Published in final edited form as:

J Ethnopharmacol. 2013 November 25; 150(2): 724–728. doi:10.1016/j.jep.2013.09.028.

***In vivo* antiprostata tumor potential of *Vernonia guineensis* Benth. (Asteraceae) tuber extract (VGDE) and the cytotoxicity of its major compound pentaisovaleryl sucrose**

Ngeh J. Toyang^{a,b,n}, Eugene N. Ateh^b, Harry Davis^b, Pierre Tane^c, Luc B. Sondengam^{d,e}, Joseph Bryant^b, and Rob Verpoorte^f

^aVirgin Botanicals & Biotech Inc., Columbia, MD, USA ^bInstitute of Human Virology, University of Maryland School of Medicine, MD, USA ^cDepartment of Chemistry, University of Dschang, Cameroon ^dVirgin Botanicals Ltd., Fundong, Boyo Division, Northwest Region, Cameroon ^eNational Polytechnic Bambui, Northwest Region, Cameroon ^fNatural Products Laboratory, Institute of Biology Leiden, University of Leiden, The Netherlands

Abstract

Ethnopharmacological relevance—*Vernonia guineensis* Benth. (Asteraceae) root decoction is used in folk medicine in Cameroon to treat some ailments including prostate cancer. The aim of this study was to validate the claimed antiprostata cancer activity of *Vernonia guineensis* Benth. *in vivo* and to investigate the cytotoxicity of a pentaisovaleryl sucrose isolated from *Vernonia guineensis* on some cancer cell lines.

Materials and methods—A crude dichloromethane extract of *Vernonia guineensis* (VGDE) was used for this study. For *in vivo* antiprostata cancer efficacy, nude mice ($n = 16$) were injected subcutaneously with prostate cancer PC-3 cells. Upon the formation of the xenograft tumors, the mice were divided into two equal groups with approximately the same mean tumor volume per group. One group was treated with VGDE orally (500 mg/kg) and the other with a vehicle control for 30 days. Body weight and tumor volumes were measured 2 × a week and on the 33rd day, the mice were euthanized and tumors harvested and weighed. For the cytotoxicity study, the WST-1 assay was used to determine the activity of pentaisovaleryl sucrose previously isolated from VGDE. The cancer cell lines used in the cytotoxicity study included breast, colon, leukemia, lung, melanoma, ovarian and prostate.

Results—Prostate cancer (PC-3) xenograft tumors treated with VGDE showed a significant decrease in tumor size ($P = 0.0295$) compared to control. Pentaisovaleryl sucrose also demonstrated cytotoxicity against various cancer cell lines with IC₅₀ values as follows: MDA-MD-231—6.66 μM; MCF-7—7.50 μM; HCT116—14.12 μM; A549—5.76 μM; HL60—6.43 μM; A375—8.64 μM; OVCAR3—9.53 μM; Capan1—7.13 μM; Mia-Paca 6.47 μM.

ⁿCorrespondence to: Institute of Human Virology, University of Maryland School of Medicine, Room S421, 725 West Lombard Street, Baltimore, MD 21201, USA. Tel.: +1 410 706 1062; fax: +1 410 706 5664. ntoyang@som.umaryland.edu, ntoyang@gmail.com (N.J. Toyang).

Conclusion—VGDE does possess *in vivo* activity against prostate tumor and has potential for development into a natural product for the treatment of prostate cancer. This study thus provides preliminary validation for the folk use of *Vernonia guineensis* against prostate conditions. Further *in vivo* studies are however required to confirm these results and to understand the mechanism of action of VGDE and the *in vivo* efficacy of pentaisovaleryl sucrose.

Keywords

Vernonia guineensis; Prostate cancer; Pentaisovaleryl sucrose; Cytotoxicity

1. Introduction

Prostate cancer is common in men worldwide and it is the second most diagnosed cancer and the sixth leading cause of cancer deaths amongst men (Ferlay et al., 2010; Jemal et al., 2011). Prostate cancer was the third most important cancer in males in sub-saharan Africa in 2002 with 26,800 new cases accounting for 10.6% of all reported cancer incidences amongst men (Lingwood et al., 2008). Developing countries especially in Africa with much less access to modern cancer diagnostic and treatment facilities are projected to account for most of the new cancer diagnosis by 2020 (Parkin et al., 2008; Jemal et al., 2012). The search for alternative and affordable treatments is thus of great significance and medicinal plants present a great opportunity for future research on new anticancer agents.

