Cytotoxic and anti-HIV activities of some Tanzanian *Garcinia* species

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Abstract: Cancer and HIV/AIDS remains the greatest public health and humanitarian challenges in the current world's health sector. For many decades now, millions of lives have been compromised by the two diseases. This study has evaluated ethanol extracts from nine *Garcinia* plant species collected in Tanzania for their *in vitro* cytotoxicity against four human cancer cell lines and for anti-HIV activity against HIV-1 viral replication in MT4 cells. Among the tested extracts, the fruit extracts of *G. livingstoneii* and *G. semseii* showed moderate to mild cytotoxic activities against A549, DU145, KB and Kbivin human cell lines with 50 % cytotoxic (CC_{50}) values ranging from 5.7-20.0 μg/ml. Furthermore, only fruit extracts of *G. livingstonei* and *G. semseii* showed significant anti-HIV-1 activity with EC_{50} values of 2.25 ± 0.51 and 0.93 ± 0.67 μg/ml respectively. This study has shown the potential of the *Garcinia* extracts to be the source of possible lead compounds and anti-HIV drug candidates currently needed for the management of HIV/AIDS. Phytochemical screening indicated dominance of phenolic compounds in *Garcinia* species while isolation of active principles from active fractions will be further undertaken.

Keywords: *Garcinia* species, Clusiaceae, extracts, cytotoxic, HIV, Tanzania

Introduction

HIV/AIDS and cancer pandemics remain the critical crisis due to both their emergent and long-term development. Cancer cases distribution shows no differences in its epidemiology in all regions of the world whereas, HIV/AIDS have affected the world disproportionately and the greatest burden being in sub-Saharan Africa (WHO, 2008). Currently, no reliable and user friendly treatment can be claimed to combat these diseases. The current anti-HIV drugs that include reverse transcriptase (RT) and protease inhibitors have experienced drug resistance with HIV strains (Boden *et al.*, 1999). On the other hand, the currently available anti-cancer chemotherapy are of limited efficacy on advanced cancer cases hence, there is a demand for more effective cancer treatments. This necessitates the demand for the development of new drugs particularly of plant origin owing to their success as sources of anticancer drugs. The use of plants for managing different diseases has become a common practice since time immemorial with most of the people in the developing world relying on traditional medicines for their primary health care including management of cancer and HIV/AIDS (WHO, 1999).

Plants of the genus *Garcinia* have been reported in the literature to display both anti-HIV and cytotoxicity activity (Tao *et al.* 2009). For instance, *G. mangostana* gave mangostin, a compound that indicated significant HIV-1 protease inhibition effect (Chen *et al.*, 1996) while *G. livingstoneii* has been reported to produce gutifferone A, being an anti-HIV compound (Gustafson *et al.*, 1992). Ethnomedically, different parts of *Garcinia* plants have been reported to exhibit many pharmacological effects. Thus, fruits of most species in this genus are edible,

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among them; those of *G. mangostana* are famous, while *G. gambogia* fruits are used in traditional medicine for treating diarrhoea and dysentery (Ambasta, 1986) and the rind of this fruit is rich in hydroxycitric acid (HCA) which has been associated with promoting weight loss, suppressing appetite, and increasing energy levels (Yamada *et al*., 2007). Other *Garcinia* species, such as *G. indica*, have oily seeds yielding more than 15% oil. Fruit extracts from *G. kola* have been claimed to be effective at stopping *Ebola* virus replication in laboratory tests and its seeds are also used in folk medicine (Yamaguchi *et al*., 2000).

In the course of our ongoing search for bioactive extracts/compounds from natural sources, nine *Garcinia* species growing in Tanzania have been screened for their cytotoxic and anti-HIV-1 viral replication activities. This paper reports the evaluation of crude extracts of nine *Garcinia* plants against HIV-1 NL4−3 viral strain on MT-4 cells and cytotoxic activities on four human cancer cell lines as well as the phytochemical screening of extracts aiming to establish the classes of compounds responsible for the biological activities noted in this study.

