Bioguided assay of polyphenols isolated from medicinal Mayan species and its activity against *Leishmania mexicana.*

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Abstract

The aim of this study underlines the *in vitro* leishmanicidal activity of the methanol extracts (MeOH), fractions, *n*-hexane (*n*-Hex), chloroform (TCM) and ethyl acetate (EtOAc), and secondary metabolites isolated from plant species used in the mayan traditional medicine. *Euphorbia hirta* and *Cedrella odorata*, showed good bioactivity with 14.81 ± 2.63 μg/mL and IC_{50} = 18.39 ± 0.88 μg/mL respectively, meanwhile *Bauhinia divaricata* not show activity and *Ruellia nudiflora* showed poor activity with IC_{50} = 92.18 ± 3.64 μg/mL, followed by *Hylocerus undatus* with IC_{50} = 122.5 ± 20.99 μg/mL, when tasted against amastigotes of *Leishmania mexicana*. The bioguided assays guided us, to the purification and isolation of four different metabolites, mainly flavonoids and structurally related compounds. The structures were stablished from ^{1}H-NMR, ^{13}C-NMR, LC-MS and FT-IR analysis, which confirmed the presence of quercetin, myricetin, kempherol and scopoletin, with IC_{50} = 2.92 ± 0.42 μM, 12.30 ± 0.57 μM, 20.22 ± 4.66 μM and 4.05 ± 0.68 μM respectively. However, their low bioavailability indicates the need for detailed structural relation activity studies, together with the development of formulations and delivery systems.

Key words: Leishmania mexicana, polyphenols, flavonoids, coumarins, NMR structural determination

1. Introduction.

The plant kingdom is in fact by far, the major factory of natural compounds, and for many people of rural areas represents a common practice for the treatment of different types of diseases. Since ancient times, the interest in the study of medicinal plant species lays down mainly on a therapeutic purpose and the probable good health state that can provide us, some of them with a specific bioactivity; moreover, are a primary health care line in accordance to the World Health Organization (WHO), that encourage the use of medicinal plants supported by scientific evidence on their biological and pharmacological effects does exists (Ankli et al., 2002; Jachak and Saklani 2007; Moo-Puc et al., 2013). In Mexico, the practice of traditional medicine has been carried out since the pre-Hispanic period, among them the Mexican Mayan population, which includes the states of Campeche, Yucatan and Quintana Roo, well known as the Yucatan peninsula. One of the ideas of ethnopharmacological knowledge is the selection of medicinal plant species, in general, based on humoral concepts, this selection was made based on their taste, smell, color, or because they showed similarities to a certain illness or body organ (Ankli et al., 1999; Can Ortíz et al., 2017). Under this premise, we look for some species cited in the holy Mayan books *Popol Vuh* and *Chilam Balam* for the treatment of a skin-like condition cutaneous leishmaniasis, a neglected tropical disease caused by *Leishmania mexicana*, with an annual mean of 637 new cases, most of them in the Yucatan peninsula (Getti et al., 2009; Vera-Ku et al., 2015). This region is particularly relevant due to high number of registered medicinal species (around 750) however, despite the variety of species only a few compounds have been tested against *leishmania mexicana* (Méndez-González et al., 2014). Although this neglected tropical disease has been studied since 1903 (Leishman 1903), today does not exist an effective chemotherapeutic agent and some of them present several asides' effects. Because of that, information regarding the use of medicinal plants has been of considerable interest in obtaining new possible pharmaceuticals; for example, some prior studies performed with plant extracts and isolated metabolites from the Mayan ethnomedicine, suggest significant leishmanicidal properties when tasted against amastigotes of *L. mexicana*, between them, the

oxylipin (3*S*)-16,17-didehydrofalcarinol isolated from *Tridax procumbens* the cholestanoid cholest-4-en-3-one isolated from *Urechites andrieuxii*, the galactolipid 1-*O*-linolenoyl-2-*O*-stearoyl-3-*O*-β-D-galactopyranosyl glycerol isolated from *Dorstenia contrajerva* with an IC_{50}= 0.54, 0.03 and 0.90 μM respectively (Carrillo-Aké et al., 2021; Martín-Quintal et al., 2009; Pan et al., 2012). This study aims to evaluate some crude extracts, fractions and if so, isolated metabolites from the Mayan traditional medicine and its biological activity against *L. mexicana* to explore new, less toxic, less costly, safe and affordable treatments of natural origin.

