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In vitro antiviral activity and phytochemical screen in the extracts of peels from four species of tropical fruits collected in Merida Yucatan, Mexico

Evaluación antiviral *in vitro* de 4 extractos de cáscaras de frutas tropicales colectadas en Mérida Yucatán, México

Chel-Guerrero LD[1], R Gómez-Cansino[2], SL Gúzman-Gutierrez[3], MG Campos-Lara[4], E Saury-Duch[1], F Díaz de León Sánchez[5], R Reyes-Chilpa[6], JA Mendoza-Espinoza[7]

Abstract. The objective of this study was to investigate extracts from the peels of *Annona squamosa* L. (purple sugar apple), *Annona reticulata* L. (custard apple), *Chrysophyllum cainito* L. (green star apple), and *Melicoccus bijugatus* Jacq. (mamoncillo), as potential sources of anti-HIV-1 agents. Methanolic extracts from the peels of the aforementioned tropical fruits were obtained by maceration. Antiviral activity was evaluated through HIV-1 RT inhibition by extracts, using a non-radioactive immuno/colorimetric assay (Lenti RT Activity Assay, Cavidi Tech). Assay was performed according to the protocol provided by the manufacturer. Extracts from the peels of *A. squamosa*, *A. reticulata*, and *C. cainito* showed high antiviral activity, with HIV-1 reverse transcriptase inhibition values of 96.45 ± 2.08%, 78.63 ± 0.97%, and 72.55 ± 2.26%, respectively. These results demonstrate that *A. squamosa*, *A. reticulata*, and *C. cainito* have significant antiviral activity in their peels and can therefore be regarded as potential sources of anti-HIV-1 agents.

Keywords: *Annona squamosa* L.; *Annona reticulata* L.; *Chrysophyllum cainito* L.; *Melicoccus bijugatus* Jacq; Antiviral activity.

Resumen. Se evaluó el potencial inhibidor de la transcriptasa inversa del VIH-1 de los extractos de las cáscaras de *Annona squamosa* L. (Saramuyo), *Annona reticulata* L. (Chirimoya), *Chrysophyllum cainito* L. (Caimito), y *Melicoccus bijugatus* Jacq. (mamoncillo). Se obtuvieron los extractos metanólicos de las cáscaras liofilizadas de las frutas antes mencionadas por maceración directa. La actividad retroviral fue determinada usando un ensayo inmuno/colorimétrico no radioactivo "el ensayo fue llevado a cabo de acuerdo con el protocolo indicado por el proveedor" (Lenti RT Activity Assay, Cavidi Tech). Los extractos de las cáscaras de *A. squamosa*, *A. reticulata*, y *C. cainito* mostraron actividad antiviral prometedora contra la transcriptasa inversa de VIH-1 obteniendo valores de inhibición de 96,45 ± 2,08%, 78,63 ± 0,97%, y 72,55 ± 2,26% respectivamente. Estos resultados demuestran que *A. squamosa*, *A. reticulata*, and *C. cainito* tienen actividad antiviral prometedora siendo candidatos interesantes para la búsqueda de compuestos antivirales, siendo este el primer reporte que los asocia a esta actividad.

Palabras clave: *Annona squamosa* L.; *Annona reticulata* L.; *Chrysophyllum cainito* L.; *Melicoccus bijugatus* Jacq; Actividad antiviral.

[1] Laboratory of Instrumental Analysis, Instituto Tecnológico de Mérida.
[2] Universidad Tecnológica de la Mixteca, Cátedra CONACyT.
[3] Institute of Biomedical Research, Universidad Nacional Autónoma de México (UNAM), Cátedra CONACyT.
[4] Hospital Infantil de México Dr. Federico Gómez.
[5] Universidad Autónoma Metropolitana, Iztapalapa.
[6] Institute of Chemistry, Universidad Nacional Autónoma de México (UNAM).
[7] Program of Natural Products, Campus Casa Libertad, College of Sciences and Humanities, Universidad Autónoma de la Ciudad de México (UACM).
* Address correspondence to: Ricardo Reyes-Chilpa. *e-mail:* chilpa@unam.mx
José Alberto Mendoza-Espinoza, *e-mail:* amendozaespinoza@gmail.com ; josealberto.mendoza@uacm.edu.mx
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INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS). HIV-1 is largely responsible for the current worldwide epidemic and is therefore most commonly referred to simply as 'HIV'. This pathogen is characterized by both its high genetic variability and high rate of mutation (Zhang et al., 2012; Rashed et al., 2012). Effective antiretroviral (ARV) drugs can control the virus and help prevent transmission so that people with HIV, as well as those at a substantial risk of infection, can continue to enjoy healthy, long, and productive lives.

