suspension was removed and mixed with an equal volume of 0.4 % trypan blue solution (Sigma Aldrich, USA). After incubation at room temperature for 5 min, the number of unstained (viable) and stained (non-viable) cells were counted using a hemocytometer. Cell viability (V) was calculated using Eq 1.

$$V = (C_v/C_t) \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where C_v is viable cell number and C_t total cell number.

MTT cytotoxicity assay

The cytotoxic activity of *C. alata* extract was assessed in OV2008 cells, while that of *P. guajava* extract was assessed in Kasumi-1 cells by MTT assay (Bioassay Systems). Cells were plated in 96-well plates (5×10^4 cells per well) in triplicate and incubated overnight at 37 °C. After 24 h, the extracts were added from a stock diluted to concentrations ranging from 100 to 180 µg/ml for *C. alata* and 100 - 500 µg/ml for *P. guajava* using dimethyl sulphoxide (DMSO, 1 %, 1000 µl, Sigma Aldrich, USA) and then serum free DMEM media at volumes ranging from 500 - 900 µl for *P. guajava* and 820 – 900 µl for *C. alata*. Final DMSO concentration was < 0.1 %. A volume of 20 µl of each concentration of *C.alata* or *P. guajava* extract was added in triplicate to selected wells, respectively. Control wells received media only (20 µl) in triplicate. The cells were then incubated for 24 h. Following incubation,
15 µl of the MTT labeling reagent was added to each well and incubated in a humidified atmosphere at 37 °C for 4 h. Following incubation, 100 µl of the solubilizing reagent, sodium dodecyl sulfate (SDS, 10 %) was added to each well and mixed gently for 1 h at room temperature. The absorbance of each well was measured at 540 nm using an ELISA reader (Lab Systems) and percent viability calculated. The mean extract concentration that was cytotoxic to 50 % of the cells (IC_{50}) was calculated from multiple runs.

HPLC analysis of C. alata extract

One milligram of *C. alata* extract was dissolved in methanol and injected into the HPLC system. The HPLC detection system

used was a Prostar 325 UV-Vis Detector. The samples were injected into LUNA C18 column (5 um particle size, 4.6 × 250 mm, Phenomenex, USA). The mobile phase consisted of acetonitrile/methanol/ammonium acetate (pH 6.8) in the ratio 25:55:20 (v/v/v) and at a flow rate of 0.5 ml/min. All chromatographic procedures were performed at 25 °C; the peaks were detected at 260 nm. Standard solutions of kaempferol (Sigma Aldrich, USA) were prepared at 5.0, 10.0, 25.0, 50.0 and 100.0 ug/ml by diluting with methanol. A sample size of 10 ul was injected for the HLPC analysis.

Statistical analysis

The results of each series of experiments (performed in triplicates) are expressed as the mean values ± standard deviation (SD). Statistical significance of the data was determined using the independent t-test; a value of $p < 0.05$ was accepted as statistically significant. Sigma plot software, version 10 (Systat Software, Inc., Richmond, CA, USA) was used to perform statistical analyses.

RESULTS

Trypan blue viability assay

The viability of the OV2008 cell line was determined to be 95% and the viability of the Kasumi one cell line was determined to be 96%.

Cytotoxic effect of C. alata extract on OV2008 cells

Figure 1 depicts the dose-dependent cytotoxic effect of the *C. alata* extract on OV2008 cancer cells. The cells were exposed to concentrations of 100, 120, 140, 160 and 180 µg/ml of *C.alata* extract for 24 h. Viability in *C. alata* treated cells was significantly ($p < 0.05$) lower than in untreated controls. IC_{50} of the *C. alata* extract was determined to be 160 ug/ml.

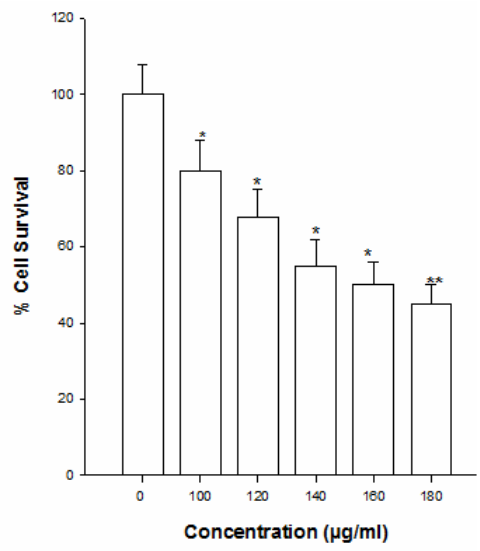

Figure 1: Cytotoxic effect of *C.alata* extract on OV2008 cells (mean ± SD (error bars , n = 3); *$p < 0.05$; **$p < 0.005$)

Cytotoxic effect of *P. guajava* extract in Kasumi-1 cancer cells

Figure 2 depicts the dose-dependent cytotoxic effect of the *P.guajava* extract on Kasumi-1 cancer cells. The cells were exposed to concentrations of 100, 200, 300, 400, 500 µg/ml of *P. guajava* extract for 24 h. Viability in *P. guajava* treated cells was significantly ($p < 0.05$) lower than in untreated controls. IC_{50} of the *P. guajava* extract was determined to be 200 ug/ml.

