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Short Communication

Inhibitory activity of *Detarium microcarpum* extract against hepatitis C virus

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ABSTRACT: Bioassay guided fractionation of the methanolic extract of *Detarium microcarpum* Guill using VLC and activity against hepatitis C virus in the Huh-7 Replicon assay was done. The active fraction MTH-1700 which demonstrated good inhibitory and selective potency (83.87%) against Hepatitis C Virus and comparable with 86.76% of the control; RS-446(2-Me-C) at a concentration of 10 µM was further fractionated by HPLC.
Keywords: *Detarium microcarpium; Anti-viral activity; Hepatitis; Cytotoxicity.*

INTRODUCTION

Hepatitis C Virus (HCV) provides the greatest risk of all the chronic liver diseases and it is the most serious hepatitis virus that leads to progressive, life-threatening chronic hepatitis, cirrhosis, liver cancer, and liver failure (Leikin & Lipsky, 2003). Hepatitis C Virus establishes a chronic infection in 50 - 80% of all reported cases (Wilber ,1995) and about 90% of the sufferers retain the indefinite evidence of it and therefore become carriers of the virus (Leikin & Lipsky, 2003). Alpha interferon (IFN-), either alone or in combination with ribavirin, is the only approved therapy for chronic hepatitis C. Anemia is the most common adverse effect associated with ribavirin treatment and neuropsychiatric adverse effects of IFN-lead to premature cessation of therapy in 10 - 20% of the patients (Collier & Chapman, 2001; Di Bisceglie *et al*. 2002). Therefore, the ongoing search for more potent antiviral compounds with fewer adverse effects should be intensified, especially among medicinal plants.

Detarium microcarpium Guill. (Fabacea) is an African leguminous medicinal plant found in the forests (Mabberley, 1987) and reported to possess antimicrobial and cytotoxic properties (Abreu *et al*. 1998; Abreu *et al*. 1999). The fruits and leaves are used traditionally in the treatment of dysentery and syphilis. (Iwu, 1993; Ikhiri & Ilagouma 1995) and the root water extract is used for leprosy (Collier, 2001). *Detarium sengalense* showed cytotoxicity at 400µg /100 ml and antiviral activity against canine parvovirus, poliovirus, astrovirus and herpes simplex viruses (Kudi and Myint, 1999).

We here report the anti-viral activity of the fractions of *D. microcarpium* using Huh-7 Replicon assay.

METHODOLOGY

All chemicals were of analytical grade. Millipore water was colleted from a Millipore Millipack® Express, 0.22 µm water purifier (Millipore; Billerica, MA, USA). Vacuum liquid chromatography (VLC) was performed on Si gel (70-230 mesh-Merck). VLC on Si gel was

Manuscript received: December 2008; Revision Accepted March 2009
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carried out with gradient elution using hexane, EtOAc, MeOH and water in the order of increasing polarity. Preparative HPLC was performed with a waters Prep LC system.

Plant material

Stem bark of *Deterium microcarpium* was collected from Ibadan, Oyo State, Nigeria. Voucher specimen was deposited in the Forestry Research Institute of Nigeria, Ibadan.

Extraction and isolation

Plant extraction was carried out with MeOH (yield: 7.4%). Bioassay guided fractionation of the methanolic extract (30.0g) was done by VLC using normal phase conditions to give ten eleven fractions. The active anti-viral fraction (MTH-1700 , weight: 6.155g) was further fractionated by HPLC (Luna C8 column 21.2 x 250 mm) using acetonitrile and water as eluents with flow rate 15ml/min. Fifteen fractions were collected and submitted for bioassay.

Antiviral assay

The anti-HCV activity was determined in the Huh-7 clone B cells containing HCV Replicon RNA system. Cells were seeded in 96-well tissue culture plates (3 000 cells/well); the compounds were tested in dose response at concentration 10μM (triplicate). After addition of test compounds the plates were incubated for 5 days (37^0C, 5%, CO_2), total cellular RNA was isolated using the Manual Perfect Pure 96 CellVac kit from 5 Prime. The replicon RNA and the internal control (TaqMan rRNA control reagent, Applied Biosystems) were amplified were amplified in a single step multiplex Real Time RT-PCR Assay. The antiviral potency of the tested metabolites was calculated by subtracting the threshold RT-PCR cycle of the test compounds from the threshold RT-PCR cycle of the negative control (ΔCtHCV). The cytotoxicity of the compounds was also calculated by using the ΔCt rRNA values. RS-446(2-Me-C) was used as the control.

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RESULTS AND DISCUSSION

Table 1 shows the observed anti-viral activity and cytotoxicity of *D. microcarpium* column fractions in the Huh-7 Replicon assay. The active fraction MTH-1700 (6.155g) which was eluted with EtOAc-MeOH (75:25) demonstrated good inhibotry and selective potency (83.87%) against Hepatitis C Virus in a dose dependent manner compared with control (86.76%) at a concentration of 10 μM. Fraction MTH-1698(171mg) that was eluted with EtOAc gave higher inhibition (94.41%) of the virus but because of the demonstrated high toxicity it is regarded as being not selective. Hence, *D. microcarpium* exhibited similar antiviral property as *Detarium sengalense* (Kudi and Myint, 1999).

Table 1:

In Vitro activity of *D. microcarpium* metabolites against HCV in Human clone B cells with host cytotoxicity

Test agents	ΔCt HCV	ΔCt rRNA	% Inhibition	
			HCV	rRNA
RS-446	2.93	-0.02	86.76	-1.27
MTH-1695	-0.64	0.07	-55.98	4.83
MTH-1696	0.48	1.16	28.23	54.98
MTH-1697	0.68	1.11	37.64	53.61
MTH-1698	4.17	3.44	94.41	90.68
MTH-1699	0.24	-0.04	15.28	-2.68
MTH-1700	**2.64**	**0.34**	**83.87**	**21.03**
MTH-1701	-0.11	0.24	-8.15	15.18
MTH-1702	0.22	0.04	14.10	2.39
MTH-1703	-0.46	-0.18	-37.42	-13.37
MTH-1704	-0.75	-0.24	-67.91	-17.90

Test compounds = 10 µg/ml RS-446 (2-Me-C) was used as the control = 10 µM

Acknowledgments

We thank the American Society for Microbiology for Fellowship to Joseph Olugbuyiro. This work was supported by the NIH grants (300222265A). We thank Dr Raymond Schinazi, Emory University, for testing the anti-HCV activity.

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