African Journal of Biotechnology Vol. 9 (12), pp. 1848-1852, 22 March, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Anti-HIV-1 protease activities of crude extracts of some *Garcinia* species growing in Tanzania

Joseph J. Magadula[1]* and Supinya Tewtrakul[2]

[1]Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, Box 65001, Dar Es Salaam, Tanzania.
[2]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, 90112, Thailand.

Accepted 11 June, 2009

Eighteenth ethanol extracts from some *Garcinia* species in the Guttiferae (Clusiaceae) family collected in Tanzania were investigated for their HIV-1 protease (HIV-1 PR) inhibitory activities using high performance liquid chromatography (HPLC). Among the tested extracts, the fruit hulls of *Garcinia semseii* showed the most potent inhibitory activity against HIV-1 PR with an IC_{50} value of 5.7 µg/ml followed by the stem bark extracts of *Garcinia edulis* and *Garcinia kingaensis* with IC_{50} values of 9.2 and 15.2 µg/ml, respectively. Phytochemical screening of extracts indicated mostly the presence of phenolic and steroidal compounds.

Key words: *Garcinia* species, clusiaceae, crude extracts, HIV-1 protease, inhibitory effect.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) crisis is one of the greatest public health and humanitarian challenges in our time. In the two decades since the disease was first diagnosed, 20 million lives have been succumbed of AIDS. Currently, over 30.8 million people are infected with HIV/AIDS and 95% of them live in the developing countries (UN-AIDS Report, 2005 and 2007). The causative organism in AIDS is the human immunodeficiency virus type 1 (HIV-1) which is the member of retrovirus family. One of the enzymes that is responsible in the life cycle of the virus is the HIV-1 protease (PR) which process viral proteins into functional enzymes and structural proteins. HIV-1 PR plays a key role in the maturity and infectivity of the virus hence it has become an important target in HIV drug development (Kohl et al., 1988). HIV-1 PR function as a protein dimer of 11 kDa each, that possess a residue of Asp-Thr-Gly as the catalytic site, and the amino acid subjected to the cleavage by protease includes Phe-Pro, Pro-Tyr and Leu-Phe in the polyprotein (Oroszlam, 1989). The investigation of Tanzanian *Garcinia* plant species as HIV-1 inhibitors has been initiated as the alternative strategy to supplement the existing HIV-PR inhibitors such as nelfinavir, amprenavir and saquinavir that have been reported to develop resistance and some side effects (Borman et al., 1996). Few *Garcinia* species reported in the literatures have been investigated for their HIV-1 PR inhibitory effects. These include *Garcinia mangostana* which gave mangostin, a compound that indicated significant HIV-1 protease inhibition (Chen et al., 1996). *Garcinia livingstoneii* has been reported to produce gutifferone A, being an anti-HIV compound (Gustafson, et al., 1992). Furthermore, other genera of the family clusiaceae showed interesting and strong activity in inhibiting the PR and RT enzymes such as coumarin derivatives (Reyes et al., 2004).

This paper reports the results of anti-HIV-1 protease activity screening of some Tanzanian medicinal plants of the genus *Garcinia* (Clusiaceae). Eighteen extracts from nine *Garcinia* species collected from different parts in Tanzania were investigated (Tables 1 and 2).

MATERIALS AND METHODS

Plant materials

The plant materials were identified by Mr. Haji O. Suleimani of the Department of Botany, University of Dar es Salaam. They include

*Corresponding author. E-mail: jmagadula@yahoo.co.uk. Tel: +255 22 2150096. Fax: +255 22 2150465.

Table 1. Phytochemical screening of the extracts from *some Garcinia* species.

S/N	Plant name	Voucher specimen	Locality in Tanzania	Part	Class of compound tested*				
					Tannins	Saponins	Flavonoids (phenolics)	Alkaloids	Steroids
1	*G. ferrea*	HOS 3425	Amani-Tanga	Root	-	-	+	-	-
				Fruit	+	-	+	-	-
				Stem	-	-	+	-	-
2	*G. edulis*	HOS 3426	Amani-Tanga	Root	-	-	+	-	+
				Stem	-	-	+	-	+
3	*G. bifasciculata*	FM 10135	Kimboza-Morogoro	Stem	-	-	+	-	+
4	*G. buchananni*	HOS 3427	Amani-Tanga	Root	+	-	+	-	+
				Stem	-	-	+	-	+
5	*G. semseii*	HOS 3422	Kihansi-Iringa	Stem	-	-	+	-	+
				Root	-	-	+	-	+
				Fruit hulls	-	-	+	-	-
				Seed	-	-	+	-	-
6	*G. volkensis*	HOS 3424	Amani-Tanga	Stem	-	-	+	-	+
7	*G. livingstoneii*	HOS 3423	Pugu Forest	Root	-	-	+	-	+
				Stem	-	-	+	-	+
8	*G. kingaensis*	HOS 3429	Lugoda-Iringa	Stem	-	-	+	-	+
9	*G. huillensis*	HOS 3428	Lugoda-Iringa	Root	+	-	+	-	+
				Stem	-	-	+	-	+

+ = Present; - = not detected.