Materials and Methods

Collection of plant materials

The plant materials were collected from different parts in Tanzania (Table 1) and identified by Mr. Haji O. Suleimani of the Department of Botany, University of Dar es Salaam. They include *Garcinia bifasculata* N. Robson, *G. buchananii* Bak., *G. edulis* Exell. *G. ferrea* Pierre, *G. huillensis* Welw. ex Oliv., *G. kingaensis* Engl., *G. livingstonei* T. Anderson, *G. semseii* Verdc and *G. volkensis*. The voucher specimens are deposited in the Herbarium at the Department of Botany, University of Dar es Salaam, Tanzania.

Table 1: Localities of *some Garcinia* plant species in Tanzania

Plant name	Voucher specimen	Place collected	Part collected
G. ferrea	HOS 3425	Amani-Tanga	Root, Fruit, Stem
G. edulis	HOS 3426	Amani-Tanga	Root, Stem
G. bifasciculata	FM 10135	Kimboza-Morogoro	Stem
G. buchananni	HOS 3427	Amani-Tanga	Root, Stem
G. semseii	HOS 3422	Kihansi-Iringa	Stem, Root, Fruit hulls, Seed
G. volkensis	HOS 3424	Amani-Tanga	Stem
G. livingstoneii	HOS 3423	Pugu Forest	Root, Stem, Fruit
G. kingaensis	HOS 3429	Lugoda-Iringa	Stem
G. huillensis	HOS 3428	Lugoda-Iringa	Root, Stem

Preparation of crude extracts

Twelve grams of the specified part of each dried plant material were soaked in ethanol (300 ml) for 48 hours at room temperature. The ethanol extracts were filtered and evaporated under vacuum on a rotary evaporator. The crude extracts (20 mg) were dissolved in DMSO (5 ml) for bioassay.

Anti-HIV assay on MT4 cells

An HIV-1 infectivity assay previously described was used in the experiments (Huang *et al.*, 2004), was performed at the Natural Products Research Laboratories, University of North

Carolina, USA. A 96-well microtiter plate was used to set up the HIV-1 viral replication assay. HIV-1 at a multiplicity of infection (MOI) of 0.01 was used to infect MT4 cells. Culture supernatants were collected on day 4 post infection for P24 assay using an ELISA kit from ZeptoMetrix Corporation (Buffalo, New York). If a test sample inhibited virus replication and was not toxic, its effects were reported in the following terms: CC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; and the Selectivity Index (SI), the ratio of the IC_{50} to EC_{50}. Azidothymidine (AZT) was used as a positive control. The definition of the anti-HIV activity used: EC_{50} < 0.5 μg/ml - strong activity; 0.5-5.0 μg/ml – moderate activity; 5.0-10 μg/ml – mild activity and EC_{50} > 10 μg/ml – inactive.

Comparison of bioactivity were calculated using the formula $= \frac{EC_{50}\text{ (Crude extract)}}{IC_{50}\text{ (AZT)}}$

Cytotoxicity assay

Cytotoxicity assay was performed at the Natural Products Research Laboratories, University of North Carolina, USA. The *in vitro* cytotoxicity screening was conducted by measuring toxicity against cancer cells using NIH-NCI protocol (Grever *et al.,* 1992; Alley *et al.,* 1988). In this study four cancer cells, A549 (lung adenocarcinoma), DU145 (prostate carcinoma), KB (nasopharyngeal carcinoma) and Kbvin (vincristine-resistant nasopharyngeal) were used. The definition of the cytotoxicity used was; CC_{50} < 1.0 μg/ml – high cytotoxicity; CC_{50} 1.0-10.0 μg/ml – moderate; CC_{50} 10.0-20.0 μg/ml – mild cytotoxicity; and CC_{50} > 20 μg/ml – non-cytotoxic.

Selectivity indices (SI) were calculated using the formula $= \frac{CC_{50}\text{ (Crude extract)}}{IC_{50}\text{ (Crude extract)}}$

Test for flavonoids, alkaloids, tannins, saponins and steroids

To test for alkaloids, tannins, flavonoids, steroids and saponins, methods developed by Trease & Evans (1983) and Harbourne (1983) were used. An amount 0.3 g of the extract was dissolved in 3 ml of methanol and heated. A small magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the presence of flavonoids or any other phenolic compounds.

About 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl and warmed on steam bath. The filtrate (1 ml) was mixed with drops of Dragendorff's reagent. Reddish orange precipitation was considered as indicative of the presence of alkaloids.