Vernonia guineensis Benth. (Asteraceae) has been reported to be used in ethnomedicine to manage prostate related problems (Noumi, 2010). Plants of the *Vernonia* genus are widely used in ethnomedicine and some have exhibited interesting activity in different bioassays (Toyang and Verpoorte, 2013). In a recent study, the crude extract of the root tubers of *Vernonia guineensis* and pentaisovaleryl sucrose isolated from this plant exhibited *in vitro* cytotoxicity and clonogenic activity against the PC-3 and DU145 prostate cancer cell lines as well as *ex-vivo* antiangiogenic activity (Toyang et al., 2012a). The carrot-like tubers of *Vernonia guineensis* are commonly used in ethnomedicine as an adaptogen to combat stress, as a stimulant, as a anthelmintic, an aphrodisiac, an antidote to poison, to treat malaria and jaundice (Iwu, 1993; Tchinda et al., 2002). The plant extracts have demonstrated bioactivity in antimicrobial, anthelmintic, antitrypanosomal, cytotoxicity and antiplasmodial assays (Tchinda et al., 2002; Donfack et al., 2012; Toyang et al., 2012b, 2013a, 2013b).

Based on the results of the preliminary results in *in vitro* cytotoxic activity tests of *Vernonia guineensis* and pentaisovaleryl sucrose on prostate cancer cell lines, this study was set up to evaluate the *in vivo* efficacy of the dichloromethane extract on prostate cancer xenografts.

2. Materials and methods

2.1. Plant collection

The plant material was collected as previously reported (Toyang et al., 2012a). Briefly, the tubers of *Vernonia guineensis* Var. *cameroonica* C. D. Adams were collected at Baicham, Boyo, Division of the North West Region of Cameroon in 2009. The sample was

authenticated at the Limbe Botanic Garden, South West Region Cameroon and a voucher specimen no. SCA 12431 was deposited at the Limbe Botanic Garden Herbarium.

2.2. Extraction and isolation

The extraction was carried out as previously reported (Toyang et al., 2012a). Briefly, about 1 kg of *Vernonia guineensis* tuber powder was extracted twice with 4 l of dichloromethane. The filtrate was dried *in vacuo* in a rotavapor to give 87 g of a brownish oily residue which was cytotoxic to PC-3 prostate cancer cell line ($IC_{50} = 67.3 \mu\text{g/ml}$).

The pentaisovaleryl sucrose (Fig. 1) used in this study was made available from a previous isolation (Toyang et al., 2012a).

2.3. Cell culture and injection

For the *in vivo* study, prostate cancer (PC-3) cells were maintained in minimum essential media (MEM) supplemented with 10% fetal calf serum (FCS), 20 mM L-glutamine, 2% penicillin–streptomycin, and 0.2% gentamicin until they were ready for injection into mice.

For the *in vitro* study, nine cancer cell lines (Breast—MDA-MB-231, Breast—MCF-7, Colon—HCT-116, Leukemia—HL-60, Lung—A549, Melanoma—A375, Ovarian—OVCAR3, Pancreatic—Mia-Paca, Prostate cancer—CAPAN-1) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were maintained in minimum essential media (MEM) supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, 2% penicillin–streptomycin, and 0.2% gentamicin or in RPMI 1640 medium supplemented with 10% FBS and 1% L-glutamine.