Garcinia bifasculata N. Robson, *Garcinia buchananii* Bak., *Garcinia edulis* Exell. *Garcinia ferrea* Pierre, *Garcinia huillensis* Welw. ex Oliv., *Garcinia kingaensis* Engl., *G. livingstonei* T. Anderson, *Garcinia semseii* Verdc and *Garcinia volkensis*. The voucher specimens are deposited in the Herbarium at the Department of Botany, University of Dar es Salaam, Tanzania.

Preparation of extracts

Ten grams (powder) of the specified part of each dried plant material were soaked in ethanol (150 ml) for 48 h at room temperature. The ethanol extract was filtered and evaporated under vacuum on a rotary evaporator. The crude extracts were dissolved in DMSO for bioassay.

Enzymes and chemicals

Recombinant HIV-1 PR, substrate peptides and acetyl pepstatin, were purchased from Sigma Chemical Co., St. Louis, USA.

Phytochemical screening test for the extracts

The methods of Trease and Evans (1983); Harbourne (1983) to test for alkaloids, tannins, flavonoids, steroids and saponins were used.

Test for flavonoids

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 flavonoids or any other phenolic compounds.

Test for alkaloids

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.

Test for tannins

The extract (1 g) was dissolved in 20 ml of distilled water

Table 2. Anti-HIV-1 protease activity of crude extracts of some *Garcinia* species.

S/N	Plant name	Plant part	% inhibition at various concentrations (µg/ml)*				Regression equation Y = A Ln(x) ±B	IC_{50} (µg/ml)
			3	10	30	100		
1	*G. ferrea*	Root	-	-22.1±4.8	29.4±2.4	87.9±6.4	Y = 47.785 Ln(x)-132.47	45.5
		Fruit	-	-4.4±2.3	3.7±5.2	94.8±1.3	Y = 43.601 Ln(x)-118.46	47.6
		Stem	-	12.1±1.1	64.8±0.2	85.5±4.3	Y = 31.643 Ln(x)-54.603	27.3
2	*G. edulis*	Root	-	-25.9±2.3	17.6±5.5	84.8±8.4	Y = 48.2 Ln(x)-140.13	51.7
		Stem	-	49.3±1.9	72.5±2.5	85.7±4.7	Y = 15.731 Ln(x)+15.11	9.2
3	*G. bifasciculata*	Stem	-	-	-	-32.7±11		>100
4	*G. buchananni*	Root	-	-	-	-6.3±4.4		>100
		Stem	-	-	-	-1.8±5.1		>100
5	*G. semseii*	Stem	-	24.9±2.0	47.7±0.5	91.2±3.5	Y = 28.911 Ln(x)-44.746	26.5
		Root	-	4.5±0.8	71.5±4.9	82.8±1.4	Y = 33.613 Ln(x)-62.571	28.5
		Fruit hulls	-	56.4±3.9	81.8±2.5	93.8±1.5	Y = 16.143 Ln(x)+21.862	5.7
		Seed	-	14.4±7.1	38.4±4.3	86.6±3.9	Y = 31.494 Ln(x)-61.758	34.8
6	*G. volkensis*	Stem	-	-13.5±0.1	-6.1±0.2	86.5±3.4	Y = 44.007 Ln(x)-128.9	58.3
7	*G. livingstoneii*	Root	-	-2.3±5.4	44.5±2.0	87.0±4.0	Y = 38.727 Ln(x)-90.012	37.2
		Stem	-	23.1±2.4	34.3±7.5	89.5±3.4	Y = 29.108 Ln(x)-51.059	32.2
8	*G. kingaensis*	Stem	-	37.6±0.6	70.8±4.1	94.4±1.0	Y = 24.587 Ln(x)-16.889	15.2
9	*G. huillensis*	Root	-	-	-	-17.5±3.2		>100
		Stem	-	-	-	20.3±6.4		>100
Acetyl pepstatin (+ve control)			50.2±1.4	70.0±0.5	82.4±0.4	88.5±0.2	Y = 10.955 Ln(x)+41.533	2.2

- = Not determined

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.

Test for saponins

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 h. A total haemolysis of the blood around the extract was indicative of saponins.

Test for steroids

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.