2. Results and Discussion.

2.1*Compounds isolation and chemical characterization.*

The total yield obtained from the MeOH crude extracts of each specie was, *Hylocerus undatus* 29.90 g (1.33%), *Bauhinia divaricata* 60.52 g (2.28%), *Euphorbia hirta* 72.59 g (2.37 %), *Ruellia nudiflora* 32.66 g (1.06 %) and *Cedrela odorata* 59.95 g (2.06 %). Only *Euphorbia hirta* and *Cedrela odorata* were further partitioned by different polarity solvents, affording in the *n*-Hex fraction (Ehw-2a and Cosl-2a) 36.85 g (50.77%) and 18.03 g (30.07%), in the TCM fraction (Ehw-2b and Cosl-2b) 0.77 g (1.06%) and 9.66 g (16.11%) finally, the EtOAc fraction (Ehw-2c and Cosl-2c) 0.19 g (0.26%) and 2.89 g (4.82 %) respectively. From all the previously mentioned fractions, only Ehw-2b was further purified by column chromatography, achieving four different isolated compounds. Based on the spectroscopic data and by comparison with those previously reported in the literature, it is proposed the structures of **1**, identified as the flavonol quercetin (Imai 2017), that has 2 benzene rings (A and B) that are connected by a 3-carbon chain to form a closed pyran ring (C), it has five hydroxyl groups, one at position 3 of ring C, two at positions 3',4' of ring B and another two at positions 5 and 7 of ring A (Saraswathi et al., 2017). **2**, identified as the flavone myricetin, substituted by hydroxy groups at positions 3, 5 and 7 and a pysrogallol B ring (Gupta 2020). **3**, recognized as the flavonol kaempferol, that contains a diphenylpropane structure, with hydroxy groups located at positions 3, 5, 7 and 4' (Devi et al., 2015). **4**, the benzopyrone scopoletin, structurally composed with two aromatic rings substituted with an

hydroxy group at position 7, a methoxy in 6 and ketone group (Figure 1 and Figure 2) (Antika et al., 2022).

2.1.1 *2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one* (**1**).

Ehw-4d1 (23 mg) yellow powder. Spectroscopic data. IR ν_{max} (KBr) 3308, 1663, 1605, 1561, 1348, 1237, 1166 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD) δ, ppm 7.66 (1H, d, *J* = 7.6 Hz, H-6'), 7.57 (1H, dd, *J* = 8.4, 2.4 Hz, H-2'), 6.84 (1H, d, *J* = 8.4 Hz, H-5'), 6.30 (1H, d, *J* = 2.0 Hz, H-8), 6.09 (1H, d, *J* = 2.3 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ, ppm 177.1 (C-4), 166.7 (C-7), 162.4 (C-8a), 158.3 (C-4'), 148.7 (C-5'), 147.8 (C-2), 146.2 (C-3), 137.1 (C-2'), 124.1 (C-5), 121.6 (C-1'), 116.2 (C-4a), 115.9 (C-3'), 104.1 (C-6), 99.6 (C-6'), 94.3 (C-8). HRMS (ESI) calcd. for $C_{15}H_{11}O_7$ [M + H^+]: 303.0499; found: 303.0499.

2.1.2 *3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one* (**2**).

Ehw-5b2 (20 mg) as a yellow powder. Spectroscopic data. IR ν_{max} (KBr) 3272, 1659, 1592, 1165, 1108 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD) δ, ppm 7.33 (2H, s, H-2', H-6'), 6.34 (1H, d, *J* = 2.0 Hz, H-8), 6.16 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ, ppm 177.2 (C-4), 166.0 (C-7), 162.4 (C-5), 158.2 (C-8a), 146.7 (C-5';3'), 137.3 (C-3), 136.9 (C-4'), 123.0 (C-1'), 108.4 (C-2';6'), 104.3 (C-4a), 99.5 (C-6), 94.6 (C-8). HRMS (ESI) calcd. for $C_{15}H_{10}O_8Na$ [M + Na^+]: 341.0267; found: 341.0266.

2.1.3 *3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one*. (**3**).

