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Research Article

# Bioguided Assay of Polyphenols Isolated from Medicinal Mayan Species and its Activity Against *Leishmania mexicana*.

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## ABSTRACT

**Objective:** This study underlines the *in vitro* leishmanicidal activity of the methanol extracts (MeOH), fractions of *n*-hexane (*n*-Hex), chloroform (TCM) and ethyl acetate (EtOAc), and compounds isolated from plant species used in the Mayan traditional medicine. **Materials and Methods**: Extracts of medicinal species collected in the Mayan Peninsula such as *Hylocerus undatus, Bauhinia divaricate, Euphorbia hirta, Ruellia nudiflora and Cedrela odorata*, were tasted in a bio guided assays against amastigotes of *Leishmania mexicana*. Different chromatographic techniques were applied in order to isolated the most active compounds. Additionally, spectroscopic experiments $^{1}$H-NMR, $^{13}$C-NMR, LC-MS and FT-IR were stablished to determine the chemical structure of the chemical compounds. **Results:** *Euphorbia hirta* and *Cedrella odorata*, showed good bioactivity with 14.81 ± 2.63 g/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*. Spectroscopic data 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. **Conclusion:** The bioguided assays guided us, to the purification and isolation of four different metabolites, mainly flavonoids and structurally related compounds, some of them show good activity, 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.

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## 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.[1-3] 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.[4,5] 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.[6,7] 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*.[8] Although this neglected tropical disease has been studied since 1903,[9] 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.[10,11,12] 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.

## MATERIALS AND METHODS

### 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

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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.

## 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 1).

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

## 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.

## 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.

**Table 1. 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 |

## 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 parasites were incubated at different concentrations of extracts, fractions, and pure compounds.[13] Amastigotes were obtained from the stationary stage of axenic culture on day 6. Followed, it was quantified $1.5 \times 10^6$ 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).

# RESULTS

## 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, [14] 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.[15] **2**, identified as the flavone myricetin, substituted by hydroxy groups at positions 3, 5 and 7 and a pysrogallol B ring.[16] **3**, recognized as the flavonol kaempferol, that contains a diphenylpropane structure, with hydroxy groups located at positions 3, 5, 7 and 4'.[17] **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).[18]

### 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (1)

Ehw-4d1 (23 mg) yellow powder. Spectroscopic data. IR $\nu_{max}$ (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.

### 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 $\nu_{max}$ (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.

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

Ehw-6b3 (16.6 mg) yellow powder. Spectroscopic data. IR $\nu_{max}$ (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.

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

Ehw-6c4 (21.7 mg) white powder. Spectroscopic data. IR $\nu_{max}$ (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.

## 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 divaricata* 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. A study, revealed the leishmanicidal activity of *E. hirta* MeOH crude extract of 68.1 μg/mL when tested against promastigotes of Leishmania donovani, meanwhile, in a similar study it was reported an activity of 51.8 μg/mL in E. hirta EtOH extracts.[19,20] 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.[21] 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 other findings of that reported an $IC_{50}$ = 6.12 ± 0.67 μg/mL in axenic amastigotes of *L. donovani*.[22] 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 some findings reporting 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 2).[23] The aforementioned leishmanicidal activities clearly indicate the good leishmanicidal activity of *E. hirta*, which confirms the presence of compounds with leishmanicidal activity (Table 2).

