

Short Communication

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Cytotoxic effect of Jamaican bissy nut extracts on various cancer cell lines**Henry I.C.Lowe^{1,2,3}, Charah T.Watson^{1,4}, Simone Badal^{1,4*}, Patrice Peart¹,
Ngeh J.Toyang^{2,3}, Joseph Bryant³**¹Bio-Tech R & D Institute, Kingston, (JAMAICA)²Educational and Scientific Corporation, Florida, (USA)³Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, (USA)⁴Natural Products Institute, University of the West Indies, Mona, (JAMAICA)E-mail: simone.badal@uwimona.edu.jm; henry.lowe@cwjamaica.com; charah_twa@yahoo.com;
simone.badal@uwimona.edu.jm; ntoyang@som.umaryland.edu; jbryant@ihv.umaryland.eduReceived: 22nd October, 2012 ; Accepted: 28th November, 2012**ABSTRACT**

Given the high incidence of cancer worldwide and one of the main sources of unearthing novel lead molecules being medicinal plants, this research undertook to investigate the possible anti-cancer activity of five bissy plant extracts (hexane, dichloromethane, acetone, methanol and water) against three human cancer cell lines; prostate (PC-3 and DU-145), breast cancer (BC) and mice melanoma (B16). Cytotoxicity was measured using the trypan blue exclusion assay. The hexane extract was the most potent, particularly against, DU-145 and PC-3 cell lines, killing 95% of the cells at 160 µg/ml. These results will prove useful for further research and so may serve as leads for the development of new drugs.

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KEYWORDSBissy;
Cola acuminata;
Prostate cancer;
Breast cancer;
Cytotoxic.**INTRODUCTION**

With just about 30% of its terrestrial plants being endemic^[1], Jamaica is known for its affluent biodiversity and its copious usage of medicinal plants as ethno medicines. Medicinal plants continue to play a critical role in drug discovery and development because of the vast structural diversity of molecules found in the plant kingdom, some of which become new drugs or leads for the development of new drugs^[2].

Bissy, *Cola* sp, is an evergreen originally endemic to West Africa; it was introduced to many tropical coun-

tries, Jamaica being one of them. It belongs to the *Sterculiaceae* family and is locally known as bissy, cola or kola nuts. The plant produces fruit pods containing seeds that are used in traditional folklore medicine to treat a range of ailments, mainly poisoning and digestive disorders^[3] though it is also known to treat asthma^[4,5] and has been shown to possess antioxidant properties^[6]. In addition, studies have shown that the crude extract of *C. acuminata* has antimicrobial properties as its bioactivity against *Staphylococcus albus* was similar to that of the known antibiotic, erythromycin^[7]. Also, active ingredients have been identified as non-steroidal

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compounds that are bioactive against prostate and breast cancer cells^[8,9].

In this study, we explore the anticancer potential of five different bissu nut extracts, in an effort to identify the most promising extract/s that demonstrate bioactivity towards the investigated cancer cell lines. Further work will lead to the isolation of the active compound(s), which could possibly be used in the pharmacological treatment for certain types of cancers and so may serve as templates for the development of new drugs.

MATERIALS AND METHODS

Plant material and extraction

The bissu nuts were obtained from a local market

and identified by Mr. Keron Stewart from the Institute of Jamaica Herbarium. The nuts were dried and grounded to a powder (110 g). This was sequentially extracted in a soxhlet apparatus using solvents; hexane, dichloromethane, acetone, methanol and water in order of increasing polarity. Each extraction was allowed to reflux for 24 h at temperatures corresponding to the boiling point of the respective solvent. Following extraction the resulting solution was filtered through a sintered glass funnel and the filtrate was reduced to dryness *in vacuo* and weighed (TABLE 1).

Cell lines and culture medium

All cell lines with their respective media and supplements were obtained from ATCC (Manassas, VA, USA).

TABLE 1 : Cytotoxicity of several extracts of *C.acuminata* at varying concentrations (40-160 µg/ml) in the presence of four cancer cell lines.

Cell Lines	Percentage of cells dead											
	Hexane			Dichloromethane			Acetone			Methanol		
	40	80	160	40	80	160	40	80	160	40	80	160
DU-145	2.4	72.5	95.4	3.6	25.9	53.4	2.4	2.4	3.4	3.54	2.38	3.6
PC-3	4.8	73.9	95.4	4.7	32.6	48.8	3.47	3.51	3.44	27.38	2.32	4.7
BC	2.4	71.6	94.3	3.6	2.5	59.4	2.4	2.3	4.6	3.5	2.3	4.7
B16	2.4	71.8	91.7	4.7	24.4	60	3.6	3.5	3.6	4.7	3.5	3.5

Cell proliferation

The cells (DU-145, PC-3, BC, and B16) were maintained in minimum essential media (MEM) supplemented with 10% foetal calf serum (FCS), 20 mM l-glutamine, 2% penicillin–streptomycin, and 0.2% gentamicin at 37°C with 5% CO₂ in Corning 75 cm³ culture flasks. Cells were trypsinized and plated at the appropriate density (80-90% confluence in growth) into 24 well plates in media after which they were exposed to varying bissu nut extracts (hexane, dichloromethane, acetone, methanol and water) at different concentrations (40-160 µg/ml) for 48 h at 37 °C in humidified atmosphere of 5% CO₂. The plant extracts were solubilized in DMSO (<0.1%). The cell suspension was centrifuged, re-suspended in media and incubated at 37 °C for 5 min after which cell death death/viability was measured by Trypan Blue exclusion assay as previously described^[10]. This relies on the modification in membrane veracity as measured by the uptake of dye by dead cells (trypan blue positive), thus giving a direct measure of cell viability. A light microscope (Nikon

Eclipse TS100) was used to count the number of viable versus dead cells mounted on a hemocytometer. The GraphPad Prism 3.0 software program (GraphPad) was used for the statistical analyses.

