African Journal of Pharmaceutical Research & Development

Vol. 6 No.1 pp.6-10 (2014)

Antihepatitis C Virus Activity of five Selected Endemic Medicinal Plants of Nigeria

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ABSTRACT

Traditional Nigerian medicinal herbs have long been used as remedies against hepatitis. The aim of the study was to investigate the claimed ethnomedicinal uses of the plants for which they are known and used. Some medicinal plants (*Persea americana* seed, PAS; *Persea americana* root, PAR; *Persea americana* leaf, PAL; *Annona muricata* stem bark, AMS; *Annona muricata* leaf, AML; *Jatropha podagrica* root, JPR and *Picralima nitida* stem bark, PNS were carefully selected using the ethnopharmacological approach. The methanol extracts were partitioned to give fractions. Evaluation for *in vitro* antihepatitis C activity against HCV RNA virus was investigated using standard and established protocols. Significant and notable cell growth inhibition against HCV RNA was observed for extracts PAR, PAS, and JPR and AMS against HCV rRNA virus. Among these extracts, AMS showed the most potent antiviral activities against HCV with EC_{50}, EC_{90} and CC_{50} values of 5.8, 33.1 and 22.6 µg/ml, respectively. In addition, the methanol extract of PNS showed a weak activity (10.75% HCV, rRNA 4.97). Taken together, these results indicate that *A. muricata* and *P. americanna* might offer a promising source of antiviral drugs against HCV. Purification of the active compound(s) would be required in the future.

KEYWORDS: *Persea americana, Jatropha podagrica, Picralima nitida,* HCV rRNA, *Annona muricata*

INTRODUCTION

Generally infections are important health problems all over the world, both in the developed and developing countries, due to their morbidity and mortality. The cure for Hepatitis C is attracting serious attention from scientists worldwide with a view to discovering new lead drugs for combating this disease. Hepatitis C virus (HCV) belongs to the family Flaviviridae, positive-stranded RNA virus [1]. It possess a 9.6 kb genome encoding a single polyprotein that is subsequently cleaved by both host and virus protease into a sub units 10 proteins, the six nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) and four structural proteins (C, E1, E2, and p7) [2]. It has been estimated that approximately 175 million people worldwide are chronically infected with HCV, which is one of the leading causes of chronic hepatitis, cancer of the liver and cirrhosis [3, 4]. About 20% of individuals infected with HCV spontaneously clear the virus in the acute phase, up to 90% develop chronic infection progressing to liver carcinoma and related diseases [5,6]. To date, no vaccine against HCV has been developed owing to the presence of large numbers of HCV genotypes and quasispecies, and lack of easily available animal models for vaccination tests [7-9]. Therefore, it is obvious that new and better drugs are required in the battle against HCV.

In our search for HCV inhibitors and rRNA inhibitors from traditional medicines, we investigated extracts and fractions from five different traditional Nigerian medicinal plants (*Jatropha podagrica*, *Persea*

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americana, Annona muricata, *Jatropha multifida,* and *Picralima nitida*), which are used in Nigerian folk medicine for the treatment of parasitic infections, cancer and hepatitis [10]. It is worthy to note that traditional medical practitioners have achieved success with the use of these plants as remedies against hepatitis.

Jatropha podagrica Hook is known locally in south western Nigeria as 'lapalapa funfun'. It is widely distributed in different parts of Nigeria, and is used in folk medicine to treat various diseases including parasitic skin infections and hepatitis. Different parts of the plant have been investigated chemically and many compounds including flavonoids, steroids, alkaloids and diterpenoids have been isolated from this plant and related species [11-13].

Picralima nitida (Stapf.) Th. & H. Durand has widely varied applications in Nigerian folk medicine as antipyretic, antimalarial, antitrypanocidal, antilesishmanial and antiparasitic (14, 15].

Jatropha multifida otherwise known as coral bush is a fast growing evergreen shrub or small tree. The roots, stems, leaves, seeds and oil of the plant have been widely used in African folk medicine for the treatment of oral candidiasis, viral diseases, gonorrhea, fever, as purgative and for wounds and skin infections [16-18].