Ehw-6b3 (16.6 mg) yellow powder. Spectroscopic data. IR ν_{max} (KBr) 3323, 1685, 1616, 1223, 1165, 883, 799 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD) δ, ppm 8.07 (2H, dd, *J* = 6.8, 2.4 Hz, H-2', H-6'), 6.89 (2H, dd, *J* = 7.2, 2.0 Hz, H-3', H-5'), 6.37 (1H, d, *J* = 2.4 Hz, H-8), 6.17 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ, ppm 177.3 (C-4), 165.5 (C-7), 162.5 (C-5), 160.5 (C-8a), 158.2 (C-4'), 148.0 (C-2), 137.1 (C-3), 130.9 (C-2'; C-6'), 124.0 (C-1'), 116.3 (C-3'; C-5'), 104.5 (C-4a), 99.2 (C-6), 94.4 (C-8). HRMS (ESI) calcd. for $C_{15}H_{11}O_6$ [M + H^+]: 287.0550; found: 287.0548.

2.1.4 *7-hydroxy-6-methoxy-2H-chromen-2-one*. (**4**).

Ehw-6c4 (21.7 mg) white powder. Spectroscopic data. IR ν_{max} (KBr) 3336, 1700, 1609, 1265, 859 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD) δ, ppm 7.83 (1H, d, *J* = 9.6 Hz, H-4), 7.08 (1H, s, H-5), 6.75 (1H, s, H-8), 6.19 (1H, d, *J* = 9.2 Hz, H-3), 3.89 (3H, s, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) δ, ppm 164.0 (C-2), 152.8 (C-8a). 151.3 (C-7), 147.0 (C-6), 146.0 (C-4), 112.5 (C-3; C-4a), 109.9 (C-5), 103.7 (C-8), 56.8 (CH_3). HRMS (ESI) calcd. for $C_{10}H_8O_4$ [M + H^+]: 193.0495; found: 193.0492.

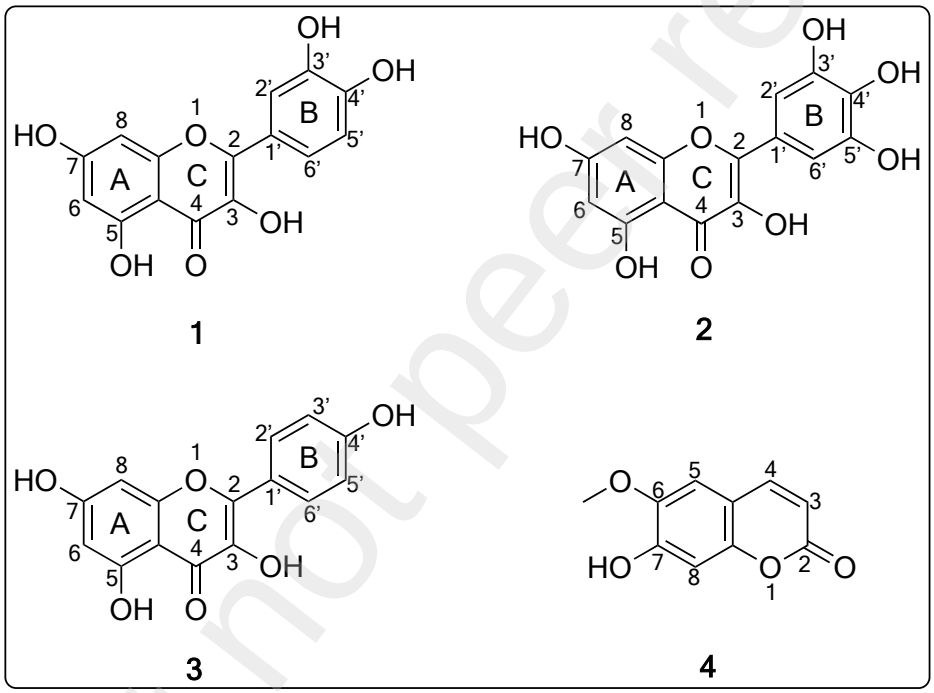

Figure 1. Isolated compounds from *Euphorbia hirta* tasted against *Leishmania mexicana.*