RESULTS AND DISCUSSION

The hexane extract showed the most potency towards the four cell lines investigated (TABLE 1 & Figure 1) with particular attention towards PC-3 and DU-145 since the effects of DMSO and media on these cell lines was negligible (TABLE 2) making it more likely that the observed bioactivity was solely due to the hexane extract.

Natural products research has long been a source for discovering new pharmaceuticals for the treatment of cancer for example vinblastine, vincristine, topotecan and the taxanes were all compounds derived from plant sources^[11]. Nature is still one of the best sources for providing new lead compounds and considering that only a small percentage have been chemically investigated^[12] there is vast potential for new discoveries.

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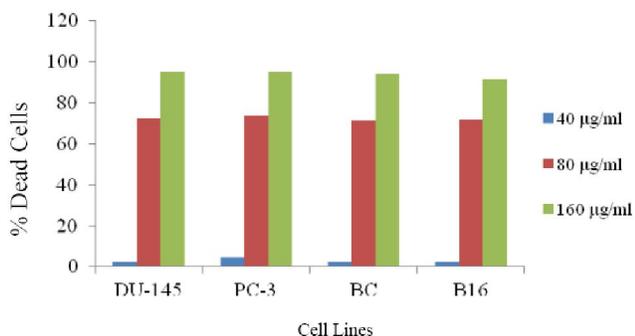


Figure 1 : Showing of non viable cells in the presence of varying concentrations of hexane *C. acuminata* extract

TABLE 2 : Cytotoxic activity of the fractionated hexane extract of *C. acuminata* against PC-3 cells.

Concentration/ µg/mL	b1	b2	b3	b4	b5	b6
	<i>Percentage of cells dead</i>					
5	38	16	1	11	-	-
10	88	42	8	0	44	0
20	44	94	3	23	100	0
40	16	100	13	7	100	0

TABLE 3 : Effects of the control on the various cell lines.

Sample	Cell Lines			
	PC-3	DU-145	BC	B16
	<i>Percentage of cells dead</i>			
DMSO	0	0	60	42.9
Media	0	0	14.3	10

Results obtained in this research are in support of previous studies in which extracts from the *Cola* sp. were active against human breast carcinoma cells^[8,13], notwithstanding, the findings presented here have never been reported elsewhere. About 100% reduction in cell viability of all the investigated cancer cell lines was observed at 160 µg/ml of the hexane extract. Fractionation of the hexane extracts (TABLE 2) and subsequent bioassay showed that fractions b1, b2 and b5 were the most active against the prostate cancer cells (PC-3). TLC analysis (data not shown) with a caffeine standard showed that the fractions did not contain any caffeine and as such the observed activity is due to some other compound(s). Guided bioassay will assist with the isolation and characterization of the active ingredients responsible for the said bioactivity and this may lead to discovery of molecules with pharmaceutical importance. The dichloromethane extract also showed promising cytotoxic activity against the investigated cell lines (TABLE 1) *albeit* less potent than the hexane, nevertheless, this warrants additional investigation.

CONCLUSION

The hexane and dichloromethane extracts of *Cola acuminata* have shown promising cytotoxic activity against four carcinoma cell lines. These results warrant further research that will aid in the identification of active ingredients that may provide the template for drug design toward the treatment of prostate and breast cancers.

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REFERENCES

- [1] S.Mitchell, M.Ahmad; West Indies Medical Journal, **55**, 243 (2006).
- [2] M.Gordaliza; ClinTranslOncol., **9**, 767 (2007).
- [3] M.Warner; Thailand, 94 (2007).
- [4] E.Odebunmi, O.Oluwaniyi, G.Awoloa, O.Adediji; Afr.J.Biotechnol., **8**, 308 (2009).
- [5] M.Simmons, H.J.Gong, D.Tashkin, K.Hui, E.Lee; Chest, **84**, 332 (1983).
- [6] S.Atawodi, B.Pfundstein, R.Haubner, B.Spiegelhalder, H.Bartsch, R.Owen; J.Agric.Food Chem., **55**, 9824 (2007).
- [7] M.Sonibare, M.Soladoye, O.Esan, O.Sonibare; Afr.J.Trad.CAM, **6**, 518 (2009).
- [8] K.Fontenot, S.Naragoni, M.Claville, W.Gray; Toxicol.Appl.Pharmacol., **220**, 25 (2007).
- [9] R.Solipuram, S.Koppula, A.Hurst, K.Harris, S.Naragoni, K.Fontenot, W.Gray; J.Toxicol., **2009**, 1 (2009).
- [10] T.Uljasz, S.Hewett; J.Neurosci.Methods, **100**, 157 (2000).
- [11] G.Cragg, D.Newman; Journal of Ethnopharmacology, **100**, 72 (2005).
- [12] S.Rates; Toxicol., **39**, 603 (2001).
- [13] S.Endrini, S.Jaska, H.Marsiati, F.Othman, A.Rahmat; J.Med.Plants Res., **5**, 2393 (2011).