Persea americana Mill commonly known as 'avocado pear' is a terrestrial, evergreen tree of 15–20 m in height. The leaves, root, seeds and other morphological parts of *P. americana* possess medicinal properties as antiparasitic, antiallergic, antihypertensive, analgesic and anti-inflammatory remedies. Traditional herbal healers use the root bark and seeds for the treatment of hepatitis [10] and other infections [19-22].

Annona muricata popularly called "soursop" is a fruit tree cultivated throughout the tropical regions of the world. The medicinal uses include antiparasitic, antidiabetic, antiprotozoal, hepatitis, cancer and cancer related diseases. The fruit is eaten raw. Systematic chemical investigation led to the isolation of more than 50 mono THF-acetogenins [23].

MATERIALS AND METHODS

Plant materials

All plant material (shown in Table 1) was collected from Edo State, Nigeria between January and June, 2013. They were identified and authenticated by Mr. Ugbogu O. A. and Shasanya O. S. of the Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimen is deposited in the herbarium.

Preparation of extracts

The powdered (100 g) material of each sample was extracted with 500 mL methanol for 48 hrs by cold maceration, filtered and the filtrate evaporated to dryness to obtain crude extracts (*Annona muricata* seed*,* AMS), (*Jatropha podagrica* leaf, JPL), (*Jatropha podagrica root,* JPR), (*Persea americana root*, PAR), (*Picralima nitida* stem) (*Persea americana* leaf, PAL) and (*Persea americana* seed, PAS). Some extracts were partitioned to obtain fractions. The dried extracts and fractions were each subjected to antihepatitis C test.

Anti HCV assay

Antiviral and cytotoxicity assays for HCV: Cells were maintained in Dulbeccos modified Eagles medium high glucose 4.5 g/L (LifeTechnologies, USA) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin and streptomycin, 1% L-pyruvate and 500 µg/mL of geneticin (G418; Invitrogen, USA). Geneticin was used to select cells permitting the HCV RNA replication. Cells were passaged every 4 days with a 1:4 ratio [24].

Cell Culture

Cells were seeded in 6-well plates at a density of 2.5 x 10^5 cells per well, 16 h before the beginning of treatment. Cells were treated with the extracts and fractions administered at different concentrations in complete medium that did not contain genticin. Administration of each extract was renewed every day for three consecutive days. Ribavirin (ICN Pharmaceuticals, USA), mycophenolic acid (Sigma, USA) and IFNa-2b (IntronA, USA) were used in the same conditions as positive controls. Total RNA was extracted at the end of treatment (24 h after the last day of treatment) with the reagent (Eurobio), which is a mix of phenol and guanidinium thiocyanate. Northern blot analysis was then performed using 6022 V NorthernMaxTM-Gly kit (Ambion, USA), following manufacturer's instructions. Five micrograms of total RNA were de-saturated in global buffer at 5^{o}C for 30 min, separated by 1.1% agarose gel electrophoresis and then transferred for 12 h onto a charged nylon membrane (Hybond^{+}, Marshal). Hybridization was carried out with three different [32P]CTP- labelled riboprobes obtained by *in vitro* transcription (Riboprobe *in vitro* transcription system; Promega). Two probes were complementary to the NS5A region of the HCV genome of negative polarity and positive polarity. A third probe was complementary to the beta-actin mRNA and obtained by *in vitro* transcription from a specific plasmid (pTRI beta actin human, reference 7424; Ambion). First, the blot was hybridized with the riboprobes directed

against the negative strand of HCV RNA and beta-actin mRNA. After one night of hybridization at 68°C, the membrane was washed, and then exposed to X-ray film and a phosphor screen (phosphorimager). This screen was then scanned and quantitative analysis was achieved using ImageQuant software. The amount of beta-actin mRNA was used as an internal loading control to standardize the amount of HCV RNA detected. The same membrane was subsequently hybridized with the negative sense riboprobe to determine the level of positive strand HCV RNA, using the same approach.