The extract (1 g) was dissolved in 20 ml of distilled water and filtered. Three drops of 10% of $FeCl_3$ were added to 2 ml of the filtrate. The appearance of blackish-blue or blackish-green colouration was indicative of tannins. Some 2 ml of the filtrate was added 1 ml of bromine water and a precipitate was taken as positive for tannins.

The 7% blood agar medium was used. The extract in methanol was applied with distilled water and methanol used as negative control while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35°C for 6 hours. A total haemolysis of the blood around the extract was indicative of saponins. About 0.5 g of the extract was dissolved in 3 ml of $CHCl_3$ and filtered. Concentrated H_2SO_4 was added to the filtrate. A reddish brown colour was taken as positive for steroid ring.

Results

Sixteen plant extracts were tested in an HIV-1 viral replication assay using MT-4 cells infected with the NL4−3 strain, which is a T-cell adapted X4 wild type HIV-1 virus. Among the species showing significant activity were the ethanol extracts of the fruits of *G. livingstoneii* and *G. semseii* that indicated anti-HIV-1 replication effects with EC_{50} values of 2.25 and 0.93 μg/ml, respectively.

Table 2: Anti-HIV replication activity of fruit extracts of *G. livingstoneii* and *G. semseii*

Plant name		HIV-1 replication inhibition EC_{50} (μg/ml)		Cytotoxicity, CC_{50}		SI	$\frac{CC_{50}}{CC_{50}\ AZT}$
	Part		N		N		
G. livingstoneii	Fruit	2.25± 0.51	4	6.1± 0.5	4	2.7	363
G. semseii	Fruit hulls	0.93± 0.67	4	3.12± 0.42	4	3.4	150

*Results are expressed as EC_{50} and CC_{50} values (μg/ml) ±SD
N = # of independent experiments;
CC_{50}, = the concentration of test sample that was toxic to 50% of the infected cells;
EC_{50}, = the concentration of the test sample that was able to suppress HIV replication by 50%
AZT (+ve control), EC_{50} = 0.0062± 0.0007μg/ml and CC_{50} value > 40 μg/ml

The cytotoxicity of the *G. livingstoneii* extract on the T cells was CC_{50} = 6.1 μg/ml with a very narrow selectivity index (SI) of 2.7 while the CC_{50} value of *G. semseii* was 3.12 μg/ml with a very narrow selectivity index of 3.4. The comparisons of EC_{50} values of the fruits of *G. livingstoneii* and *G. semseii* with that of standard drug revealed 362.9 and 150.0 fold far lower anti-HIV activity as compared to the standard drug, AZT (Table 2) respectively. The rest of the crude extracts were not active (EC_{50} > 20 μg/ml) on this assay and having very narrow SI < 2 (Table 2).

Table 3: Cytotoxicity results of Tanzanian *Garcinia* species against four human cancer cell lines

Extract	Part	MW	Cytotoxicity (CC_{50} (μg/ml)*			
			A549	DU145	KB	Kbvin
G. livingstoneii	Fruit		8	8.2	5.7	12
G. semseii	Fruit hulls		7.87	9.1	7.81	8.8
	Seed		16.5	14	20	>20
	Stem		9.2	12.1	>20	>20
Taxol (up~0.1 μM)		853.91nM	11nM	8.92 nM	5.8 nM	>100nM

* CC_{50} values >20 μg/ml ; not considered to be significant and not calculated.
A549 = lung adenocarcinoma; DU145 = prostate carcinoma; KB = nasopharyngeal carcinoma; Kbvin = vincristine resistant nasopharyngeal

In the cytotoxicity assay, eighteen *Garcinia* plant extracts were screened for cytotoxicity against A549, DU145, KB and Kbvin cell lines (Table 3). Among the tested extracts, only the fruit extracts of *G. livingstoneii* and *G. semseii* showed moderate to mild cytotoxic activities against four human cell lines with CC_{50} values ranging from 5.7-12.0 μg/ml. The extracts from fruit hulls, seed and stem bark of *G. semseii* showed marginal activity to all four cancer cells with CC_{50} values ranging from 7.8-20 μg/ml.