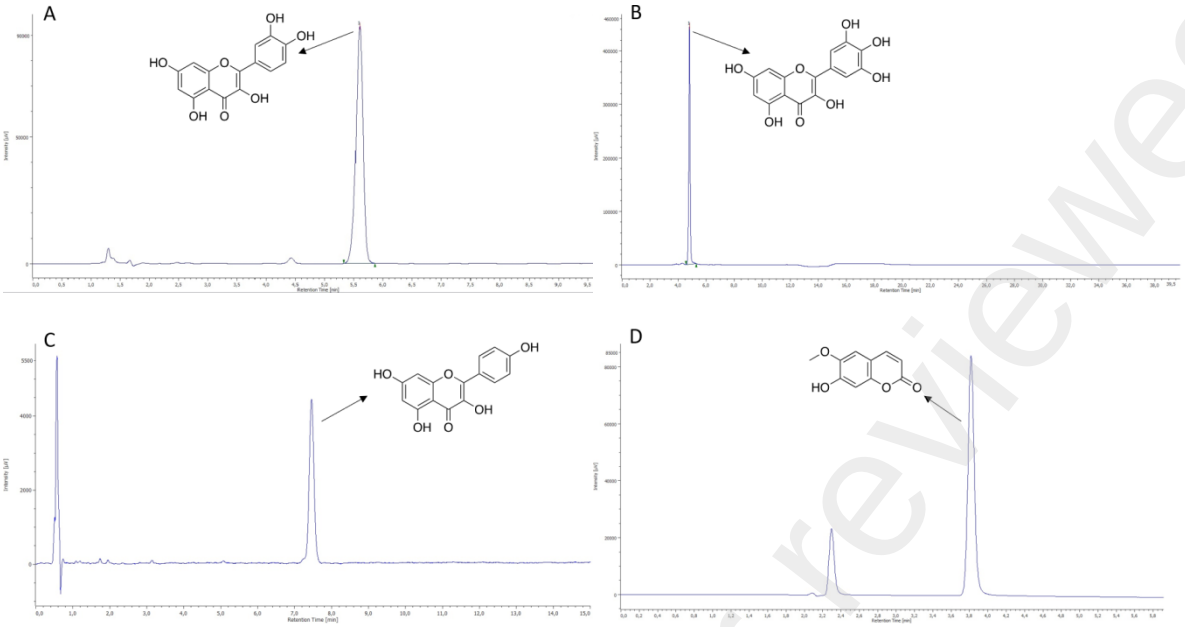

Figure 2. HPLC-chromatograms of the isolated compounds. A) Quercetin, B) Myricetin, C) Kaempferol and D) Scopoletin.

2.2 *Bio-guided leishmanicidal bioactivity of extracts and fractions.*

The MeOH extracts of five different traditional medicinal species were investigated and their leishmanicidal activity was evaluated, from which only *Euphorbia hirta* (IC_{50} = 14.81 ± 2.63 µg/mL) and *Cedrela odorata* (IC_{50} = 18.39 ± 0.88 µg/mL) showed–good bioactivity, meanwhile, *Bauhinia divaricate* not show activity*, Hylocerus undatus* and *Ruellia nudiflora* showed poor activity with IC_{50} = 122.5 ± 20.99 µg/mL and 92.18 ± 3.64 µg/mL, respectively. Sharma, 2020, revealed the leishmanicidal activity of *E. hirta* MeOH crude extract of 68.1 µg/mL when tested against promastigotes of *Leishmania donovani*, meanwhile, Singh, et al., 2011, reported an activity of 51.8 µg/mL in *E. hirta* EtOH extracts. A significant reduction in the number of parasites was observed when used Ehw-1a, these differences obey to the different stage of parasite, cell line, and the part of plant used in the assays, since it is well known that different metabolites are expressed in accordance with the plant physiology and environment changes (Qaderi et al., 2023). When the bio guided assay was performed with the apolar fraction of *E. hirta* (Ehw-2a), it was not observed activity against *L. mexicana* amastigotes, while the medium polarity fractions (Ehw-2b and Ehw-2c), presented a bioactivity with IC_{50}= 1.71 ± 0.58 µg/mL and 2.7 ± 0.89 µg/mL, respectively. Furthermore,