For cell viability assays, cells were seeded in 96-well plates at a density of 12,500 cells per well. They were treated by the different extracts with the same concentrations conditions as those used for the antiviral assays. Then, cell viability was measured by neutral red assay. Neutral red which specifically colours lysosomes and its accumulation depends on cellular membrane integrity. The yield of neutral red incorporated in cells is proportional to the number of living cells. At the end of treatment, culture medium was removed; cells were washed by PBS and then coloured with neutral red at 0.005% for 3 h at 37 °C. Cells were then fixed for 1 min by formol calcium and lysed by treatment with a 10% v/v mixture of acetic acid and ethanol. After 15 min incubation, absorbance was read at 490 nm [24].

RESULTS AND DISCUSSION

The results of the study indicated that *A. muricata* stem bark (AMS) and leaf (AML) exhibited the inhibitory effect on HCV antigen and rRNA (84.24% and 78.23% for leaf, 89.71% and 60.24% for stem) at a concentration of 10 µg/mL (Table 2). Treatment of HCV cells with AMS at various concentrations (1, 3, 10 and 33 µg/mL) resulted in significant percentage inhibitory reduction of HCV and rRNA secretion in a concentration-dependent manner (Table 3). AML inhibited HCV and rRNA secretions by 84.24% and 78.23% at non-cytotoxic concentration of 10 µg/mL. From all indications, *A. muricata* stem (AMS) bark showed the most potent antiviral activity against HCV with EC_{50}, EC_{90} and CC_{50} values of 5.8, 33.1 and 22.6 µg/mL, respectively. This probably could be due to the presence of acetogenins with multi-hydroxy groups in the plant extract. EC_{50} and CC_{50} values of *Annona muricata* stem (AMS), suggest a promising future for this extract as anti HCV. The other extracts (JML, JMR, PALH) demonstrated moderate activity against HCV virus (Table 2). *P. americana* root (PAR) also showed promising activity against HCV in a dose-dependent manner at concentrations of 1, 3, 10, and 33 µg/mL (Table 3). Previous phytochemical investigation of PAR revealed the presence of alkene gamma lactone and long chain fatty acids [25], and the marked inhibitory activity against HCV of the plant extract could be attributed to these constituents. The inhibitory activity of PAR, PAS and PARP were 89.15, 85.80 and 94.74% respectively. The weakest activity was demonstrated by *P. nitida* giving 10.75 % and 4.97 % inhibition for HCV and rRNA respectively. *P. nitida* have been reported to contain copious indole alkaloids, also suggesting that these alkaloids lack HCV activity.

In this preliminary study, it is obvious that *A. muricata* is a good candidate for anti HCV agents. *Annona muricata*, *J. podagrica* and *P. americana* class have never been evaluated for their anti HCV activity, interesting activity profile of these extracts and fractions, opens up a new class of anti HCV metabolites.

Further purification of these herbal extracts and isolation of the active metabolites may identify new lead molecules, which could be developed into anti HCV drugs.

CONCLUSION

In conclusion, we have demonstrated that the extracts of *A. murcata stem* and *P. americana* root possess a significant inhibitory effect on HCV replication. These results, showing in particular an interesting anti HCV activity, confirm the relevance of the investigation on the therapeutic potential of plants used by rural communities.

ACKNOWLEDGEMENT

This work was in part supported by a US- Senior Fulbright Award granted to Dr. A. Falodun for study at University of Mississippi, USA, CIESCs for the Fulbright award. USDA Agricultural Research Service Specific Cooperative Agreement 58-6408-1-603 and TETFUND grant 2013, URPC (VC 23 University of Benin) is also highly acknowledged.