Chromatogram of *C. alata* extract and kaempferol standard

The peak for kaempferol in the *C. alata* extract (Figure 3a) was identified by comparing the relative retention time (RRT) with that of the chemical standard kaempferol (Figure 3b). The relative retention time (RRT) were 6.895 (Peak-1) and 6.882 (Peak-2) minutes for extract and standard, respectively.

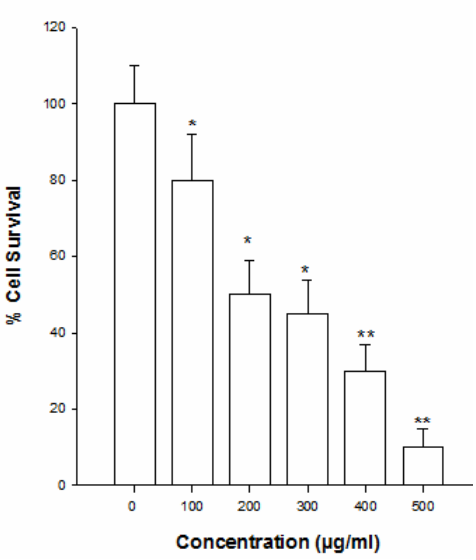

Figure 2: Cytotoxic effect of *P. guajava* extract in Kasumi-1 cells (mean ± SD (error bars , n = 3); *$p < 0.05$; **$p < 0.001$)

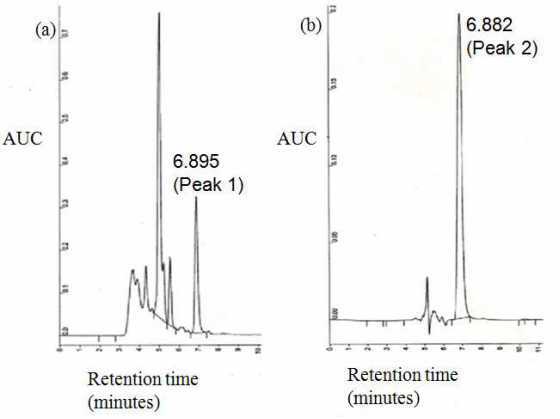

Figure 3: HPLC chromatograms of *C. alata* extract (a) and Kaempferol standard (b) detected at 260 nm

DISCUSSION

A trend towards increased screening of plant derived material for anti-cancer activity has been rapidly developing in recent years. Generally, driven by the ethno-traditional uses of different herbal remedies, the screening of plant extracts for cytotoxic potential towards cancer cells is usually accomplished by routine colorimetric assays such as the MTT assay. The MTT test produces sufficient preliminary data to confirm or discredit the ability of an extract to kill cancer cells and as such will validate the need for further studies.

In the present study, *P. guajava* extract demonstrated significant cytotoxic activity in the Kasumi-1 leukemia cancer cells. The IC_{50} of 200µg/ml obtained in our study was comparable to the IC_{50} of 250 µg/ml obtained by Chen et al [19] in the human prostate carcinoma DU-145 cell line treated with aqueous *P. guajava* extract. It has been suggested that this anti-cancer activity may be due to the presence of polyphenolic compounds such as gallic acid and flavonoidsin the extract [19]. Although a hexane extract of the leaves was used in our study, gallic acid as well as flavonoids such as quercetin and kaempferol have also been identified in hexane extracts of *P. guajava* leaves [20]. Further, both kaempferol and quercetin have been reported to exhibit cytotoxic activities in various cancer cell lines [21]. Therefore, the cytotoxic activity of *P. guajava* extract against kasumi-1 cells could be attributed to the presence of these compounds. In further studies we will examine the chemical constituents of the extract to substantiate this claim.

The *C. alata* extract used in our study also showed significant cytotoxic acitivity in OV 2008 cancer cells. These preliminary results could be justified by the cytotoxic activity of the flavonoid, kaempferol, which is present in *C.alata* species [22]. The cytotoxic activity of kaempferol has been reported in other ovarian cancer cell lines [23]. Preliminary analysis of our *C.alata* extract using HPLC identified kaempferol as a constituent of the leaf extract (Figure 3). While the compound rhein from *C. alata* has been shown to inhibit the viability of MCF-7 and MDA-MB-435s breast cancer cells [9], our study is the first to report the cytotoxic activity of crude *C. alata* extract in a cancer cell line such as OV2008.

The implication of these results is noteworthy in the light of the fact that plants have for a long time been a copious source of therapeutic agents for the treatment of cancers [24]. The Caribbean Herbal Pharmacopeia, however, is quite diverse, and there is still a significant number of medicinal plants whose entire active constituents have not yet been fully investigated [25] . Therefore, in further studies we intend to elucidate the mechanism via which both *C. alata* and *P. guajava* extracts produced cytotoxicity in these cancer cells and to characterize the bioactive chemical principles in each extract via activity-directed separation. We will examine apoptosis as a route by which the extracts might be inducing cell death since generally, compounds rich in flavonoids and other phenolics have a tendency towards activation of the apoptotic pathway [26]. Furthermore, kaempferol has been shown to induce apoptotic death of cancer cells via activation of the intrinsic pathway [23].

CONCLUSION

C. alata and *P. guajava* leaf extracts exhibit cytotoxic activity in OV2008 and Kasumi-1 cells, respectively. The results suggest that further chemical analysis and investigations of mechanisms should be conducted on both extracts to elucidate active chemical principles and to validate their potential uses for anticancer properties. Such properties could be of significant therapeutic and economical value, especially in countries where these plants are found.

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