2.4. Anti-proliferation study

The inhibitory efficacy of pentaisovaleryl sucrose (Fig. 1) previously isolated from *Vernonia guineensis* was investigated *in-vitro* using the WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) (Roche) colorimetric assay (Ngamwongsatit et al., 2008). Briefly, on the day the experiment is initiated, cells are trypsinized and plated into 96 well plates in 50 μl of media. Test compounds are added approximately 18 h after plating. Cells are plated at a density so that 72 h postdrug addition, the cells are in log phase (500–2000 cells/well). The compounds are solubilized in DMSO at a concentration of 100 mM, aliquoted and stored at -20°C . Following drug addition, the cells are allowed to proliferate for 72 h. The experiment is terminated using WST-1 (Roche) 10 μl per well and absorbance is read at 450 nm/690 nm. The effect of drugs on growth was assessed as percent of cell viability. The IC_{50} values were determined from the compound dose versus control growth curves using Graphpad Prism software. All experiments were carried out at least in duplicate and the mean results determined.

2.5. Drug preparation and treatment

The dried crude dichloromethane extract of *Vernonia guineensis* was formulated in DMSO, Tween 80 and normal saline (2:2:6). The drug concentration was 50 mg/ml and each mouse administered 0.2 ml for mouse weighing 20 g giving a dose of 500 mg/kg. The vehicle was prepared using the same diluent concentrations without the drug.

2.6. In vivo MTD study

All mice used were maintained in a pathogen-free environment in the Institute of Human Virology Animal Facility at the University of Maryland, School of Medicine in accordance with the *Institutional Animal Care and Use Committee (IACUC)* guidelines on the handling of research animals. To determine the maximum tolerable dose (MTD) for the experiment, 12 nude mice were divided into three equal groups. Two of the groups were respectively treated with 500 mg/kg and 1000 mg/kg orally using a crude VGDE extract daily for 5 days and the third group was treated with the vehicle control. None of the animals died or showed any signs of adverse reaction to the drug or vehicle during the 5 days of the treatment. It was thus determined that it was safe to use any of the two concentrations in the efficacy study.

2.7. In vivo efficacy study

All mice were maintained as described above. Twenty two nu/nu (NIH) mice, 4–6 weeks old were inoculated with 3×10^6 PC-3 prostate cancer cells in 33% matrigel/67% media with no FCS. Mice were monitored daily for tumor growth. When tumors reached $\sim 100 \text{ mm}^3$, the mice were divided into two groups ($n = 8$) so that the mean tumor volume was similar. Dosing was initiated on day of sorting (day 1). Mice were dosed every day for a total of 30 days orally. The body weight of mice, were taken alongside tumor volumes 2× a week and the tumor volumes were determined using the following formula:

$$LW^2=2(L=\text{tumor length}; W=\text{tumor width})$$

Mice were euthanized by CO₂ asphyxiation on the 33rd day and tumors collected and weighed. Major organs including heart, liver, lungs, kidneys and pancreas were collected and examined for any abnormalities.

2.8. Statistical analysis

The antiproliferation assay experiments were run at least in duplicate with each experimental concentration quadrupled during each run. The average mean of the different assays was calculated and the data represented as mean $\pm$ STD. For the *in vivo* study, statistical analysis was carried out by analysis of variance (ANOVA) followed by Unpaired *t*-test analysis. $P > 0.05$ was considered as indicative of significance, as compared to the control group. All data were analyzed in Graphpad Prism (Graphpad Software, La Jolla, CA) software.

3. Results

3.1. Effect of VGDE on PC-3 tumor xenograft

VGDE extract demonstrated a significant reduction in tumor volume ($P = 0.0295$) compared to the control. Fig. 2 presents a graph comparing tumor weights at the end of the experiment while Fig. 3 presents the tracking of tumor progression during the 33 day treatment period. Organ examination did not reveal any abnormalities or lesions in the mice. Fig. 4 presents the comparative antiproliferation activity of VGDE and its major compound pentaisovaleryl sucrose against the PC-3 cell line.

3.2. Effect of VGDE on body weight

The extract of VGDE at 500 mg/kg for 30 days caused a statistically insignificant (P value = 0.1043) drop in the body weight of mice in the treatment group while there was a slight gain in body weight in the control group. Fig. 5 presents the body weight change measurements during the 33 day trial.

3.3. Antiproliferation assay

The results of the antiproliferation activity of pentaisovaleryl sucrose conducted against nine cancer cell lines utilizing the WST-1 cell proliferation reagent are presented in Table 1. The compound showed activity against all nine cell lines with the most potent activity observed against the lung cancer cell line (A549) with IC_{50} of 5.00 μ M while it was least active against the colon cancer cell line (HCT-116) with IC_{50} of 14.12 μ M. The IC_{50} values of pentaisovaleryl against the other cell lines were less than 10 μ M.

