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VIRAL HEPATITIS IN THE PERUVIAN AMAZON: ETHNOMEDICAL CONTEXT AND PHYTOMEDICAL RESSOURCES

Vincent Roumy[a], Lastenia Ruiz[b], Juan Celidonio Ruiz Macedo[c], Andrea-Luz Gutierrez- Choquevilca[d], Jennifer Samaillie[a], Leonor Arévalo Encinas[b], Wilfredo Ruiz Mesia[b], Hivelli Ericka Ricopa Cotrina[b], Céline Rivière[a], Sevser Sahpaz[a], Simon Bordage[a], Guillaume Garçon[e], Jean Dubuisson[f], Sebastien Anthérieu[e], Karin Séron[f], Thierry Hennebelle[a].

[a]Univ. Lille, INRA, YNCREA, Univ. Artois, Univ. Littoral Côte d'Opale, EA 7394-ICV-Institut Charles Viollette, F-59000 Lille, France.
[b]Laboratorio de Investigación de Productos Naturales Antiparasitarios de la Amazonia (LIPNAA), Universidad Nacional de la Amazonía Peruana (UNAP), Centro de Investigaciones de Recursos Naturales de la Amazonía (CIRNA), Nuevo San Lorenzo, Iquitos, Perú.
[c]Herbarium de la Amazonía Peruana Amazonense de la Universidad Nacional de la Amazonía Peruana (UNAP), Nanay con Pevas, Iquitos, Perú.
[d]Ecole Pratique des Hautes Etudes, EPHE PSL, Laboratoire d'anthropologie sociale (UMR 7130, Collège de France, 75005 Paris, France).
[e]Univ. Lille, CHU Lille, Institut Pasteur de Lille, EA 4483-IMPECS-IMPact de l'Environnement Chimique sur la Santé humaine, F-59000 Lille, France.
[f] Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 – CIIL, Center for Infection and Immunity of Lille, F-59000 Lille, France.

Abstract

Ethnopharmacological relevance

An extensive ethnopharmacological survey was carried out in the Peruvian Amazonian district of Loreto with informants of various cultural origins from the surroundings of Iquitos (capital city of Loreto) and from 15 isolated riverine Quechua communities of the Pastaza River.
A close attention was paid to the medical context and plant therapy, leading to the selection of 35 plant species (45 extracts). The extracts were tested for antiviral activity against HCV with counting of Huh-7 cellular death in case of toxicity, and cytotoxicity was evaluated in HepG2 cells.

Aim of the study

The aim of the study was to inventory the plants used against hepatitis in Loreto, then to evaluate their antiviral activity and to suggest a way to improve local therapeutic strategy against viral hepatitis, which is a fatal disease that is still increasing in this area.

Materials and methods

An ethnographic survey was carried out using “participant-observation” methodology and focusing on plant therapy against hepatitis including associated remedies. 45 parts of plant were extracted with methanol and tested *in vitro* for anti-HCV activity in 96-well plate, using HCV cell culture system with immunofluorescent detection assisted by automated confocal microscopy. Toxicity of plant extracts was also evaluated in microplates on hepatic cells by immunofluorescent detection, for the Huh-7 nuclei viability, and by UV-absorbance measurement of MTT formazan for cytotoxicity in HepG2 cells.

Results

In vitro assay revealed interesting activity of 18 extracts (50% infection inhibition at 25 µg/mL) with low cytotoxicity for 15 of them. Result analysis showed that at least 30% of HCV virus were inhibited at 25 µg/mL for 60% of the plant extracts. Moreover, the ethnomedical survey showed that remedies used with low and accurate dosing as targeted therapy against hepatitis are usually more active than species indicated with more flexible dosing to alleviate symptoms of hepatic diseases.

Conclusion

Together with bibliographic data analysis, this study supported the traditional medicinal uses of many plants and contributed to a better understanding of the local medical system. It also permitted to refine the therapeutic plant indications regarding patients’ liver injuries and vulnerability.

Only 2 of the 15 most active plant species have already been studied for antiviral activity against hepatitis suggesting new avenues to be followed for the 13 other species.

Keywords: Antiviral activity; Hepatitis C; Peru; Medicinal plant; Traditional use.

Introduction

Since 2017, viral hepatitis B and C have been considered as a major public health concern affecting 325 million people and leading to around 1.5 million deaths every year (WHO, 2017). Approximately 1.5-2 million people are newly infected with viral hepatitis C every year, bringing the total number of the population chronically ill to 71 million in 2019 (for 150 million infected people), and causing around 400 000 deaths (Carrat et al., 2019; Petruzziello et al., 2016; Séron et al., 2018).
The estimated HCV prevalence in Latin America was around 1.4% in 2018 (MINSA, 2018), with higher prevalence in tropical area such as Peruvian Amazonia, where this study was carried out. Contrary to hepatitis B, whose prevalence has reduced over the past decade, hepatitis C cannot be easily prevented by vaccination or available treatments and it became one of the most common causes of death among Amerindian populations. Moreover, the Peruvian health sector policy promotes community-based and self-supported health care services in the isolated indigenous communities. Therefore, traditional medicine remains the first recourse for treating health problems but still needs scientific evaluation (PAHO, 2019; Mejia and Rengifo, 2000).
In this context, attention has been paid to plant species used in traditional medicine against hepatitis and associated liver diseases in order to contribute to the “*Materia medica* for infectious diseases in the Amazonian district of Loreto” (Consensus Statement on Ethnopharmacological Field Studies - ConSEFS Heinrich, 2018). This study took place in the northern region of Peru called Loreto with a high level of both biodiversity and traditional knowledge. The bioprospecting was multisite and performed in Iquitos city surroundings or in Quechua/Jivaro communities along the Pastaza River. These 2 different areas were chosen to obtain more extensive data. The first one, located around Iquitos, is characterized by mixed-blood native people’s ethnomedical system including neighbouring Amerindian knowledge and more “conventional” medicines. The second one consisted of the riverside indigenous populations of the

Pastaza, mainly composed of Quechua, Jivaro (Shuar, Ashuar...) and Candoa (Candoshi, Shapra) groups. In this more remote area, hepatitis is a major concern and herbalism is still the most effective treatment (Duke and Vasquez, 1994; FORMABIAP and AIDESEP, 2008).

Despite many centuries of colonisation and contemporary petroleum or forestry exploitations, the Peruvian Amazonian territory, nearly 60% of the country's area, is still inhabited by remote indigenous populations. The Pastaza River Wetland is located in Loreto and covers 3.8 million hectares, the largest tropical area of Peru. The Pastaza River begins in the Ecuadorian Andes and the mixture of this spring water with the black one from the flooded forests contributes to a very high and peculiar biodiversity. This area includes over 300 indigenous communities mainly composed of Quechua-speaking population (more than 6000 people) mixed with around 7500 Jibaroan speakers (C. Germaná, L. Lozano, 2013). Their subsistence economy relies on slash-and-burn horticulture, hunting, fishing and gathering, which contributes to the maintenance of large forest areas. Their elementary conception and exchange with their natural environment is based on the animism. It consists in the attribution of a common subjectivity or a "soul" to every being, including plants or animals and lead to a social structuration of the relations between human beings and natural species (Descola, 2013). Sickness in many ways reveals a

disruption affecting the individual's soul and its relation with otherness, humans or non-humans. Accordingly to this belief, although this population uses many medicines, ill people mostly attend a Shaman ("yachak" in Quechua or "curandero" in Spanish; Roumy et al., 2007).

Materials and methods

Ethnopharmacological survey

The ethnobotanical study was conducted during humanitarian missions from June to August 2013, 2014 and 2016.The survey was carried out with 32 informants of various cultural origins from the surroundings of Iquitos (capital city of Loreto), and in 15 riverine Quechua communities of the Pastaza River from Santa Ana (river mouth of Pastaza) to Sabalo Yaccu (upstream remote community situated 250 km North, Fig.1). Cultural heterogeneity of informants permitted to gather exhaustive data on local phytotherapies. Close attention was paid to the plant therapy used against hepatitis including associated remedies (e.g. special plant diets, symptomatic treatment for jaundice and liver-related ailments). These biomedical uses of plant-derived remedies belonged to different categories according to the International Classification of Primary Care (ICPC), mainly infections and gastrointestinal disorders (Staub et al, 2015).