In 2016, there were approximately 36.7 million people infected with HIV worldwide, and in that same year, 1.8 million new infections were also reported (WHO, 2016). Due to the sheer number of HIV-infected people and the rapid emergence of drug-resistant strains, the demand for new antiviral therapeutics against HIV-1 remains high (Cos et al., 2002). In this context, the development of safe, effective, and low-cost anti-HIV drugs is among the top global priorities for drug development – with one promising strategy being the identification of compounds with anti-HIV activity from natural sources (Rege et al., 2015). Many of such compounds have additionally been found to inhibit HIV at nearly all stages of its life cycle. Among them can be found certain alkaloids, polyphenolics, flavonoids, coumarins, phenolics, tannins, triterpenes, lactones, iridoids, lignans, saponins, and xanthone (Sharma & Rangari, 2016).

For many years, the search for plant-derived bioactive agents has focused on the use of leaves, flowers, and bark as the principal sources for such compounds; only recently have studies sought to explore the medicinal use of fruits and their peels (Chel et al., 2018). Fruit peels are generally considered waste, yet they have the potential to be used as sources of cheap and readily available bioactive compounds for certain applications in the food and pharmaceutical industries (Deng et al., 2012).

The peels of *Annona squamosa L.*, *Annona reticulata* L., *Chrysophyllum cainito* L. and *Melicoccus bijugatus* Jacq., all tropical species cultivated in Mexico´s Yucatan Peninsula, represent between 10 and 44% of the total fresh weight of these fruits (Can et al., 2017). Additionally, they have also been shown to contain many phenolic compounds (chlorogenic acid, 4-hydroxybenzoic acid, synapic acid, caffeic acid, ellagic acid, transcinnamic acid, ferulic acid and gallic acid) as well as certain flavonoids (myricetin, catechin, epicatechin and quercetin) (Kaladhar & Rayavarapu, 2014; Can et al., 2017). Some of these compounds have been proven to have anti-HIV activity (Sha, 2013; Nutan et al., 2013; Pasetto et al., 2014) such as the diterpenoids isolated from the pulp of *A. squamosa* (Wu et al., 1996) however, no study has yet reported on the presence of similar activities in the peels from the four-aforementioned species of tropical fruits.

Thus, the objective of this study was to investigate the peels from *Annona squamosa L.*, *Annona reticulata* L., *Chrysophyllum cainito* L., and *Melicoccus bijugatus* Jacq., as potential sources of anti-HIV-1 agents.

MATERIALS AND METHODS

Biological material. Approximately 10 kg of each fruit were purchased at commercial maturity from local markets in the city of Merida (state of Yucatan), Mexico in 2014. Commercial maturity was determined by the firmness of the fruit and the easiness of handling (Sivakumar & Korsten, 2008). The fruit species collected were: *Annona squamosa* L. or purple sugar apple, *Annona reticulata* L. (Annonaceae) or custard apple, *Chrysophyllum cainito* L. (Sapotaceae) or green star apple, and *Melicoccus bijugatus* Jacq. (Sapindaceae) or mamoncillo. These fruits are locally known as saramuyo, anona, caimito verde, and huaya, respectively. The fruit material was authenticated by two taxonomists, one from the Universidad Autónoma de la Ciudad de México and the second from a Mexican government agency (Centro de Investigaciones Regionales del Sureste, INIFAP, México).

Preparation of peels and extraction procedure. The dried peels from each fruit and their corresponding methanolic extracts were obtained according to the methodology reported by Can et al. (2017) which is briefly described as follows:

The fruits were first washed and dried, and their peels manually removed. These were then freeze-dried at -50 °C under a 5 mtorr (9.67 x 10^{-5} psi) vacuum for 48 h in a Labconco Freeze Dry-5 dryer (Labconco, MO, USA). The dried peels were subsequently grounded and stored at -20 °C in hermetic plastic bags until solvent extraction could be perfomed. At such time, 5 g of the lyophilized peel from each fruit was macerated, dissolved in 50 mL of methanol, and then stirred at room temperature for 24 h at 160 rpm using a Labnet shaking incubator, Model 211DS before finally being filtered through a Whatman No. 2 paper. The solvent was then evaporated under a vacuum at 40 °C using a rotary evaporator (Büchi model R-3). Lastly, the dried peel extracts were stored at -20 °C until further analysis (Can et al., 2017).