4. Discussion

The results of the present investigation demonstrate the *in vivo* antitumor activity of the dichloromethane root extract of *Vernonia guineensis* against the PC-3 prostate tumor. This study provides preliminary *in vivo* validation of the use of preparations of this plant in folk medicine to manage prostate related problems (Noumi, 2010). The absence of any noticeable signs of toxicity in the treated mice is also an indication that the VGDE extract can be considered to be reasonably safe. This is confirmed by the fact that there was statistically no significant difference ($P > 0.05$) in the body weight of the treated versus the untreated group as well as the absence of any negative morphological changes in the organs of all the mice in both groups.

The results of this study also suggest that the pentaisovaleryl sucrose previously obtained from the dichloromethane extract of *Vernonia guineensis* through bioactivity guided isolation with activity against three prostate cancer cell lines (Toyang et al., 2012a) may also be responsible for the *in vivo* activity observed. By *in vitro* comparison, the bioactive pentaisovaleryl sucrose isolated from VGDE is at least 10 $\times$ more active than the parent extract (Fig. 4).

Pentaisovaleryl sucrose demonstrated cytotoxic activity against the nine cancer cell lines tested (Table 1). The lung cancer cell line A549 was most sensitive while the colon cancer cell line HCT-116 was least sensitive to pentaisovaleryl sucrose. The cytotoxic effect of pentaisovaleryl sucrose against other cell lines and its Multi Drug Resistant (MDR) modulating effect has previously been reported (Murakami et al., 2002; Toyang et al., 2012a). Sucrose esters are generally known to exhibit diverse behavior and are used mainly in the food and cosmetic industry as emulsifiers, antibacterial agents, crystallization inhibitors, and as permeation enhancers (Garti et al., 2000; Mutoh et al., 2007; Csóka et al., 2007). Due to their good solubilizing properties, there is an interest to use sucrose esters as pharmaceutical excipients for nasal drug delivery amongst other applications (Kürti et al., 2012). Apart from reported cytotoxic and antibacterial activity of sucrose esters, studies have also shown that sucrose ester have anti-inflammatory properties with potency levels

reaching those of indomethacin (Pérez-Castorena et al., 2010). Sucrose esters have also found use as insecticides and are thought to contribute to the insecticidal properties associated with tobacco leaves (Chortyk et al., 1996; Simonovska et al., 2006). The most interesting finding supporting the outcome of this study is that investigations have shown that tobacco leaf surface sucrose esters and some synthetic disaccharide esters have potential to be used in cancer prevention (Okabe et al., 1999). This supports our claim that pentaisovaleryl sucrose may indeed be responsible for antitumor activity demonstrated by VGDE.

5. Conclusion

This study confirms that the dichloromethane root extract of *Vernonia guineensis* does possess *in vivo* activity against prostate tumors and has potential for development into a natural product for the treatment of prostate cancer. Further studies are however required to confirm these results as well as the *in vivo* efficacy of pentaisovaleryl sucrose found in this extract and its mechanism of action.

Acknowledgments

The authors are grateful to Alosyius N. Toyang, Yua Eric and Therese Toyeng for assisting with the collection and processing of the plant material. The technical support of the Translational Core, Greenebaum Cancer Center at the University of Maryland School of Medicine; an NCI Comprehensive Cancer Center are acknowledged.