Information presented here was compiled through general conversations, informal interviews, rainforest walks and other activities shared with local people (harvesting, weeding, hunting, nursing...) adopting a "participant-observation" methodology. Depending on their willingness, origin and availability, the contribution of each informant was so variable that we preferred not to take the numbers of citations into account here (number of citation are just mentioned Table 1).

Plant material

The plants collected were vouchered, deposited and identified at the Herbarium of the Universidad Nacional de la Amazonia Peruana by the botanist Juan Celidonio Ruiz Macedo, (UNAP, Iquitos) and according to regional floras (Duke and Vasquez, 1994; Rutter, 1990; Velasquez, 1990). This project was realized in accordance with the Universidad Nacional de la Amazonía Peruana guidelines (Laboratorio de Investigación de Productos Naturales Antiparasitarios de la Amazonia LIPNAA, UNAP) pertaining to ethnopharmacological studies, and ethical approvals with signature from each informant were obtained before investigations.

Plant samples were dried, powdered and extracted three times with methanol for 24 h and filtered (10 g of powdered plant in 100 mL of MeOH). Extracts were taken to dryness at 40 °C under vacuum and weighed. The processing of the plants performed in this study was different from the traditional preparations (Table 1). Therefore, it was not an exact replication of the traditional knowledge but it was admitted that methanolic plant extracts provided more consistent antimicrobial activities (Murphy Cowan, 1999). Moreover, methanol also provided a more complete extraction, including fewer polar compounds, and was more representative of the traditional preparations than other chemical solvents.

Cytotoxicity on HepG2 cells

Methanolic dried extracts were suspended in DMSO to obtain an original concentration of 120 mg/mL. Cells were subcultured in 96-well plates with 3500 cells/well in 100 µL of growth medium (Dulbecco's Modified Eagle Medium, DMEM, supplemented with 10% foetal bovine serum 50 U/mL penicillin and 50 µg/mL streptomycin). After 24 h of incubation (in humidified atmosphere, 5% CO_2 at 37 °C), the medium was discarded and replaced with 5 dilutions of the plant extracts in DMEM (at 240, 120, 60, 30, and 15 µg/mL) in quadruplicate. Cellular growth control was performed using medium with 0.2% DMSO. Then, after an incubation period of 48 h, the medium was discarded and replaced with DMEM containing 0.5 mg/mL MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After 1 h 30 of incubation at 37°C and 5% CO_2, the water-insoluble formazan was dissolved in 100 µL DMSO and the absorbance was measured at 550 nm using a UV-spectrometer (Tecan, Spark 10M). The cytotoxicity of the crude extracts and standard (camptothecin: IC_{50}: 0.9 ± 0.1 µg/mL) was determined by comparing the absorbance of treated cells with the absorbance of control cells cultured in 0.2% DMSO. Data were expressed as a percentage of inhibition calculated according to the formula (% Cell viability = Abs treated cells x 100 / Abs control cells) and analysed by linear regression.

Anti-HCV activity

We used HCV cell culture system (HCVcc) to perform the antiviral screening. A modified HCV strain (JFH1, genotype 2a) containing cell culture-adaptive mutations (Delgrange et al., 2007; Goueslain et al., 2010) was used. JFH1 was kindly provided by T. Wakita (National Institute of Infectious Diseases, Tokyo, Japan). The viral stocks were produced in human hepatoma Huh-7 cells.
For antiviral assay, Huh-7 cells, 6000/well, seeded in 96-well plate the day before infection were inoculated with HCVcc at a multiplicity of infection (MOI) of 0.8 during 2 h at 37°C in the presence of plant extracts at 25 µg/mL. Epigallocatechin-3-gallate (EGCG, Calbiochem) from green tea extract, an HCV entry inhibitor, was used as a control, inhibiting here 99.5% of HCV infection at 25 µg/ml (Calland et al., 2012). Then, the inoculum was removed and cells were overlaid with fresh medium containing plant extracts at 25 µg/mL for 28 h at 37°C. After 30 h, cells were fixed with ice-cold methanol and were subjected to immunofluorescent detection of E1 envelope protein as described (Rouillé et al., 2006). Nuclei were stained with 1 µg/mL of DAPI (4′,6-diamidino-2-phenylindole), and infected cells were detected by immunofluorescent labeling of E1 envelope glycoprotein (A4 epitope). Confocal images were recorded using an automated confocal microscope IN Cell Analyzer 6000 (GE Healthcare Life Sciences) using a 10× objective with exposure parameters 405/450 nm and 561/610 nm. Six fields per well were recorded. Each image was then processed using the Colombus image analysis software (Perkin Elmer) (Calland et al., 2015). The ratio of infected cells over total cells represents the infection rate. Infection rates in DMSO controls were expressed as 100%.

Fig. 1: Maps of the North of Peru (Loreto) with studied areas.

Results

Ethnobotanical study: local uses of medicinal plants in case of "hepatitis"

The ethnobotanical survey led to the collection and identification of 35 plant species providing 45 extracts. All the remedies were not specifically indicated in case of viral hepatitis infection but rather against hepatic injury and associated symptoms (Table 1). In such cases of infectious diseases with digestive symptoms, useful medicine against hepatitis does not necessarily have antiviral activity. Moreover, it is relevant to show how food and medicinal plant are interrelated. This ethnobotanical survey with related publications indicated that it is usual in Peruvian Amazon to find dietary and other restrictions with plants, such as sexual abstinence, or social seclusion (Sanz-Biset and Cañigueral, 2011). This dietary restriction called "*dieta*" in Spanish and "*sasina*" in lowland Quechua consists of stopping food like meat, fat, eggs, salt, sugar, spices, alcohol, until healing, and progressive reestablishment of normal diet with boiled plantains, manioc and fish. During this survey, restricted diets were mentioned for chronic or important diseases such as hepatitis and associated digestive affections. This practice also depended on plant species, preparation and administration route, or particular uses. Stronger diets may also be observed for "teaching plants", such as *Aspidosperma excelsum*, *Hura crepitans*, *Jatropha curcas*, *Mansoa alliacea*, *Maytenus macrocarpa, Phyllanthus* sp., *Pouteria caimito.* Indeed, these species are also used in apprenticeships of traditional medicine that need a ritual gathering. These particular and well-structured traditional medicinal practices are usually characterized by an important symbolic significance (Jauregui et al., 2011).

In this context, a comparative study of local uses allowed an *a posteriori* classification of most of the plant remedies in broadly 3 interconnected categories (A, B, C):

- A: Preparations with low and accurate dosing for targeted therapy against aetiology of liver diseases. These remedies were usually characterized by a narrow therapeutic range, associated with a special food diet and strictly contraindicated in combination with "modern medication": *Aspidosperma excelsum*, *Hura crepitans*, *Jatropha curcas*, *Mansoa alliacea*, *Maytenus macrocarpa, Phyllanthus* sp., *Pouteria caimito*, *Pseudelephantopus spiralis*. Most of these species were considered as "teaching plants"as mentioned above.

- B: Preparations with flexible dosing usually indicated to alleviate symptoms of hepatitis and liver injuries such as jaundice, functional digestive disorders, inflammation, ascites...: *Bixa orellana*, *Cajanus cajan*, *Carica papaya seed, Crescentia cujete*, *Curcuma longa*, *Desmodium adscendens*, *Eryngium foetidum, Gossypium barbadense, Malachra ruderalis, Physalis angulata, Senna reticulata, Tessaria integrifolia, Urera baccifera.*

- C: Preparations used without particular dosing as substitutes for food or "functional food" in order to replace contraindicated unpalatable nutrients such as animal proteins and lipids, or to drain the body: *Costus erythrocoryne*, *Gynerium sagittatum, Cecropia ficifolia,* hearts of palms (Arecaceae such as *Astrocarium* spp., *Oenocarpus bataua*), preparations called "agua de tiempo" ("drunk at any time" e.g. *Scleria microcarpa, Carica papaya* leaves, *Persea americana*).