Cedrella odorata crude extract exhibited a good leishmanicidal activity comparable with the findings of Vásquez-Ocím, et al., 2018, who reported an $IC_{50} = 6.12 \pm 0.67$ µg/mL in axenic amastigotes of *L. donovani*. Moreover, when *C. odorata* was partitioned, a better active fraction was obtained with Cosl-2a, 3.3 µg/mL; Cosl-2b 3.5 µg/mL and Cosl-2c (no activity), which not show activity, with a noticeable difference when compared with the findings of González-Coloma, et al., 2012, who reported 95.9 ± 0.5 µg/mL in the *n*-Hex fraction and 100.0 ± 0.1 µg/mL in the TCM fraction when tested against promastigotes of *L. infantum* at 100 µg/mL (Figure 3). The aforementioned leishmanicidal activities clearly indicate the good leishmanicidal activity of *E. hirta*, which confirms the presence of compounds with leishmanicidal activity (Table 1).

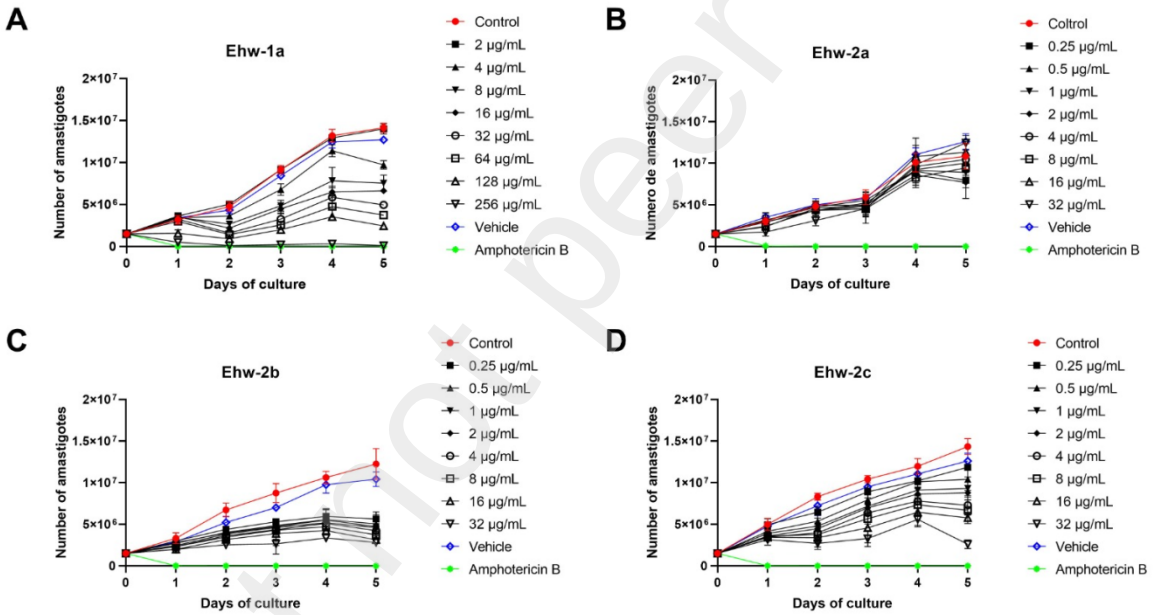

Figure 3. Leishmanicidal activity of **A**) Ehw-1a, **B**) Ehw-2a, **C**) Ehw-2b and **D**) Ehw-2c, over the growth of axenic amastigotes of *Leishmania mexicana*. The amastigotes were incubated at different concentrations. The viability was determined by daily counting. Data are presented as the mean (±) of the standard error (SE) ($n = 4$).

Table 1. Leishmanicidal activity of extracts, fractions and isolated compounds of studied species.

Key extract	Yield (%)	IC_{50} (µg/mL)	Key fraction	Yield (%)	IC_{50} (µg/mL)	Key compound	IC_{50} (µM)

Hus-1a	1.33	122.5 ± 20.99	-	-	-	-	-
Bdl-1a	2.28	No activity	-	-	-	-	-
Ehw-1a	2.37	14.81 ± 2.63	Ehw-2a	50.70	No activity	-	-
			Ehw-2b	1.06	1.7	**1** **2** **3** **4**	2.92 ± 0.42 12.30 ± 0.57 20.22 ± 4.66 4.05 ± 0.68
			Ehw-2c	0.26	2.7 ± 0.87	-	-
Rnw-1a	1.06	92.18 ± 3.64	-	-	-	-	-
Cosl-1a	2.06	18.39 ± 0.88	Cosl-2a	4.82	3.04 ± 0.2	-	-
			Cosl-2b	16.11	2.04 ± 0.56	-	-
			Cosl-2c	30.07	No activity	-	-