References

- Chortyk OT, Pomonis JG, Johnson AG. Syntheses and characterizations of insecticidal sucrose esters. *Journal of Agriculture and Food Chemistry*. 1996; 44:1551–1557.
- Csóka G, Marton S, Zelkó R, Otomo N, Antal I. Application of sucrose fatty acid esters in transdermal therapeutic systems. *European Journal of Pharmaceutics and Biopharmaceutics*. 2007; 65:233–237. [PubMed: 16963242]
- Donfack ARN, Toyang NJ, Wabo HK, Tane P, Awoufack MD, Kikuchi H, Tamokou JDD, Kuate JR, Oshima Y. Stigmatane derivatives from the root extract of *Vernonia guineensis* and their antimicrobial activity. *Phytochemistry Letters*. 2012; 5:596–599.
- Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *European Journal of Cancer*. 2008; 46:765–781. [PubMed: 20116997]
- Garti N, Aserin A, Fanun M. Non-ionic sucrose esters microemulsions for food applications. Part 1. Water solubilization. *Colloids and Surfaces A*. 2000; 164:27–38.
- Iwu, MM. *Handbook of African Medicinal Plants*. London: CRC Press; 1993.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: A Cancer Journal for Clinicians*. 2011; 61:69–90. [PubMed: 21296855]
- Jemal A, Bray F, Forman D, O'Brien M, Ferlay J, Center M, Parkin DM. Cancer burden in Africa and opportunities for prevention. *Cancer*. 2012; 118:4372–4384. [PubMed: 22252462]
- Kürti L, Veszelka S, Bocsik A, Dung NTK, Ózsvári B, Puskás LG, Kittel Á, Szabó-Révész P, Deli M. The effect of sucrose esters on a culture model of the nasal barrier. *Toxicology In Vitro*. 2012; 26:445–454. [PubMed: 22274662]
- Lingwood RJ, Boyle P, Milburn A, Ngoma T, Arbuthnott J, McCaffrey R, Kerr DJ. The challenge of cancer control in Africa. *Nature Reviews Cancer*. 2008; 8:398–403.
- Murakami N, Tamura S, Iwata E, Aoki S, Akiyama S, Kobayashi M. Facilely accessible multidrug resistance modulator derived from sucrose. *Bioorganic and Medicinal Chemistry Letters*. 2002; 12:3267–3270. [PubMed: 12392729]

- Mutoh T, Kubouchi H, Noda M, Shiinoki Y, Matsumura Y. Effect of oil soluble emulsifiers on solidification of thermally treated creams. *International Dairy Journal*. 2007; 17:24–28.
- Ngamwongsatit P, Banada PP, Panbangred W, Bhunia AK. WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic *Bacillus* species using CHO cell line. *Journal of Microbiological Methods*. 2008; 73:211–215. [PubMed: 18417231]
- Noumi E. Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon. *African Journal of Pharmacy and Pharmacology*. 2010; 4:793–805.
- Okabe S, Suganuma M, Tada Y, Ochiai Y, Sueoka E, Kohya H, Shibata A, Takahashi M, Mizutani M, Matsuzaki T, Fujiki H. Disaccharide esters screened for inhibition of tumor necrosis factor- α release are new anti-cancer agents. *Japanese Journal of Cancer Research*. 1999; 90:669–676. [PubMed: 10429660]
- Parkin DM, Sitas F, Chirenje M, Stein L, Abratt R, Wabinga H. Cancer in indigenous Africans—burden, distribution, and trends. *The Lancet Oncology*. 2008; 9:683–692. [PubMed: 18598933]
- Pérez-Castorena AL, Martínez M, Maldonado E. Labdanes and sucrose esters from *Physalis sordid*. *Journal of Natural Products*. 2010; 73:1271–1276. [PubMed: 20575572]
- Simonovska B, Srbinska M, Vovk I. Analysis of sucrose esters—insecticides from the surface of tobacco plant leaves. *Journal of Chromatography A*. 2006; 1127:273–277. [PubMed: 16820155]
- Tchinda AT, Tsopmo A, Tane P, Ayafor JF, Connolly JD, Sterner O. Vernoguinoesterol and vernoguinoside, trypanocidal stigmastane derivatives from *Vernonia guineensis* (Asteraceae). *Phytochemistry*. 2002; 59:371–374. [PubMed: 11830150]
- Toyang NJ, Verpoorte R. A review of the medicinal potentials of plants of the Genus *Vernonia* (Asteraceae). *Journal of Ethnopharmacology*. 2013; 146:681–723. [PubMed: 23395623]
- Toyang NJ, Wabo HK, Ateh EN, Davis H, Tane P, Kimbu SF, Sondengam LB, Bryant J. In vitro anti-prostate cancer and ex vivo antiangiogenic activity of *Vernonia guineensis* Benth. (Asteraceae) tuber extracts. *Journal of Ethnopharmacology*. 2012a; 141:866–871. [PubMed: 22465590]
- Toyang NJ, Ateh EN, Keiser J, Vargas M, Bach H, Tane P, Sondengam LB, Davis H, Bryant J, Verpoorte R. Toxicity, antimicrobial and anthelmintic activities of *Vernonia guineensis* Benth. (Asteraceae) crude extracts. *Journal of Ethnopharmacology*. 2012b; 144:700–704. [PubMed: 23107821]
- Toyang NJ, Wabo HK, Ateh EN, Davis H, Tane P, Sondengam LB, Bryant J, Verpoorte R. Cytotoxic and antiangiogenic sesquiterpene lactones from the leaves of *Vernonia guineensis* Benth. (Asteraceae). *Journal of Ethnopharmacology*. 2013a; 146:552–556. [PubMed: 23376285]
- Toyang NJ, Krause MA, Fairhurst RM, Tane P, Bryant J, Verpoorte R. Antiplasmodial activity of sesquiterpene lactones and a sucrose ester from *Vernonia guineensis* Benth. (Asteraceae). *Journal of Ethnopharmacology*. 2013b; 147:618–621. [PubMed: 23542146]