It should be noted that a same species could belong to different categories depending on its preparation.

Fig. 1: Maps of the North of Peru (Loreto) with studied areas.

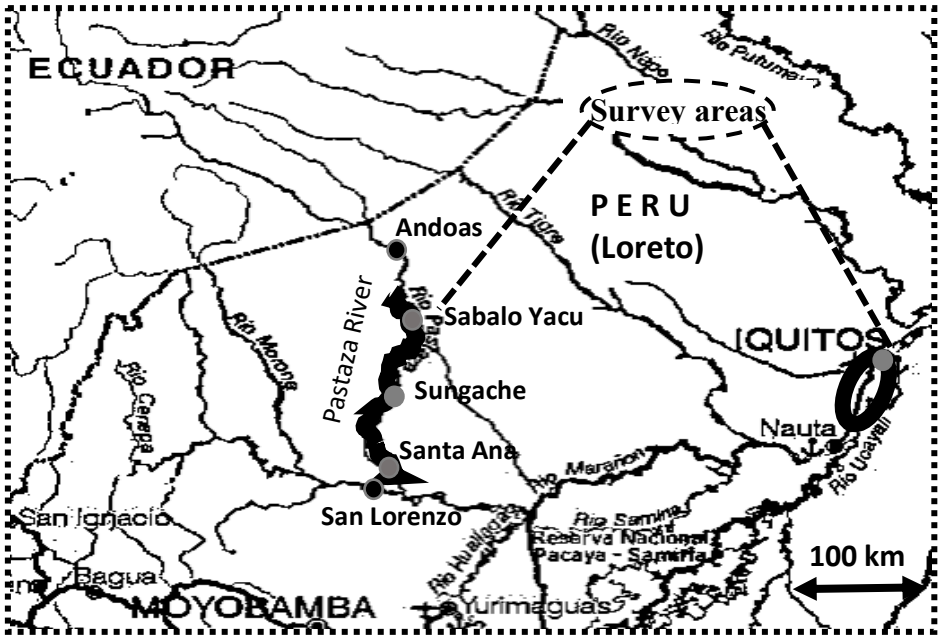

ECUADOR
Río Napo
Río Putumayo
Survey areas
P E R U
(Loreto)
Río Tigre
Andoas
Pastaza River
Río Pastaza
Sabalo Yacu
IQUITOS
Río Morona
Río Corrientes
Sungache
Nauta
Santa Ana
Río Marañon
Río Ucayali
San Ignacio
San Lorenzo
Río Huallaga
Río Samiria
Reserva Nacional
Pacaya - Samiria
100 km
Bagua
MOYOBAMBA
Yurimaguas

Table 1: Alphabetic list and traditional uses of plant species to treat liver diseases

***Scientific name*, Family**	**Vernacular name in spanish (and quechua), *voucher specimen***	**Part used**	**Traditional uses to treat liver diseases (category of use:** A, B or C**)**
Aspidosperma excelsum Benth., Apocynaceae	Remo caspi, (kawina anku), *035867*	B	Few drops of resin diluted in water or root decoction are drunk one a day in the morning with special diet [a]. (A)
Astrocaryum jauari Mart., Arecaceae	Asawachi (wiririma), *023361*	L R	Heart of palm are boiled during 15 minutes. The decoction is drunk and the palm heart is eaten 3 times a day with dietary restrictions [a], to "make urine pale". (C)
Bixa orellana L., Bixaceae	Achiote, (mantru), *026053*	L	Decoction of leaves is drunk against digestive symptoms of hepatitis (10 leaves in 1 litre / day). (B)
Caesalpinia pulcherrima (L.) Sw., Fabaceae	Chanca piedra (ambiguous denomination), *035563*	L	See *Phyllanthus stipulatus.*(NC)
Cajanus cajan (L.) Huth Fabaceae	Puspo poroto, (purutu), *041695*	AP	Leaves decoction is drunk during the day, and cooked fruits are eaten to reduce liver pain. (B)
Carica papaya L., Caricaceae	Papaya (papayu), *033863*	L S	Root decoction is drunk during all the day as "agua de tiempo [b]". (C) Pulverised seeds are eaten in the morning to purge the body, associated with special diet[a]. (B)
Carludovica palmata Ruiz & Pav., Cyclanthaceae	Bombonaje, (lisand tullu), *035546*	HP L	Heart of palm and buds are boiled and drunk during the day with dietary restrictions [a]. (C)
Cecropia ficifolia Warb. ex Snethl., Urticaceae	Cetico (sitica, shuruna), *035552*	L	The sap is extracted from roots or aerial parts, then diluted in water and drunk every morning until recovery. (C)
Chamaesyce thymifolia (L.) Millsp., Euphorbiaceae	Cocodrilo (confused with chanca piedra), *025312*	R AP	See *Phyllanthus stipulatus.* (NC)
Costus erythrocoryne K. Schum., Costaceae	Caña agria, *032256*	AP	The fresh exudate from the stem is drunk in the morning. (C)
Crescentia cujete L., (Bignoniaceae)	Huingo, (mati), *033852*	L	The infusion is drunk against digestive symptoms of hepatitis. (B)
Curcuma longa L., Zingiberaceae	Guisador, *033866*	R	The decoction (about ¼ kg in one litre of water) is drunk during the day with special diet, sometimes added to flowers of *Senna reticulata*. The juice is expressed from the rhizome and drunk (3 spoonsful per day for children). Ablution with expressed leaves are realised for younger children. (B)
Desmodium adscendens (Sw.) DC., Fabaceae	Amor seco, *037518*	AP	Decoction of aerial parts (3 times a day). (B)
Eryngium foetidum L., Apiaceae	Sacha culantro, *033895*	EP	Used as condiment in food. (B)
Euterpe precatoria Mart., Arecaceae	Huasay, *026315*	R	Decoction of crashed roots against liver disorder, drunk during the day with dietary restrictions [a]. (C)
Gossypium barbadense L., Malvaceae	Algodón blanco, (utku), *026673*	Fl L	The infusion of few flowers (one handful, sometimes with leaves) is drunk 3 times a day. (B)
Gynerium sagittatum (Aubl.) P. Beauv., Poaceae	Caña brava, *040671*	AP	The fresh exudate from the stem is drunk in the morning. (C)
Hura crepitans L., Euphorbiaceae	Catahua (catawa), *035787*	B	The bark decoction is drunk against viral infections such as HIV (once a day) but the treatment mustn't exceed one month, associated with strict restrictions (food diet [a], sexual abstinence), without sun exposure (risk of blindness). (A)