2.3 *Leishmanicidal activity of isolated compounds.*

Pure compounds isolated from *E. hirta* demonstrated a dose-dependent manner, herein a promising result when tasted against axenic amastigotes culture of *L. mexicana* being more susceptible to compound **1**, resulted to be the most active chemical between the isolated molecules with an IC_{50}= 2.92 ± 0.42 μM after 72 h of culture. There are numerous antileishmanial activity findings of some promising pure compounds isolated from plants, but only a few of them are focused on *L. mexicana*. De Sousa et al., 2015, reported an inhibitory effect of **1** against the rCPB2.8 proteinase (IC_{50}= 18.03 μM) from *L. mexicana*. In another study, Manjolin, et al., 2013, finds an IC_{50}= 10 μM of **1** over amastigotes of *L.*

amazonensis. Similar to the previous published by Montrieux, et al., 2014 and Fonseca-Silva, et al., 2011, who demonstrated the bioactivity of **1**, both of them with IC_{50}= 4.3 μM. In our study, **1** showed a higher bioactivity than previously reported, the aforementioned, could be related to the strain of Leishmania used and the different experimental conditions. The reaction mechanism could be due to the dihydroxy group between the A-ring, the *o*-dihydroxy group of B, the $\Delta^{2(3)}$ and 4-carbonyl of the C-ring being the active groups in **1**. The biological activity of **1** is largely attributed to these active phenolic hydroxyl groups and double bonds. B-ring in flavonoids is the main active site for antioxidant and reactive oxygen species scavenging (Marín, et al., 2009). By its part, in our assay, **2** showed an IC_{50}= 12.3 μM ± 0.57, meanwhile Tasdemir, et al., 2006, reported an IC_{50}= 1.3 μM in *L. donovani* amastigotes; on the contrary, Torres-Mendoza, et al., 2006, could isolate three flavonol arabinosidoes with myricetin skeleton but none of them showed bioactivity with *L. mexicana*. The leishmanicidal activity of flavonoids, mainly is attributed to the number of hydroxy substituents present. **2** with its six hydroxy moieties could be expected to have a strong radical scavenging activity. Xie et al., 2013 revealed that the hydroxy group in the C-4′ position plays the biggest role in the activity of myricetin against lipid peroxide radical $CH_3OO\cdot$. The bioactivity of the 4′-hydroxy site is perhaps due to weak H-bonding interactions between the oxygen radical of the reactive hydroxy group and the adjacent hydroxy group in the B-ring, additionally, the presence of the 3′,4′-catechol moiety in the B-ring was linked to a strong scavenging activity (Semwal, et al., 2006); disrupting the mitochondrial function on the parasites, and most likely inhibit different enzymes, including shock proteins, topoisomerases and kinases, among others. They could also show indirect activity through the induction of microbicidal responses, for example, the production of various cytokines and the production of nitric oxide (Dodson, et al., 2011). The poorest bioactivity was found in compound **3**, with an IC_{50}= 20.22 ± 4.66 μM, lower than the findings by Alotaibi, et al., 2021, who isolated and tried 4′,7-dimethoxykaempferol against the same cell line (IC_{50}= 12.9 ± 3.7 μM) in addition, Marín et al., 2009, could evaluate the bioactivity of **3** reporting an IC_{50}= 30.49 μM against amastigotes of *L. brazilensis*. Overall, the lack of activity of **3**, could be due to the hydroxyl group in flavonoid ring B, playing an important role in the hydrogen atom transfer reactions, converting the B ring in a stable structure, with no hydrogens to donate. The rate of reaction in **3** with one hydroxyl group in B is faster than

1, having 3',4'-OH groups. Furthermore, the 3-OH substitution in ring C and the torsion angle for ring B and C, provide a crucial stability to **3** (Halder et al., 2017). According to Soares-Vilanova, et al., 2013, reported an IC_{50}= 44.2 ± 0.25 µM of **4** with a methoxy substituted group in C-7, meanwhile, when tasted against the same cell line of *L. mexicana*, **4** showed a much better activity with an IC_{50}= 4.05 ± 0.68 µM (Figure 4). The afore mentioned clearly indicates that the hydroxyl group in C-7, plays an extremely important role as a leishmanicidal chemical group. In general, the influence of the isolated compounds over the amastigotes, could be associated with indirect events, such as anti-inflammatory properties, antioxidant or immunostimulatory activities.