Anti-HIV activity. Antiviral activity was evaluated through inhibition of HIV-1 reverse transcriptase (RT) activity by the peel extracts using a non-radioactive, immuno/colorimetric assay (Lenti RT Activity Assay, Cavidi Tech) (Shao et al., 1997). The assay was performed according to the protocol provided by the manufacturer. All extracts were first tested at 50 µg/mL with a final DMSO concentration of 0.5% v/v. Reported values are the means of 7 replicates ± SEM. Nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), was used as a positive control at concentrations ranging from 0.01 µM to 1 mM using 1-log increments.

A mixture of all reagents minus RT was used as the buffer blank. The anti-HIV-1 activity from the peel extracts was determined using the following equation: Inhibition of HIV-1 RT (%) = (absorbance of each sample – absorbance of buffer blank) / (absorbance of negative control – absorbance of buffer blank) x100.

RESULTS

Three of the extracts tested exhibited a high percentage of HIV-1 RT inhibition (Table 1), with the one obtained from the peels of *A. squamosa* presenting the highest value (96.45 % ± 2.08), followed by those from *A. reticulata* (78.63% ± 0.97) and *C. cainito* (72.55% ± 2.26). On the other hand, the extract from the peels of *M. bijugatus* failed to present similarly high values of anti HIV activity.

Table 1. Inhibitory effect of the methanolic extracts from the peels of the four species of tropical fruits evaluated on the activity of HIV-1 reverse transcriptase (RT).
Tabla 1. Efecto inhibitorio de los extractos metanólicos de las cáscaras de las cuatro especies de frutos tropicales evaluados sobre la actividad de transcriptasa inversa (RT) del HIV-1.

Extract [a]	Inhibition of HIV-1 RT (%)
A. squamosa	96.45 ± 2.08 A
A. reticulata	78.63 ± 0.97 B
C. cainito	72.55 ± 2.26 C
M. bijugatus	46.87 ± 3.50 D
Nevirapine [b]	93.94 ± 4.11 A

[a] Used at concentrations of 50 μg/mL.
[b] Used as a positive control.
Values are five replicates ± standard deviation. The similar capital letters are not significantly different amount each peels extracts to the hypothesis testing "means-two independent samples, $\alpha = 0.005$"

DISCUSSION

The reverse transcriptase from HIV-1 (HIV-1 RT) is the enzyme responsible for transcribing viral RNA into DNA, which is later integrated into the host cell´s genome as it carries the information necessary for the synthesis of new viral particles. Due to this key function, it is also one of the main drug targets of treatments seeking to inhibit the reproduction of HIV (Silprasit et al., 2011). In the search for HIV-1 RT inhibitors, the screening of plant extracts constitutes a valuable method for the discovery of compounds potentially useful in the development of new therapeutics (Huerta et al., 2004).

In the present study, we measured the ability of extracts from the peels of four species of tropical fruits to inhibit HIV-1 RT, finding that three of them displayed potent activity against HIV-1 RT. *A. squamosa* exhibited the highest percentage of inhibition, with a value that was similar to the one observed for the positive control (nevirapine). Thus, in accordance with the criterion of Tan, (1991), *A. squamosa* (saramuyo), *A. reticulata* (anona), and *C. cainito* (caimito) can all be considered anti-HIV agents because they exhibit percentages of inhibition >70%. To our knowledge, this work is likely the first to report such activities from compounds in peels.

Further investigations should be undertaken to identify the active agent(s) responsible(s) for this inhibiting effect, which could subsequently be used for the development of new anti-HIV drugs. In the case of *Annona squamosa*, the diterpenoid 16β, 17-dihydroxy-entkauran-19-oic acid has previously been reported as the principal antiviral metabolite (Wu et al., 1996).

CONCLUSION

Extracts from the peels of *A. squamosa*, *A. reticulata*, and *C. cainito* are effective inhibitors of the HIV-1 RT enzyme, with values that justify their consideration as promising sources of new anti-HIV agents.

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CONFLICT OF INTEREST

No conflict of interest is associated with this work.

Contribution of Authors

"We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". José Alberto Mendoza Espinoza, Ricardo Reyes Chilpa and Enrique Sauri Duch, conceived and designed the study. Lilian Dolores Chel Guerrero, Rocío Gómez Cansino, Silvia Laura Guzmán Gutiérrez, and Maria Guadalupe Campos Lara, carried out the experiments, and collected and analyzed the data.

Lilian Dolores Chel Guerrero and José Alberto Mendoza Espinoza wrote the manuscript. All authors read and approved the manuscript for publication.

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