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Table 1: Plant part, place of collection and voucher specimen numbers of plants used

Plant	Voucher - specimen no.	Family	Locality	Part tested	Extraction yield
Jatropha multifida Linn	FHI109573	Euphorbiaceae	Owan west	Root, Leaf	15.98%
Persea americana Mill	FHI 107767	Lauraceae	Ovia North east Ekosodin	Leaf, Root, seed	40.68%
Jatropha podagrica Hook	FHI 93265	Euphorbiaceae	Owan West	Leaf, Root	7.02%
Annona muricata	FHI 39154	Annonaceae	Oredo Benin City	Stem, Leaf	12.34%
Picralima nitida	FHI109429	Apocynaceae	Ikpoba-Okha	stem	16.32%

Table 2: Anti HCV activities of extracts and fraction from the medicinal plants

Treatment		Concentration (µg/mL)	DCt HCV	DCt rRNA	% inhibition	
					HCV	rRNA
AM						
		10	2.67	2.21	84.24	78.23
AML						
	AMS	10	3.29	1.34	89.71	60.29
JP						
	JPL	10	0.37	0.50	22.62	29.21
	JPR	10	2.53	1.14	82.61	54.47
JM						
	JML	10	0.47	0.57	27.62	32.40
	JMR	10	-0.38	-0.41	-30.23	-32.45
PA						
	PALH	10	0.58	1.77	33.23	70.63
	PAR	10	3.21	0.62	89.15	34.70
	PARP	10	4.26	4.36	94.74	95.07
	PAS	10	2.82	5.43	85.80	97.66
PN						
	PNS	10	0.16	0.07	10.75	4.97

PAS: *Persea americanna* seed
PARP: Petroleum ether fraction of *Persea americana* root bark
PALH: Hexane fraction of *Persa americana* leaf

Table 3: EC_{50} and EC_{90} values of fraction

Treatment	Concentration (µg/mL)	DCt HCV	DCt rRNA	% inhibition		EC values(µg/mL)		
				HCV	rRNA	[a]EC_{50}	[b]EC_{90}	[c]CC_{50}
AMS	33	3.31	1.21	89.86	56.71	5.8	33.1	22.6
	10	1.37	0.79	61.20	41.93			
	3	0.80	0.33	42.33	20.21			
	1	0.38	0.02	22.92	1.37			
PAR	33	0.55	0.06	31.46	4.28	ND	ND	ND
	10	-0.73	-0.31	-	-24.17			
	3	-0.10	-0.45	-7.15	-36.79			
	1	0.09	-0.13	6.03	-9.65			

ND, not Determined.
[a]EC_{50}: The concentration that affords 50% inhibition of viral growth.
[b]EC_{90}: The concentration that affords 90% inhibition of viral growth
[c]CC_{50}: concentration of the 50% cytotoxic effect