Jatropha curcas L., Euphorbiaceae	Piñon blanco (yura piñon), *033695*	L	The sap is expressed from the leave, then diluted in water and drunk 3 times a day, with dietary restrictions [a]. (A)
Malachra ruderalis Gürke, Malvaceae	Malva (malwa), *015674*	AP	Infusion macerated all the night and drunk during the following day, against digestive symptoms of hepatitis. (B)
Mansoa alliacea (Lam.) A.H. Gentry, Bignoniaceae	Ajo sacha, (ahu sacha), *035204*	L	3-4 Leaves or bark decoction are drunk against viral infections (HIV, hepatitis), with special food diet [a]. (A)
Maytenus macrocarpa (Ruiz & Pav.) Briq., Celastraceae	Chuchu washa, *036978*	B	Maceration in alcohol or decoction, drunk against viral infections such as HIV, with dietary restrictions [a]. (A)
Oenocarpus bataua Mart., Arecaceae	Ungurahui, (Unkurahui), *033549*	Fr R	Edible fruit are eaten during the food diet. Heart of palm or roots are infused, then macerated all the night and drunk during the following day, against digestive symptoms of hepatitis. (C)
Persea americana Mill., Lauraceae	Palta, *033683*	L	Decoction of leaves or crushed roots are drunk during all the day (as "agua de tiempo[b]"). (C)
Phyllanthus stipulatus (Raf.) G.L. Webster, Phyllanthaceae	Chanca piedra (Aya kiwa puca), *041971*	AP R	Decoction of the whole crushed plant is drunk against hepatitis and its digestive symptoms (3 times a day), with dietary restrictions [a]. (A)
Phyllanthus urinaria L., Phyllanthaceae	Chanca piedra, *024633*	EP	Similar use as described for *Phyllanthus stipulates.* (A)
Physalis angulata L., Solanaceae	Bolsa mulaca (putiuntiu, mullaca), *033878*	AP ±GFr	Roots and aerial parts decoction used to reduce symptoms of hepatitis (3 times a day). (B)
Phytelephas macrocarpa Ruiz & Pav., Arecaceae	Cabeza de mono, yarina, *026484*	L	Heart of palm or leaves are infused and drunk 3 times a day against digestive symptoms of hepatitis (with special diet [a], and avoiding sun exposure). (C)
Pouteria caimito (Ruiz & Pav.) Radlk., Sapotaceae	Caimito, (apiyu panka), *033388*	L	The ecoction of a handful of leaves in 1 L of water is drunk in the morning during one week with strict restrictions (food diet [a], and sexual abstinence). (A)
Pseudelephantopus spiralis (Less.) Cronquist, Asteraceae	Mata pasto (millaypa kiwa), *033943*	AP	Decoction of a handful of dried plant in 1 L of water. The preparation is drunk 2 times a day until recovery. The treatment is associated with dietary restrictions [a]. (A)
Scleria microcarpa Nees ex Kunth, Cyperaceae	Cortadera (Chitiaru kayu, kuchurikiwa), *033427*	AP R	Root decoction of 5 roots in one litre of water is drunk during all the day (as "agua de tiempo [b]") with dietary restrictions [a]. (C)
Senna reticulata (Willd.) H.S. Irwin & Barneby, Fabaceae	Retama (Kucha kaspi, aya kaspi), *035336*	Fl L	The infusion of leaves is drunk during few days. The infusion of flower (about 4 handfuls per litre of water) is drunk alone or mixed with *Curcuma longa* (with special food diet [a]). Used against hepatitis and associated symptoms (yellowing of eyes and skin). (B)
Socratea exorrhiza (Mart.) H. Wendl., Arecacea	Casha pona (kawitu), *033315*	L	Heart of palm are boiled and eaten every morning. (C)
Tessaria integrifolia Ruiz & Pav., Asteraceae	Pájaro bobo (chillka), *025845*	AP R	Infusion or decoction are drunk against hepatitis with fever. (B)
Urera baccifera (L.) Gaudich. ex Wedd., Urticaceae	Lagarto caspi (lagartu kaspi), *039990*	AP	The infusion is drunk against digestive symptoms of hepatitis. (B)

AP: aerial parts, B: stem bark, Br: branch, EP: entire plant, Fr: fruit (G: green), HP: heart of palm (interior part of the trunk), L: leaves, R: root., NC: not classified (plant adulteration).

[a] Special diet: dietary restrictions without meat, fish, salt, spices, fat, sweet, alcohol, unpalatable foods.

[b] "Agua de tiempo": which means "water at any time", can be drunk all day long.

Ethnomedical study: The Amerindian situation with respect to illness and hepatitis

Despite favourable weather conditions, the daily life reality of Amerindian people living in rainforests is often a perpetual fight for good health. In fact indigenous people suffer from many difficulties related to their particular environment, socioeconomic context, history, culture, or governmental disregard and social discrimination, which results in a reduction of their life expectancy (Montenegro and Stephens, 2006). This high mortality depends on various risk factors whose control could improve the management of diseases such as hepatitis as we described below:

Physical and material factors:

- Location of population. Geographic and rural isolation of indigenous communities contribute to increasing diseases morbidity by different ways: low access to health facilities (in case of emergency such as malaria crisis, ante/post-natal care, infected ascites due to hepatitis, *etc.*), lack of safe drinking water, dangerous work (woodcutting, hunting, fishing), hostile environments (predators, animal bites, drowning, wounds causing tetanus, mosquito or insect bites causing infections such as malaria), and other rural lifestyles that can expose populations to higher health risks. Solutions should consist in the development of transport logistics (boats with motors and fuel dedicated to emergencies) and protection devices (lyophilized antiserums, mosquito nets, clothing and safety equipment such as gloves and rubber boots).

- Evictions from ancestral lands. The Amerindian way of life is directly linked to their natural resources and this equilibrium can be destabilised by the intervention of modern society through oil or gas industries, dam or road building, woodcutting, trafficking in protected species, and other projects leading to the deforestation and dispossession of indigenous territories. In the oil concessions 1AB/192, including a part of the Pastaza river basin, the surface area deforested or covered by crude oil spills was estimated at 10 538 ha (Martínez et al., 2007). These environmental dislocations lead to brutal changes of daily life and disturb subsistence activities such as hunting, fishing, harvesting and collecting clean water from springs. It also weakens the indigenous cohesion and identity of the community. Moreover it deeply affects individual health with stress, diseases, hunger and poverty. It would seem important to moderate the external access to the rainforest and its exploitation.

- Material goods from modern society. Even though technology and new medications from the industrial world can be useful for indigenous communities, some imported goods remain deleterious for health e.g. fake or outdated medicines, strong spirit ("aguardiente", i.e. fermented juice of sugar cane), expired or "synthetic food" (freeze-dried milk), batteries and other harmful pollutants. Numerous cases of diseases due to the environmental impact of industries were reported in this area: acute cases of poisoning, cutaneous cancer, allergies, spontaneous abortions, malaria (due to the creation of large standing water basins leading to the proliferation of mosquitoes), leishmaniasis (because of proliferation of Phlebotominae in timber farms); this has contributed to increasing mortality in the neighbouring communities (Martínez et al., 2007; San Sebastián et al., 2001, 2002). Environmental exposure and human health parameters studied in the communities along the Pastaza river revealed high and toxic concentrations of mercury (41% of inhabitants with hair mercury level > 10μg/kg, and 4% of fish were considered as toxic with [Hg] > 0.5 μg/g). Similar results were obtained for barium, lead and chlorides as consequences of the petroleum exploitations (Webb and Mainville, 2009). It should be pointed out that an average of 2 spills a year are officially reported in Pastaza river, and that the high level of heavy metal contamination of the soils suggested other unreported leaks (OSINERG, 2007). Protection of these areas from foreign intrusion, and shifting of policies in the direction of environmental sustainability, with reparation of the past damages and prevention of further destructions, should be considered as parts of a reasonable health policy for these areas (e.g. water borehole drilling and water treatment systems).

- Housing and living area. In the Amazonian communities, an indigenous family usually lives in the same house, in quite a limited area, and sometimes shares the same mosquito net during the night. This proximity can be reinforced during a travel, usually by boat, or a stay in town, and enhances the spread of contagious diseases. More resources could be given to local trainings on prevention in health and safety.

- Community infrastructure. Waste disposal (sludge, septic and solid waste), safeguard water resources, electricity for cold chains (permitting conservation of vaccines, venom antiserum, interferon against hepatitis), collective transport dedicated to emergency usually lacked in isolated areas, aggravating the impact of infectious diseases. Assistance for the development of these defective or nonexistent infrastructures is still lacking (only 1 motorised medical boat was available for 2-5 communities).

Socio-cultural factors:

- Children's schooling and health education. School or health and safety training are the initial steps for limiting the spread of illnesses, particularly for current contagious diseases such as syphilis, AIDS, hepatitis and tuberculosis. In remote villages or poor neighbourhoods, the language barrier and environmental hostility block the achievement of these pedagogic supports and contribute to worsening the health situation of these underprivileged areas. Intensive bilingual trainings at school on prevention in health would sustainably reduce the incidence of contagious diseases among the younger population.