Assay of HIV-1 PR inhibitory activity

This assay was modified from the previously reported method (Tewtrakul et al., 2003). In brief, the recombinant HIV-1 PR solution was diluted with a buffer composed of a solution containing 50 mM of sodium acetate (pH 5.0), 1 mM ethylenediamine disodium (EDTA.2Na) and 2 mM 2-mercaptoethanol (2-ME) and mixed with glycerol in the ratio of 3:1. The substrate peptide, Arg-Val-Nle-(pNO_2-Phe)-Glu-Ala-Nle-NH_2, was diluted with a buffer solution of 50 mM sodium acetate (pH 5.0). Two microliters of plant extract and 4 ml of HIV-1 PR solution (0.025 mg/ml) were added to a solution containing 2 ml of 50 mM buffer solution (pH 5.0) and 2 ml of substrate solution (2 mg/ml), and the reaction mixture (10 ml) was incubated at 37°C for 1 h. A control reaction was performed under the same conditions but without the plant extract. The reaction was stopped by heating the reaction mixture at 90°C for 1 min. Subsequently, 20 ml of sterilized water was added and an aliquot of 10 ml was analyzed by HPLC using RP-18 column (4.6 x 150 mm I.D., Supelco 516 C-18-DB 5 mm, USA). Ten microlitres of the reaction mixture was injected

to the column and gradiently eluted with acetonitrile (15-40%) and 0.2% trifluoroacetic acid (TFA) in water, at a flow rate of 1.0 ml/min. The elution profile was monitored at 280 nm. The retention times of the substrate and p- NO_2-Phe-bearing hydrolysate were 11.25 and 9.72 min, respectively. The inhibitory activity on HIV-1 PR was calculated as follows:

$$\% \text{ inhibition} = (A_{control} - A_{sample}) \times 100 / A_{control}$$

whereas A is a relative peak area of the product hydrolysate. Acetyl pepstatin was used as a positive control.

Statistical analysis

The results of anti-HIV-1 PR activity were expressed as means ±SD of three determinations. The IC_{50} values were calculated using the Microsoft Excel programme at which the logarithmic regression equations were obtained (Table 2).

RESULTS AND DISCUSSION

The phytochemical screening conducted on *Garcinia* extracts collected in Tanzania revealed the presence of mainly flavonoids (phenolic compounds) and some steroids (Table 1). These compounds are widely reported in the genus *Garcinia* having novel and known molecular structures (Oliveira et al., 1999, Mbwambo et al., 2006, Nyemba et al., 1990). It is therefore believed that the potential bioactivities noted in this study may have been contributed by these two classes of compounds.

Furthermore, investigation of ethanol extracts from some *Garcinia* species for their HIV-1 protease (HIV-1 PR) inhibitory activities using high performance liquid chromatography (HPLC) was carried out. Among the tested samples, the fruit hulls of *G. semseii* showed the most potent inhibitory activity against HIV-1 PR with an IC_{50} value of 5.7 µg/ml, followed by the stem bark extracts of *G. edulis* and *G. kingaensis* with IC_{50} values of 9.2 and 15.2 µg/ml, respectively (Table 2). Other crude extracts exhibited moderate to mild anti-HIV-1 PR activity (IC_{50} = 26.5->100 µg/ml). Acetyl pepstatin, a positive control, possessed anti-HIV-1 PR activity with an IC_{50} value of 2.2 µg/ml. Calanolide A, a kind of coumarin, isolated from *Calophyllum lanigerum* (Clusiaceae family) has been reported to possess anti-HIV-1 activity (Ma et al., 2008) as well as the compounds from *C. braziliense* leaves (Reyes et al., 2004). Therefore the plants in this study which are all in the Clusiaceae family may have potential to be developed as anti-HIV-1 agents, especially *G. semseii* (Fruit hulls), *G. edulis* (stem) and *G. kingaensis* (stem). Regarding chemical constituents of *Garcinia* species, it has been recently reported that polyisoprenylated benzophenones, semsinones A-C, have been isolated from the stem bark of *G. semseii* (Magadula et al., 2008). Some polyisoprenylated benzophenone derivatives isolated from *Clusia torresii* such as clusianone and 7-*epi*-clusianone have been reported to exhibit marked anti-HIV-1 activity (Piccinelli et al., 2005). Since *G. semseii* showed the most potent anti-HIV-1 PR effect in the present study (IC_{50} = 5.7 µg/ml), prenylated benzophenone derivatives might be responsible for anti-HIV-1 PR activity of this plant. Hence, these plants are the potential candidates as sources of ingredients for drug formulations for the treatment of HIV/AIDS and other related illness. The isolation of active compounds from *G. semseii* fruit hulls and other active extracts will be further investigated.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Haji O. Suleiman of the Botany Department, University of Dar es Salaam, Tanzania for collection and identification of the plant materials and the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand, for laboratory facilities. This study was supported by the International Foundation for Sciences (IFS), Stockholm, Sweden and the Organisation for the Prohibition of Chemical Weapons (OPCW), the Hague through a grant number F/4572-1 extended to JJM.

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