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

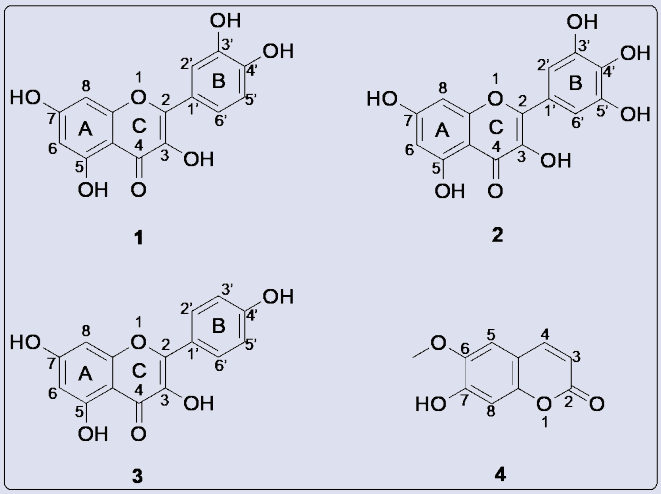


**Figure 1**. Isolated compounds from *Euphorbia hirta* tasted against *Leishmania mexicana*.

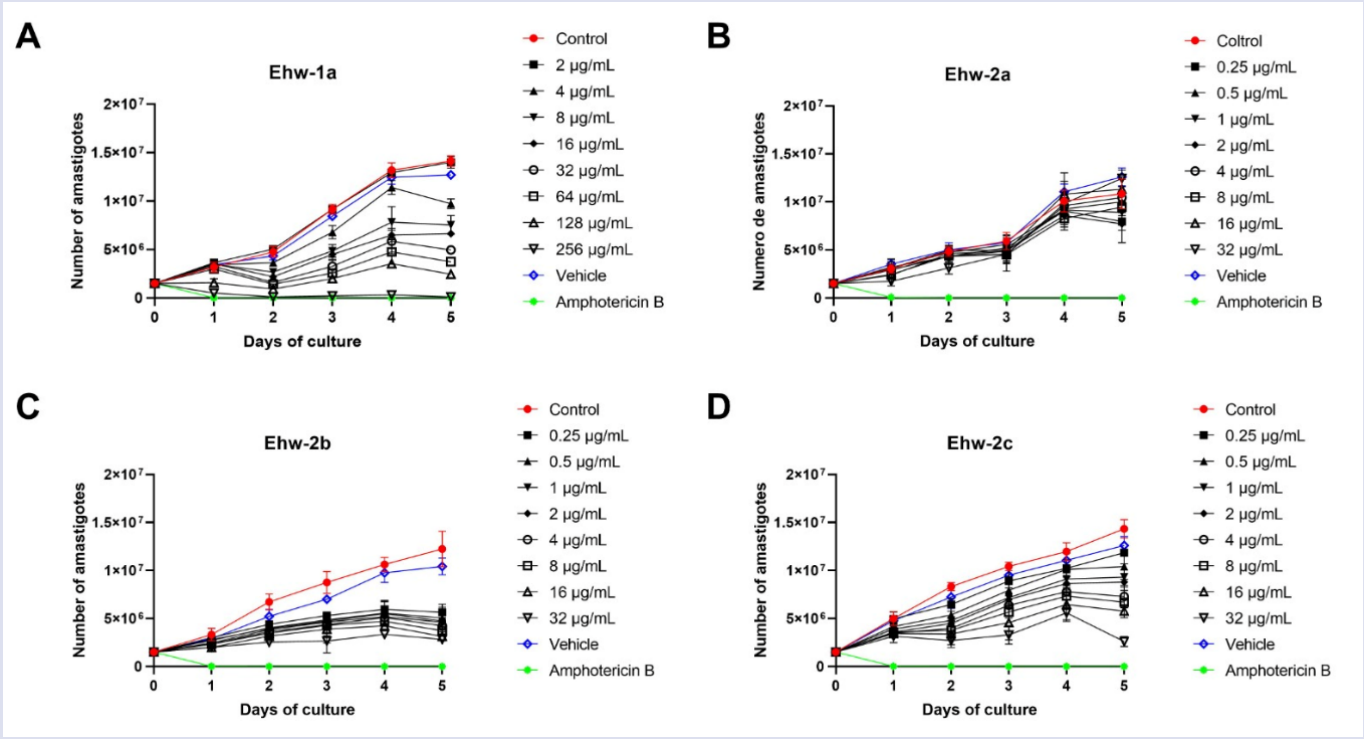


**Figure 2.** Leishmanicidal activity of **A**) Ehw-1a, **B**) Ehw-2a, **C**) Ehw-2b and **D**) Ehw-2c, over the growth of axenic amastigotes of *Leishmania mexicana*. The amastigotes were incubated at different concentrations. The viability was determined by daily counting. Data are presented as the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