- Cultural practices among Amerindian populations. Some particular cultural practices can enhance the likelihood of transmission of infectious diseases as hepatitis or exacerbate symptoms of illnesses, e.g. ways of feeding (without cutlery, irregular schedule and supply of food depending on weather conditions) or drinking (for instance “masato” is a usual Amazonian drink prepared from masticated manioc, usually mixed with water from the river, and distributed in the same cup to every visitor or members of a ceremony), delousing, sexual practices (sometimes polygamy), tattooing, piercing or lip perforations with unsterilized tools (Coimbra et al., 1996). This point highlights once again the need for bilingual trainings on prevention in health, with supplying of preventive goods like vitamin supplements, condoms, disinfectants *etc*.

- Cultural impact of modern society. Despite numerous projects for Amazonian conservation, and even thought traditional knowledge is cumulative and dynamic, the modern society contributes to accelerating the changing value, which implies a rapid loss of the “pre-existing know-how” and traditional therapies (Heinrich et al., 2009). Ethnographic surveys, especially those dedicated to ethnomedicine, ethnobotany, ethnoscience and other types of written testimonies or audio-video recordings are good ways to preserve traditional knowledge, especially when they are performed by the members of indigenous communities themselves.

- Employment or income. Indigenous citizens living near a city or an industrial establishment may have opportunities to get a paid work, but employment rates and incomes are usually lower for them than for unskilled workers (Rakibul and Mashhood, 2010). This disparity is all the more detrimental to these new employees as they live far from their plentiful forest, leading to gradual poverty and health decline. Moreover, numerous cases of prostitution and sexual abuse are observed for indigenous women living near such establishments, with introduction of new diseases by the foreign workers, e.g. HIV and hepatitis (Martínez et al., 2007). The compensation per indigenous employee should be normalized, avoiding any conflict of interest, and child prostitution or sexual abuse should be severely punished.

- Lack of data. Low access of health brigade and other assistance mandated for vaccination campaigns, disease eradication or prevention, epidemiologic survey, emphasised by cultural Amerindian specificities (e.g. poor written languages, absence of birth or death registrations, impossibility to gather all the members of a community) raise the difficulty of data compilation required for the improvement of health

implementation plans. In this context, epidemiologic data on hepatitis are all the more incomplete as for this infectious disease the diagnosis assessment is logistically impossible and sick persons usually live in seclusion. New census campaigns aiming at completing national registrations and epidemiological data, with routine screening for viral diseases such as HIV and hepatitis, should be more widely undertaken.

Physiological and medical factors:

- Genetic determinism. Genetic and epigenetic heritage with poor diversity, due to the long-time isolation of these communities, makes these populations more sensitive to new diseases emerging from the modern society (e.g. viral or bacterial diseases like the flu or whooping cough were introduced by foreigners). Immunological studies exhibited the negative effects of homozygosity on health of Amerindians which are often infested with intestinal macroparasites. These chronical exposures lead to the overexpression of gene encoding for Ig,E, and results in a decrease of defence against other infections such as hepatitis (Salzano, 1990). Moreover, fieldwork data collection also showed that those isolated populations are more predisposed to develop side effects with some modern medication (intolerance or hyper-reactivity to vaccines, antibiotics, psychotropics), which can overcomplicate the cure of diseases (Hurtado et al., 2001). Epidemiological monitoring and research on indigenous susceptibility to drug and disease must be carefully considered and registered. Specific study of local iatrogenic effects and plant-drug interactions should require more attention from both researchers and medical staff.

- Discrepancy of healing concepts. Modern medicine is based on universal scientific practices adapted to large populations with standard ethical regulations, while traditional Amazonian medicines are influenced by various popular beliefs (here related to the animistic ontology, Descola, 2013; Luna, 1986) and depend on both practitioner and patient culture (Lévi-Strauss, 1949). In the case of the traditional medicine of Quechua people from Loreto, different aetiological categories of illnesses may be distinguished such as: "*Unkuy*", due to natural causes (excess of water, wind, heat...); "*Pahu*", relating to supernatural origins such as spirits from the forest; and "*Wiruti*" that referred to an invisible dart or a smoke blown by a shaman for therapy and sorcery in case of human conflicts (Roumy et al., 2007). The traditional curer can also include some "modern" aetiological factors such as the inter- or intra-specific contagiousness of diseases. In this context, modern medication is usually locally underestimated or proscribed and considered as primarily preventative, whereas traditional medicines are "curative". For example, administration of tablets and other pharmaceutical forms are often contraindicated with traditional medications and considered as ineffective against the aetiology of the disease. Compliance with prescriptions and medical advice must be explained and supervised while always considering possible interactions with local medicine.

These complex interactions between different medical systems may sometimes create a mutually beneficial relationship but also generate medical incompatibilities leading to the loss of medication compliance and treatment efficacy, especially for chronic diseases such as viral hepatitis.

More specific factors affecting viral hepatitis:

In case of hepatitis B or C in Amazonian communities, other aggravating factors of morbidity are present such as: the need for health centres or medical devices for diagnosis (the existing ones are far away and expensive), the non-assessment of vaccination due to the household head willing or for administrative reason (undocumented patient), the local unawareness of transmission routes, the cost and conservation of medication or vaccines, the non-respect of nutritional- hygienic precautions, the material or cultural incompatibilities with condom protection, *etc*.

The clinical context is also a major determinant for the spread of disease. Healthy carriers may contaminate their relatives without wariness (Alberti et al., 1992). Patients with associated cutaneous infections are more likely to be contaminated by viral hepatitis (31.5% of inhabitants have dermatoses in Peruvian Amazon; Coimbra et al., 1996; Gutierrez et al., 2010). Clinical observations also exhibited that cumulated liver damages due to other diseases such as malaria and other parasitoses also exacerbated the hepatitis symptoms, and mutually, chronic viral hepatitis may be a risk factor for severe malaria (an overall prevalence of HBsAg of 23.8% have already been reported in Vietnamese patients hospitalized for malaria ; Barcus,et al., 2002). These factors and clinical data could be better controlled through bilingual trainings on transmission of viral infections in indigenous communities.

For all the above reasons or aggravating factors, and despite casual medical supports, hepatitis is still one of the first causes of illness and mortality in Amazonia. Nonetheless, this analysis of indigenous situation will permit a better adjustment of health strategy in these communities and other tropical areas. It also justified the ethnopharmacological work dedicated to the collect of cultural data for their preservation and the improvement of local treatments.

Results of biological assays and interpretation.

In vitro assays revealed interesting activities (> 50% inhibition of HCV infection at 25 µg/mL) for 18 plant extracts corresponding to 15 species: *Bixa Orellana* (L), *Euterpe precatoria* (R), *Scleria microcarpa* (R and AP), *Astrocaryum jauari* (R), *Phyllanthus stipulatus* (R and AP), *Oenocarpus bataua* (Fr), *Cecropia ficifolia* (L), *Pouteria caimito* (L), *Persea americana* (L), *Physalis angulata* (APGFr), *Chamaesyce thymifolia* (R and AP), *Mansoa alliacea* (EP), *Maytenus macrocarpa* (B), *Curcuma longa* (R), *Caesalpinia pulcherrima* (L). The same assays showed that 6 other extracts had low activities (30% to 50% of HCV inhibition): *Aspidosperma excelsum* (B), *Gossypium barbadense* (Fl), *Hura crepitans* (B), *Jatropha curcas* (L), *Malachra ruderalis* (AP), *Senna reticulata* (Fl) (see Table 2; Fig. 2). Moreover, the remedies from category A (preparations with low and accurate dosing as targeted therapy against hepatitis) were moderately more active than plants from categories B or C (80% of activity, > 30% inhibition at 25 µg/mL, for category A, instead of 37.5% or 47.1% for categories B or C), which attested the previous classification assumed in the ethnobotanical study (Fig. 3) and corroborated biological activities described in previous study for “teaching plants” from category A (Jauregui et al., 2011). Huh-7 cell nuclei number analysis showed that only 4 of the most active plants were also toxic on Huh-7 cells: *Persea americana* (L), *Physalis angulata* (APGFr), *Curcuma longa* (R) and *Aspidosperma excelsum* (B), which demonstrated a potential toxicity of these species. Nevertheless, this narrow therapeutic range did not forbid the use of species like *Curcuma longa* or *Physalis angulata* which have specific toxicity against hepatoma cell lines (HepG2 or Huh-7) without effects on healthy human cells (Salama et al., 2013). Moreover, cytotoxic assays realised on HepG2 cells (see Table 2), did not reveal important growth inhibitions except for *Curcuma longa* (as previously cited) and *Aspidosperma excelsum* (a species carefully used for the antimalarial activity of its indole alkaloids, and characterized by a moderate anti-HCV activity).