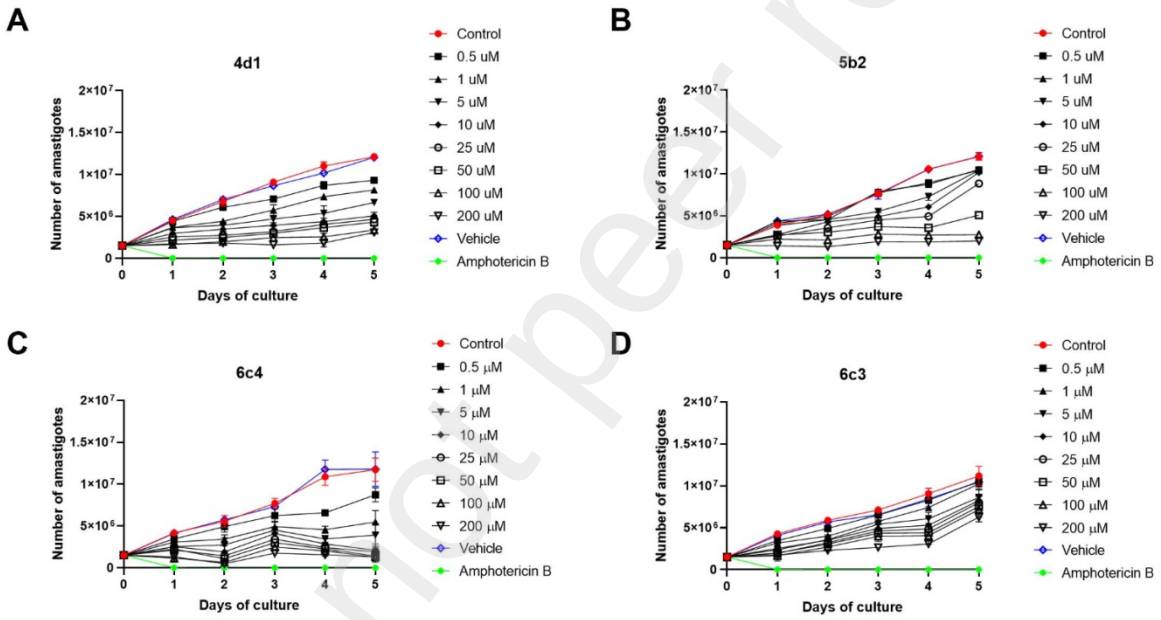

Figure 4. Leishmanicidal activity of **A**) Ehw-4d1, **B**) Ehw-5b2, **C**) Ehw-6c4 and **D**) Ehw-6c3, over the growth of axenic amastigotes of *Leishmania mexicana*. The amastigotes were incubated at different concentrations. The viability was determined by daily counting. Data are presented as the mean (±) of the standard error (SE) (n = 3).

3. Experimental

3.1 *Chemical procedures.*

Purification was observed by Thin Layer Chromatography (TLC) (Kieselgel 60 F254 precoated plates, E. Merck®, Germany) and the spots were detected due to the exposure to

UV light at λ 254 nm coloration with sprays of 10% phosphomolybdic acid in ethanol and heating the plate. Purification by flash column chromatography was performed on Merck® 60 silica gel (0.063-0.2 mesh). ^{1}H-NMR and ^{13}C-NMR spectra were obtained in a Varian-Mercury® 400 MHz (400 MHz for ^{1}H and 50 MHz for ^{13}C). The spectra were measure in CD_3OD, chemical shifts (δ) are given in ppm and coupling constants in (*J*) in Hertz. Infrared Spectroscopy (FT-IR) was performed in a Perkin Elmer® Spectrum Two. High resolution mass spectra (HRMS) were obtained by electron spray ionisation-mass spectrometry (ESI-MS) technique (5 kV) on a QSTAR XL® mass spectrometer.