PentaIsovaleryl sucrose

Fig. 1.
PentaIsovaleryl sucrose.

Fig. 2. Nude mice ($n = 8$) implanted with PC-3 cells (3×10^6 /mouse) by subcutaneous injection were treated for 30 days orally with VGDE (500 mg/kg). Treatment started approximately 10 days after cell injection with the average tumor volume of about 100 mm^3 for the treatment and control groups. The mice were euthanized on the 33rd day and tumors collected and weighed. Treatment with VGDE resulted in a 37.59% reduction in tumor weight compared to the control group.

Fig. 3. Nude mice ($n = 8$) implanted with PC-3 cells (3×10^6 /mouse) by subcutaneous injection were treated for 30 days orally with VGDE (500 mg/kg). Treatment started approximately 10 days after cell injection with the average tumor volume of about 100 mm^3 for the treatment and control groups. Tumor volumes 2 $\times$ a week and the tumor volumes were determined using the formula $LW^2/2$ (L = tumor length; W = tumor width). VGDE extract demonstrated a significant reduction in tumor volume ($P = 0.0295$) compared to the control.

WST-1 Cell Proliferation Assay against prostate cancer PC-3 cells (72 Hours)

Fig. 4. Dose response curves for *in vitro* WST-1 assay antiproliferation activity of pentaisovalerylsucrose and the dichloromethane extract (VGDE) of *Vernonia guineensis* against the PC-3 prostate cancer cell line. The dose response curves and IC₅₀ values were determined from the compound dose versus control growth curves using the Graphpad Prism Software. All experiments were carried out at least in duplicate and the mean $\pm$ STD determined. Comparatively, the pure sucrose ester was 10 $\times$ more active than the crude VGDE ($P > 0.0001$).

Fig. 5.

Nude mice of age 4–6 weeks and weighing 20–30 g were used in the experiment. The body weight of mice was recorded 2× a week. VGDE extract at 500 mg/kg for 30 days caused a slight drop (1.22%) in the body weight of mice in the treatment group compared to the weight of the same mice at the start of the experiment. The mice in the control group experienced a small gain (2.14%) in body weight compared to the weight at the start of the experiment.

Table 1

In vitro cytotoxicity activity of pentaisovaleryl sucrose against selected human cancer cell lines in the WST-1 antiproliferation assay.

Cancer cell line	IC ₅₀ in μ M
Breast—MDA-MD-231	6.667 1.17
Breast—MCF-7	7.5171.84
Colon—HCT-116	14.1276.16
Leukemia—HL-60	6.3970.37
Lung—A549	5.0070.01
Human melanoma—A375	8.6471.56
Ovarian—OVCAR3	9.5373.76
Pancreas—Mia-Paca	6.4770.96
Prostate—Capan1	7.1370.09