Discussion

Phytochemical analysis

Bibliographical review exhibited less studies on anti-HCV plant properties than on anti-HBV. However, some publications related the antiviral activity of plant extracts and natural products extracted from plants on HCV *in vitro* (Séron et al 2018). These compounds target the different steps of the viral infectious cycle, entry, replication, cell-to-cell spread and assembly. Our groups have recently identified dehydrojuncusol from *Juncus maritimus* as a new inhibitor of HCV replication (Sahuc 2019). Interestingly, the target of the molecule is the viral NS5A protein, which is one of the main target of the drugs used in hepatitis C therapy. In this study, some similarities were observed between plant extract already described in literature for their anti-HBV activities, and plant extracts with anti-HCV capacity identified here (*Phyllanthus* spp, *Euterpe precatoria*, *Curcuma longa* are active against both hepatitis virus).
A search in the literature indicated that phytochemical antiviral agents of some of the 15 most active plants have already been characterized but the majority of them are unknown, as described below:

Bixa orellana (leaves): No antiviral activity had been reported, but antimicrobial terpenes and phenolics compounds have already been identified from leaves without observed toxicity (Stohs, 2014. Moreover, hepatoprotective activity had been characterized in rats with liver damage induced by CCl_4 (Ahsan et al.,2009).

Euterpe precatoria (roots): Stem extract *E. precatoria* previously tested exerted a reduction in HBV replication at 100 mg/mL (measured by PCR, Quintero et al., 2011). Regarding the root of this species, antioxidant and hepatoprotective flavonoids (quercetin, catechin, epicatechin, rutin and astilbin) were isolated. Catechin, epicatechin and rutine did not show any activity against HCV, whereas quercetin activity against HBV has already been described (partial inhibition of HBsAg secretion at 0.2 mmol/mL, Galotta et al., 2008; Calland et al., 2012; Yang and Chen, 2008). Therefore, the presence of quercetin could partially explain anti-HCV activity observed for the root of this species.

Scleria microcarpa and *Astrocaryum jauari*: No previous data on compounds and biological activity of these plants have been published.

Phyllanthus stipulatus (roots or aerial parts): Despite absence of previous study of this species the genus *Phyllanthus* is one of the most active botanical group against viral hepatitis all over the world. Lignans and gallic derivatives isolated from various species of Phyllanthus (*P. amarus, P. multiflorus, P. tenellus, P. virgatus*) were characterized by anti-HBV activities. Other previous study showed that *Phyllanthus urinaria* grown in different areas in China exhibited different anti-HBV activity, nonetheless this species is now commercially produced in China for clinical use (Huang et al., 2003). Additionally, liquid chromatography coupled to mass spectrometry (LC–IT-MS^n) of *P. stipulatus* showed presence of phenolic compounds as quercetin and gallic acid derivatives potentially involved in anti-HCV and antioxidant activities (Da Fontoura Sprenger and Cass, 2013).

Oenocarpus bataua (fruits): Antioxidant phenolic compounds have been identified using UPLC-API-IT-MS^n (stilbenes, hydroxycinnamic acid, procyanidin and anthocyanin derivatives) but anti-HCV activity has never been described before (Rezaire et al., 2014).

Cecropia ficifolia (leaves): This species has never been studied for its chemical compounds or biological activity but other species of the genus *Cecropia* (*C. palmata* and *C. obtusa* leaves) have been described to contain anti-HCV triterpenic compounds as oleanolic acid (Schmidt et al., 2018; Kong et al., 2013).

Pouteria caimito (leaves): Antioxydant proanthocyanidin and anti-inflammatory terpenes (spinasterol) have already been identified in leave extract, but no antiviral activity has been mentioned before (França et al., 2016).

Persea americana (leaves): Antiviral activity of the infusion of *P. americana* leaves against herpes simplex virus (HSV-1), adenovirus (AD3…), and human immunodeficiency virus 1 (HIV-1) have been previously reported, and may be induced by synergistic effect between flavonoids, chlorogenic acid, and other unidentified compounds (De Almeida et al., 1998; Wigg et al., 1996). Nevertheless, the leave extract toxicity observed in this study was also previously demonstrated on livestock and mice (on myocardium or mammary gland, at 60-100 mg/kg and caused by the fatty acid "persin"), prohibiting the use of this plant by pregnant/nursing woman and patients with heart weakness (Oelrichs et al., 1995).

Physalis angulata (aerial parts with green fruits): No previous study reported antiviral activity of this plant species. Even if cytotoxicity was observed in this screening on HepG2 cells, previous data showed that cytotoxic effect did not affect normal mouse liver cells. Furthermore, hydro-ethanolic extract has been shown to possess potent anti-inflammatory and anti-hepatoma activities due to steroid compounds as physalins, and flavonol glycosides such as myricetin derivatives (Wu et al., 2004; Chiang et al., 1992; Ismail and Alam, M. 2001).

Chamaesyce thymifolia (roots or aerial parts): Moderate antiviral activity on HSV-1 and bovine viral diarrhea virus (BVDV) with low selectivity has been described for phenolic compounds (quercetin and galloyl esters of glucose derivatives) isolated from the aerial parts, but antiviral activity against hepatic viruses has never been studied (Amaral et al., 1999; Lin et al., 2002). Even if anti-inflammatory and hepatoprotective activities have been characterized for this species, dermatotoxicity (due to phorbol derivatives) and low selectivity observed for this plant do not allow chronic or important use of this remedy (Mali and Panchal, 2013; Muthumani et al., 2016).

Mansoa alliacea (entire plant): Even if this well-known traditional remedy is rich in organosulfur compounds with anti-inflammatory and anti-infectious properties, its organoleptic properties and its cytotoxic naphthoquinones may limit the recommended dosage (Itokawa et al., 1992; Pires et al., 2016; Towne et al., 2015).

Maytenus macrocarpa (bark): Terpenic derivatives from the leaves (di and triterpens) exhibited marginal cytotoxic activity and antiviral activity against HIV (triterpenonic acids), but no antiviral activity against hepatic viruses has already been published (Chavez et al., 1999; Piacente et al., 2006).

Curcuma longa (roots): The polyphenolic compound "curcumin" has been shown to inhibit HCV entry and replication, and HBV replication, and is considered to be a potentially important

chemo-preventive agent against a variety of cancers, including liver cancer, without observed toxicity (Kim et al., 2010; Salama et al., 2013). This study and previous data demonstrated that *Curcuma longa* could be used as a complementary therapy for HCV, while considering low oral bioavailability of curcumin, it should be administered with piperin (from Pieraceae or other botanical families; Shoba et al., 1998).

Caesalpinia pulcherrima (leaves): Quercetin flavonoids and isoflavons were responsible of anti-inflammatory and antiviral activities (against HSV or adenovirus), but no previous data were recorded on hepatic viruses (Akram et al., 2018; Chiang et al., 2003). Nonetheless, a slight toxicity on brine shrimp had been reported (26% mortality at 1000 µg mL^{-1}), that could limit the recommended dosage for pregnant women or young children (Chanda and Baravalia, 2011; Rao et al., 2005).