Table 4: Comparative cytotoxicity results of *Garcinia* extracts against cancer cell lines

Plant name	Part	CC_{50} A549 CC_{50}Taxol	CC_{50} DU145 CC_{50}Taxol	CC_{50} KB CC_{50}Taxol	CC_{50} Kbvin CC_{50}Taxol
G. livingstoneii	Fruit	851	1076	1151	>140.5
G. semseii	Fruit hulls	837	1194	1578	>103.1
	Seed	1755	1837	4040	>234
	Stem	978	1588	>4040	>234

The comparison of the bioactivity of the crude extracts to that of a standard showed to be far lower cytotoxic than that of a standard drug (Table 4). The rest of the crude extracts were not active on this assay showing CC_{50} values > 20 μg/ml. In this test, taxol was used as a positive control. All *Garcinia* extracts were subjected to phytochemical screening methods that revealed the presence of mainly phenolics and some steroidal compounds (Table 5).

Table 5: Phytochemical screening of the extracts from *some Garcinia* species

Plant name	Part	Class of compound tested*				
		Tannins	Saponins	Phenolics	Alkaloids	Steroids
G. ferrea	Root	-	-	+	-	-
	Fruit	+	-	+	-	-
	Stem	-	-	+	-	-
G. edulis	Root	-	-	+	-	+
	Stem	-	-	+	-	+
G. bifasciculata	Stem	-	-	+	-	+
G. buchananni	Root	+	-	+	-	+
	Stem	-	-	+	-	+
G. semseii	Stem	-	-	+	-	+
	Root	-	-	+	-	+
	Fruit hulls	-	-	+	-	-
	Seed	-	-	+	-	-
G. volkensis	Stem	-	-	+	-	+
G. livingstoneii	Root	-	-	+	-	+
	Stem	-	-	+	-	+
	Fruit	-	-	+	-	+
G. kingaensis	Stem	-	-	+	-	+
G. huillensis	Root	+	-	+	-	+
	Stem	-	-	+	-	+

* +: present; -: absent

Discussion

The present study has shown that some crude extracts from the *Garcinia* plant species growing in Tanzania have both cytotoxic and anti-HIV-1 activities. Out of sixteen extracts tested in an HIV-1 viral replication assay, two extracts (fruit extracts of *G. semseii* and *G. livingstoneii*) showed moderate to mild anti-HIV activities, respectively. The literature indicated that some polyisoprenylated benzophenone derivatives isolated from *Clusia* (Piccinelli *et al.,* 2005) and *Garcinia* (Gustafson *et al.,* 1992) genera exhibited potent anti-HIV-1 activities. Similar findings have been reported by Gustafson, *et al.,* 1992 of the isolation of guttiferones, benzophenone compounds with anti-HIV activities from *Garcinia* plants. Recent study on the stem bark of *G. semseii* reported the isolation of three novel prenylated

benzophenones (Magadula *et al*, 2008), which were not tested due to the small amount of pure compounds obtained. Therefore, this class of compounds may be responsible for the activity indicated by extracts of *G. livingstoneii* and *G. semseii* hence phytochemical work on these two plants have to be undertaken.

Our results also show that, four extracts indicated moderate to mild cytotoxic activities with CC_{50} in between 5.7-20.0 μg/ml on four human cancer cell lines. The phytochemical screening indicated the presence of phenolic and steroidal compounds. These compounds are widely reported in the genus *Garcinia* (Nyemba *et al.,* 1990; Oliveira *et al.,* 1999) displaying significant cytotoxic activities. For instance, three polyisoprenylated benzophenones, isogarcinol, garcinol and xanthochymol were isolated from the pericarps of *G. purpurea* and evaluated to show growth inhibition in four human leukemia cell lines (NB4, HL60, U937 and K562) (Matsumoto *et al.,* 2003). Furthermore, two benzophenones, gutifferone H and gambogenone isolated from fruits of *G. xanthochymus* indicated cytotoxicity activity in the SW-480 colon cancer cell line with CC_{50} values of 12 and 188 μM respectively (Baggett *et al.,* 2005). Hence, there is a need to investigate phytochemically, the active fractions from these plants in order to establish chemical constituents responsible for the cytotoxic and HIV-1 activities.

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