2.2 *Plant material.*

Between 2 – 3 Kg of dry material was collected in the Yucatan peninsula. The collect and identification were carried out by an expert ethnobotanic and taxonomist. A sample of each specie was deposited at the herbarium *U Najil Tikin Xiw* from the Yucatan Scientific Research Center. A voucher number was given to each specie. (Table 2).

Table 2. Evaluated species for its leishmanicidal bioactivity.

Specie name	Common name	Key	Collect coordinates	Voucher No.
Hylocerus undatus	Pitahaya	Hus	20º18’50.6’’N; 89º22’55.4’’W	399
Bauhinia divaricata	Pata de venado	Bdl	20º18’58.9’’N; 89º22’35.7’’W	311
Euphorbia hirta	Hierva de pollo	Ehw	20º18’50.5’’N; 89º22’14.5’’W	529
Ruellia nudiflora	Cabal	Rnw	20º18’22.4’’N;89º21’47.0’’W	1718
Cedrela odorata	Cedro	Col	20º18’12.5’’N;89º21’45.7’’W	2296

Final letters of keys stand for, s: stem, l: leaves and w: whole plant.

2.2 *Samples extraction and fractionation.*

After collection, all samples were dried in an oven FED 720 (Binder® GmbH, Tuttlingen, Germany) at 40 ºC to avoid degradation of any thermolabile compounds and milled for its further exhaustive extraction by maceration with methanol (MeOH) (Conquimex®, Ecatepec, Mexico) (3×) overnight at room temperature. After solvent evaporation *in vacuo* (R-100, Büchi®, Switzerland) the crude extracts were dissolved in water 1:1 and then, in solvent 1:3,

partitioned with *n*-hexane (*n*-Hex), chloroform (TCM) and ethyl acetate (EtOAc) (Merck®, Darmstadt, Germany), followed by solvent evaporation by low pressure was afforded the different polarity fractions.

2.3 *Isolation and identification of compounds*.

Only the fraction that showed the best bioactivity was further purified by column chromatography; for that end, fraction Ehw-2b was subjected to a stepwise gradient purification (10% *n*-Hex/EtOAc), 94 subfractions were collected that in accordance to its partition profile were combined given 7 subfractions (Ehw3a – Ehw3g). Subfraction Ehw-3a was longer purified (20% *n*-Hex/EtOAc), 62 subfractions were collected in conformity to its partition profile and were combined, yielding to 5 subfractions (Ehw-4a – Ehw4d). From subtraction Ehw-3d (30% *n*-Hex/EtOAc), were obtained 50 subfractions that, once combined gave 4 subfractions (Ehw-5a – Ehw-5d). Finally, from subfraction Ehw-3g (40% *n*-Hex/EtOAc), 28 subfraction were collected, affording 4 subfractions (Ehw-6a – Ehw-6d). The presence of a single spot was detected by means of the TLC analysis, which reveals the existence of a pure compound; Ehw-4d1, Ehw-5b2, Ehw-6b3 and Ehw-6c4. The purity of compounds and its chemical characterization was established by spectroscopical experiments. The spectroscopic data were compared with those previously reported in the literature.

2.4 *Biological activity assay of extracts, fractions, and pure compounds.*

In order to determine the effect of extracts and fractions over the *L. mexicana* amastigotes strain MHOM/MX/2011/Lacandona (Escalona-Montaño et al., 2016), parasites were incubated at different concentrations of extracts, fractions, and pure compounds. Amastigotes were obtained from the stationary stage of axenic culture on day 6. Followed, it was quantified 1.5×10^6 parasites/mL, which were incubated at 33 ºC in 5 mL tubes that contain between 2-256 µg/mL, 0.25-32 µg/mL y 0.5-200 µM for extracts, fractions, and pure compounds, respectively. As a negative control, it was added one condition without treatment; Amphotericin B was used as a positive control at 1 µM. Extracts and fractions were diluted in ethanol as vehicle. It was added one condition with the vehicle to discard that

the solvent was not killing the parasites. The number of parasites was evaluated daily by Neubauer chamber. The IC_{50} at 72 h was calculated using the software GraphPad® 8.0 (San Diego, CA, USA).

Declaration of competing interest

The authors declare no competing financial or personal interest that could influence the results reported in this paper.

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