The drug extracts characterized by lower anti-HCV activity than species mentioned above may have other interests against hepatitis. For instance, they may be efficient against other hepatitis viruses (non-evaluated in this study) or may reduce liver degeneration and fibrosis, thereby contributing to alleviate hepatitis disease as observed for the biological activities of species cited below:

- Anti-oxidant and/or anti-inflammatory properties: *Crescentia cujetes* (Parvin et al., 2015), *Eryngium foetidum* (Paul et al., 2011), *Jatropha curcas* Leaves (Thomas et al., 2008), *Senna reticulata* (Navarro et al., 2017), *Tessaria integrifolia* (Peluso et al., 1995), *Urera baccifera* (Gindri et al., 2014).
- Hepatoprotective activities: *Desmodium adscendens* (François et al., 2015), *Cajanaus cajan* (Ahsan et al., 2009), *Carica papaya* (leaf and seed: Pandit et al., 2013; Adeneye et al., 2009).
- And other pathway as fighting hepatitis symptoms (diuretic against ascites, choleretic against icterus, digestive …) or improving bioavailability of antiviral remedies.

Nonetheless, some of the selected species have previous toxicological reports that could contraindicate their dispensation for chronic use in case of pregnancy or childhood:

Carica papaya: Seeds extract caused concentration-dependent tocolysis of uterine strips rats (Adebiyi et al., 2003).

Aspidosperma excelsum: Toxicity of the bark methanolic and dichloro-methane extracts were observed on brine shrimp (LC_{50}: 764 and 85 µg/mL, Desmarchelier et al., 1996).

Gynerium sagittatum: The high content of isoflavonoids makes this plant root an important source of phytoestrogen that could contraindicated the chronic use of the plant in case of pregnancy (Benavides et al., 2007).

Jatropha curcas: Leaf extract exerted cardiovascular effects (beta-blockers) on guinea pigs. Moreover, the presence of the toxin toxalbumin (curcin) may contraindicate a chronic use of this plant by pregnant women or young children (Thomas et al., 2008).

Pseudelephantopus spiralis: An important cytotoxicity of leaf extract has already been described on VERO cells and macrophage (CC_{50} :1-5 µg/mL) and may contraindicate its chronic use (Girardi et al., 2015).

Socratea exorrhiza: The leaf extract cytotoxicity evaluated in this study on HepG2 cells (IC_{50}: 39.3 ± 18.3 µg/mL) highlighted potential side effects of this drug in chronic use.

Fig. 2: Antiviral activity of plant extracts (HCV inhibition > 30% at 25 µg/mL) and cytotoxicity (% of viability at 25 µg/mL)

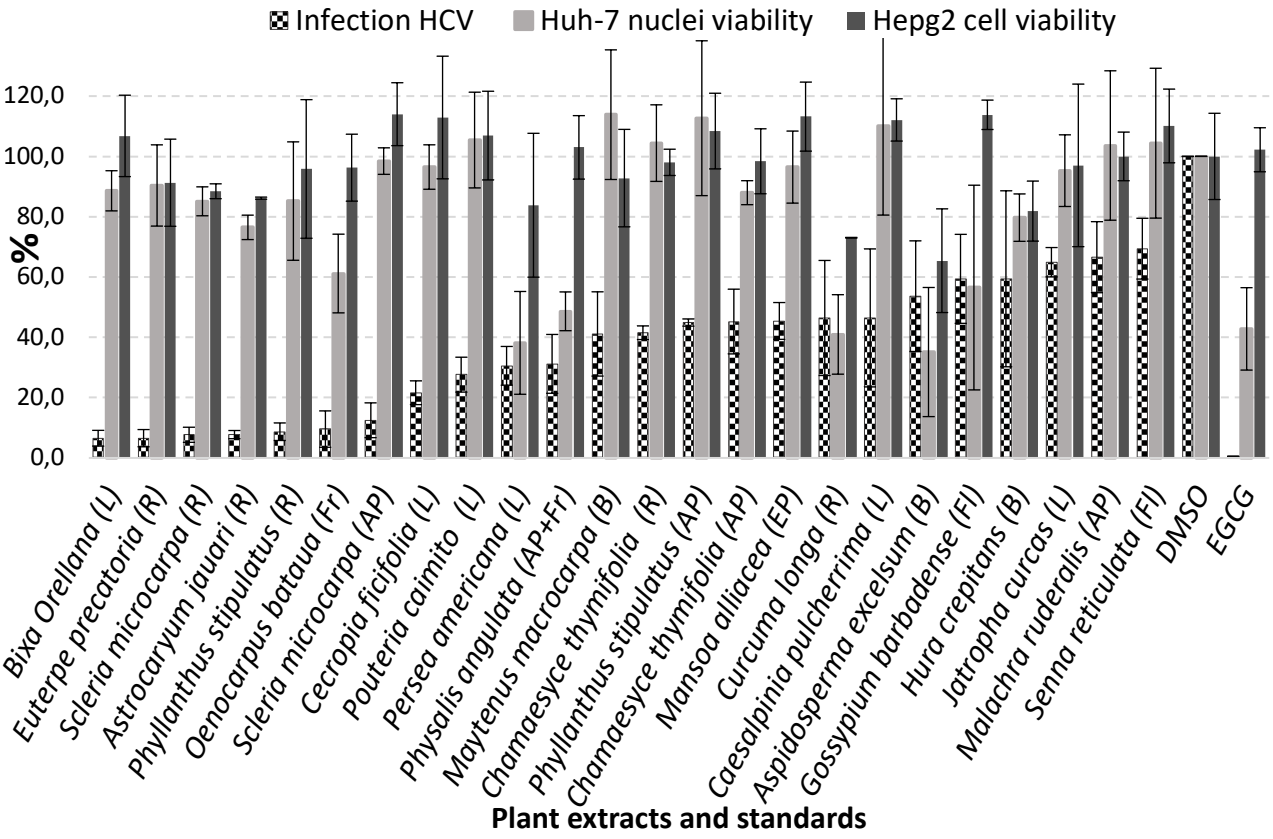
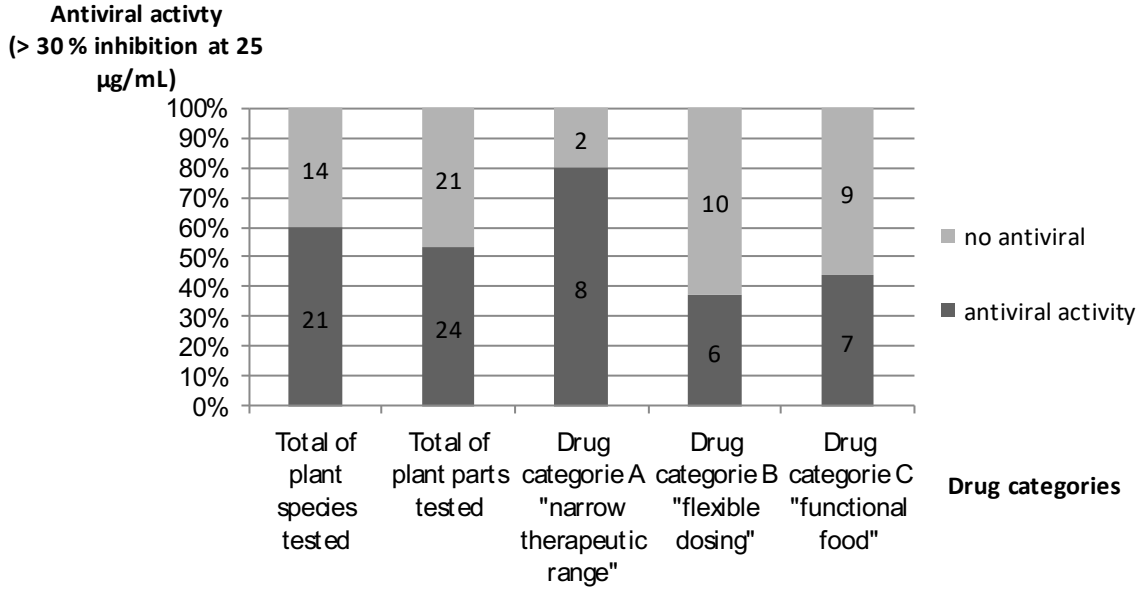


Fig. 3 Anti HCV activity at 25 µg/mL (>30% infection inhibition) and its repartition by drug category



Conclusion

This study exposed the results of a bioprospecting leading to the collect and identification of 35 plants with assessment of their antiviral or cytotoxic activities, and may contribute, with the ethnomedical analysis of indigenous situation, to a better adjustment of the health strategy against hepatitis in Peruvian Amazon.