REFERENCES

1. Lindenbach, B.D. and Rice, C.M. Unraveling hepatitis C virus replication from genome to function. Nature, 436, **2005**, 933-938.
2. Lin, K.; Perni, R.B.; Kwong, A.D. and Lin, C. VX-950, a novel hepatitis C virus (HCV). NS3-4A protease inhibitor, exhibits potent antiviral activities in HCV replicon cells. Antimicrobial Agents Chemotherapy, 50, **2006**, 1813-1822.
3. Mao, H.X.; Lan, S.Y.; Hu, Y.W.; Xiang, L. and Yuan, Z.H. Establishment of a cell-based assay system for hepatitis C virus serine protease and its primary applications. World Journal of Gastroenterology, 9, **2003**, 2474–2479.
4. Lever, M. Viral Hepatitis and liver cancer: the case of Hepatitis C. Oncogenesis, 25, **2006**, 3834-3847.
5. Saito, I.; Muyimura, T.; Obhayashi, O.A.; Harada, H.; Kikuchi, S.; Watanabe, Y.; Koi, S.; Onji, M. and Ohta, Y. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proceedings of the National Academy of Sciences, 87, **1990**, 6547–6549.
6. Seeff, L.B. Natural History of Hepatitis C. Hepatology, 26, **1997**, 21S-28S.
7. Shepard, C.W.; Finelli, L. and Alter, M.J. Global epidemiology of hepatitis C virus infection. The Lancet, 5, **2005**, 558–567.
8. Jens, B. A critical role for the chimpanzee model in the study of Hepatitis C. Hepatology, 39, **2004**, 1469-1475.
9. Desjeux, P. and Alvar, J. Leishmania/HIV co-infection: epidemiology in Europe. Ann Tropical Medicine and Parasitology, 97, **2003**, 4–15.
10. Gills, L.S. Ethnomedical uses of plants in Nigeria. University of Benin press, Nigeria. **1992**, Pp 276.
11. Odebiyi, O.O. Antibacterial property of tetramethylpyrazine from the stem of *Jatropha podagrica*. Planta Medica, 38, **1980**, 144–146.
12. Aiyelaagbe, O.O.; Adesogan, E.K.; Ekundayo, O. and Gloer, J.B. Antibacterial diterpenoids from *Jatropha podagrica* Hook. Phytochemistry, 68, **2007**, 2420–2425.
13. Aiyelaagbe, O.O.; Adesogan, E.K.; Ekundayo, O. and Hassanali, A. Antifeedant activity of *Jatropha podagrica* roots. Fitoterapia, 69, **1998**, 175–176.
14. Wosu, L.O. and Ibe, C.C. Use of extracts of *Picralima nitida* bark in the treatment of experimental trypanosomiasis: A preliminary study. Journal of Ethnopharmacology, 25, **1989**, 263–268.
15. Fakeye, T.O.; Itiola, O.A. and Odelola, H.A. Evaluation of the antimicrobial property of the stem bark of *Picralima nitida* (Apocynaceae). Phytotherapy research, 14, **2000**, 368–370.
16. Dehgan, B. *Proc Fla State.* Horticulture Society, 95, **1982**, 277–280.
17. Kayode, J. and Omotoyinbo, M. A. Research Journal of Botany, 3, **2008**, 107-115.
18. Aiyelaagbe, O.O. Antibacterial activity of *Jatropha multifida* roots. Fitoterapia, 72, **2001**, 544–546.
19. Adeyemi, O.O.; Okpo, S.O. and Ogunti, O.O. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia, 73, **2002**, 375–380.
20. Antia, B.S.; Okokon, J.E. and Okon, P.A. Hypoglycemic activity of aqueous leaf extract of thoracic rat aorta. *Persea americana* Mill. India Journal of Pharmacology, 37, **2005**, 325–326.
21. Adeboye, J.O.; Fajonyomi, M.O.; Makinde, J.M. and Taiwo, O.B. A preliminary study on the hypotensive activity of *Persea americana* leaf extracts in anaesthetized normotensive rats. Fitoterapia, 70, **1999**, 15–20.
22. Owolabi, M.A.; Jaja, S.I. and Coker, H.A.B. Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated rat uterus. Fitoterapia, 76, **2005**, 567-573.
23. Feng, E.; Zeng, L.U.; Zhe-Ming, G.U.; Geng-Xian, Z.; Yean, Z.; Jon, T.; Schwedler, J.L. and McLaughlin, J.L. New Bioactive monotetrahydrofuran Annonaceous Acetogenins, Annomuricin C and Muricatocin, from the leaves of *Annona muricata*. Journal of Natural Products, 58, **1995**, 909-915.
24. Schinazi, R.F.; McMillan, A.; Cannon, D.; Mathis, R.; Lloyd, R.M.; Peck, A.; Sommadossi, J.P.; St. Clair, M.; Wilson, J.; Furman, P.A.; Painter, G.; Choi, W.B. and Liotta, D.C. Antimicrobial Agents and Chemotherapy, 36, **1992**, 2423–2431.
25. Falodun, A.; Nadja, E.; Udo K.; Barbara N. and Langer, P. Novel anticancer alkene lactone from *Persea americana*. Pharmaceutical Biology, 51(6), **2013**, 1-7**.**