45 plant extracts were tested for antiviral activity against HCV with counting of cellular death in case of toxicity and cytotoxicity evaluation in HepG2 cells. *In vitro* assay revealed interesting activity of 18 extracts (> 50% infection inhibition at 25 µg/mL) with a selectivity index > 2 for 15 of them. Lower antiviral activities were also observed for 6 other drug extracts (30% to 50% infection inhibition at 25 µg/mL).

Result analysis showed an interesting global level of activity (60% of plant extract inhibited more than 30% of HCV virus at 25 µg/mL). This supported the traditional medicinal uses of these plants and highlighted the relevance of the ethnobotanical approach. Moreover, drugs from the category A were more active against HCV than plants from categories B or C, that reinforced the tripartite drug classification assumed during the survey, and permitted to justify the cautious but efficient use of these plants against viral hepatitis. Nonetheless, drugs from other categories (B or C) may also occasionally have anti-HCV activities and are still helpful to prevent collateral effects of hepatitis.
On the other hand, bibliographical report and cytotoxicity evaluations permitted to refine the therapeutic indications regarding to patient's vulnerability (in case of pregnancy or childhood), specially for *Socratea exorrhiza* leaves and *Gynerium sagittatum* aerial parts, which were usually indicated for daily use (category C) whereas potential toxicity had been characterized. The bibliographic study of the 15 most active plants (> 50% inhibition at 25 µg/mL) also indicated that only 2 of them have already been studied for antiviral activity against hepatitis (*Curcuma longa* and *Socratea exorrhiza*), suggesting new avenues to be followed for the 13 other species.
More generally, these results will permit to improve the medical administration of the different plant species regarding their traditional uses and considering their antiviral or cytotoxic activities, with a better understanding of their preparation and posology in case of acute hepatitis (punctual administration), liver dysfunctions (regular treatment), or incorporated in a diary food diet against digestive troubles.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary data

Table 1: HCV inhibition at 25 µg/mL and cytotoxicity of the 45 plant extracts

Biological activities (*n=3*) / Plant extracts	Infection HCV (% at 25 µg/mL) ± SD	Huh-7 nuclei viability (% at 25 µg/mL) ± SD	Cytotoxicity on HepG2 cells IC_{50} (µg/mL) ± SD
Bixa Orellana (L)	6.5 ± 2.6	88.6 ± 6.7	196.5 ± 9.1
Euterpe precatoria (R)	6.6 ± 2.9	90.4 ± 13.5	163.6 ± 17.8
Scleria microcarpa (R)	7.7 ± 2.5	85.1 ± 4.8	> 250
Astrocaryum jauari (R)	7.7 ± 1.4	76.5 ± 4.1	87.4 ± 10.0
Phyllanthus stipulatus (R)	8.7 ± 2.9	85.2 ± 19.6	192.6 ± 10.3
Oenocarpus bataua (Fr)	9.6 ± 6.0	61.2 ± 13.1	109.2 ± 23.5
Scleria microcarpa (AP)	12.5 ± 5.8	98.4 ± 4.4	178.7 ± 18.2
Cecropia ficifolia (L)	21.6 ± 3.9	96.5 ± 7.4	221.9 ± 24.1
Pouteria caimito (L)	27.6 ± 5.8	105.4 ± 15.9	250.8 ± 11.4
Persea americana (L)	30.6 ± 6.4	38.2 ± 17.0	116.5 ± 19.7
Physalis angulata (AP+Fr)	31.2 ± 9.8	48.6 ± 6.4	182.2 ± 7.4
Maytenus macrocarpa (B)	41.1 ± 14.0	113.8 ± 21.5	108.9 ± 7.0
Chamaesyce thymifolia (R)	41.5 ± 2.3	104.4 ± 12.7	193.2 ± 8.6
Phyllanthus stipulatus (AP)	44.9 ± 1.2	112.7 ± 25.7	98.4 ± 4.2
Chamaesyce thymifolia (AP)	45.2 ± 10.7	88.0 ± 4.0	159.7 ± 21.4
Mansoa alliacea (EP)	45.4 ± 6.1	96.5 ± 11.9	218.4 ± 14.8
Curcuma longa (R)	46.4 ± 19.1	41.0 ± 13.2	36.2 ± 6.2
Caesalpinia pulcherrima (L)	46.4 ± 22.9	110.0 ± 29.5	> 250
Aspidosperma excelsum (B)	53.6 ± 18.3	35.1 ± 21.4	42.6 ± 21.2
Gossypium barbadense (Fl)	59.4 ± 14.8	56.5 ± 33.9	170.0 ± 24.2
Hura crepitans (B)	59.4 ± 29.2	79.7 ± 7.9	>250
Jatropha curcas (L)	64.9 ± 4.9	95.3 ± 11.9	176.2 ± 3.3
Malachra ruderalis (AP)	66.6 ± 11.8	103.6 ± 24.8	> 250
Senna reticulata (Fl)	69.4 ± 10.1	104.4 ± 24.8	> 250
Gossypium barbadense (L)	75.6 ± 7.4	113.8 ± 19.7	217.8 ± 5.8
Phyllanthus urinaria (EP)	75.6 ± 15.2	97.9 ± 8.3	174.8 ± 12.4
Carludovica palmata (L)	75.8 ± 6.6	72.5 ± 8.0	243.4 ± 24.1
Cajanus cajan (AP)	76.7 ± 10.7	100.1 ± 26.0	86.2 ± 31.0
Oenocarpus bataua (R)	80.3 ± 15.4	105.6 ± 20.9	> 250
Desmodium adscendens (AP)	81.4 ± 13.2	99.3 ± 18.8	> 250
Socratea exorrhiza (L)	82.1 ± 6.0	83.7 ± 17.3	39.3 ± 18.3
Astrocaryum jauari (L)	85.7 ± 11.7	75.7 ± 17.5	176.9 ± 17.7
Tessaria integrifolia (R)	86.7 ± 2.5	78.6 ± 8.3	170.0 ± 12.5
Gynerium sagittatum (AP)	88.7 ± 5.4	99.2 ± 7.8	226.5 ± 5.6
Pseudelephantopus spiralis (AP)	89.5 ± 21.9	87.4 ± 18.3	151.9 ± 8.8
Carica papaya (S)	91.0 ± 7.0	96.4 ± 10.6	> 250
Eryngium foetidum (EP)	93.0 ± 16.8	73.8 ± 10.7	192.0 ± 17.0
Costus erythrocoryne (AP)	95.9 ± 13.3	97.2 ± 14.4	> 250
Tessaria integrifolia (AP)	96.4 ± 6.7	94.1 ± 9.9	108.1 ± 11.9
Urera baccifera (AP)	98.3 ± 7.7	78.8 ± 8.2	> 250
Senna reticulata (L)	99.7 ± 18.3	100.0 ± 21.2	213.2 ± 12.2
Carica papaya (L)	102.5 ± 10.8	101.2 ± 16.8	> 250
Phytelephas macrocarpa (L)	103.2 ± 31.7	100.3 ± 11.6	188.6 ± 25.3
Crescentia cujete (L)	103.3 ± 18.9	104.2 ± 21.3	224.2 ± 6.3
Carludovica palmata (HP)	105.4 ± 8.1	106.2 ± 19.7	> 250