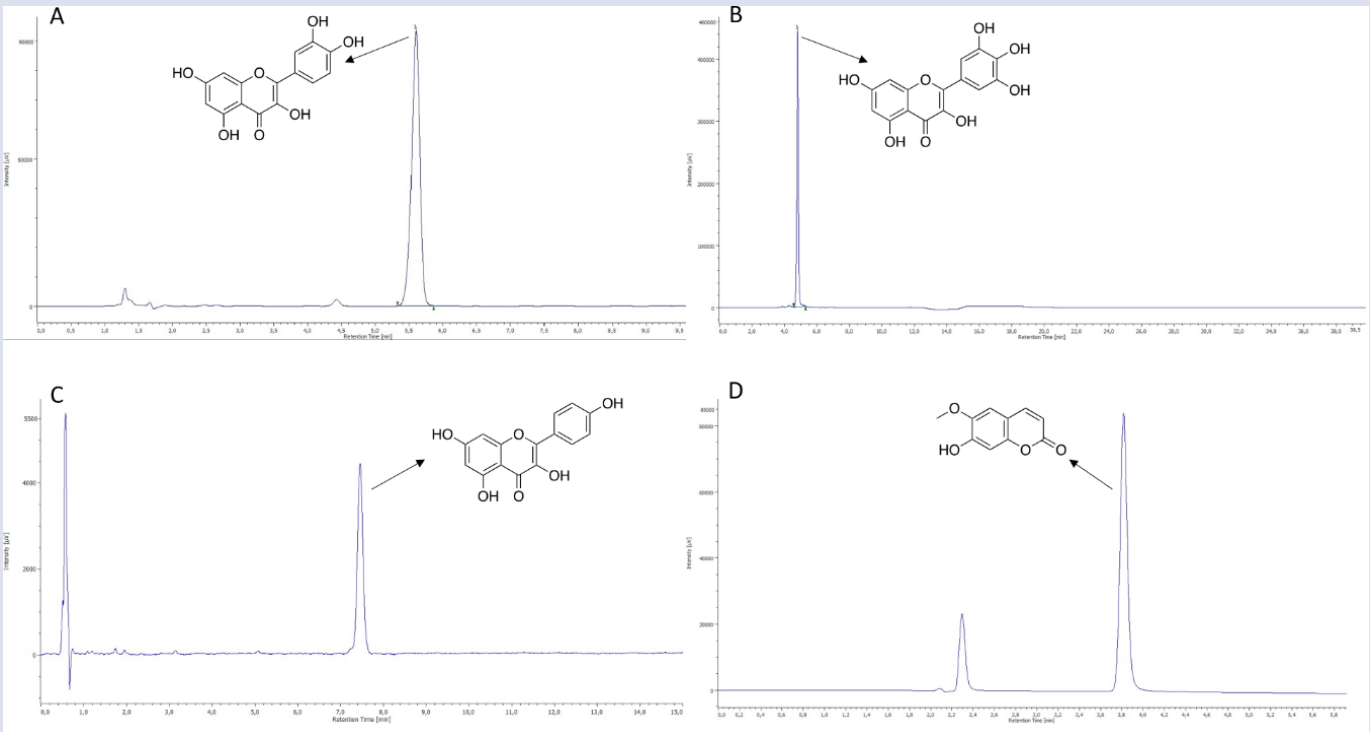


**Figure 3.** HPLC-chromatograms of the isolated compounds. A) Quercetin, B) Myricetin, C) Kaempferol and D) Scopoletin.

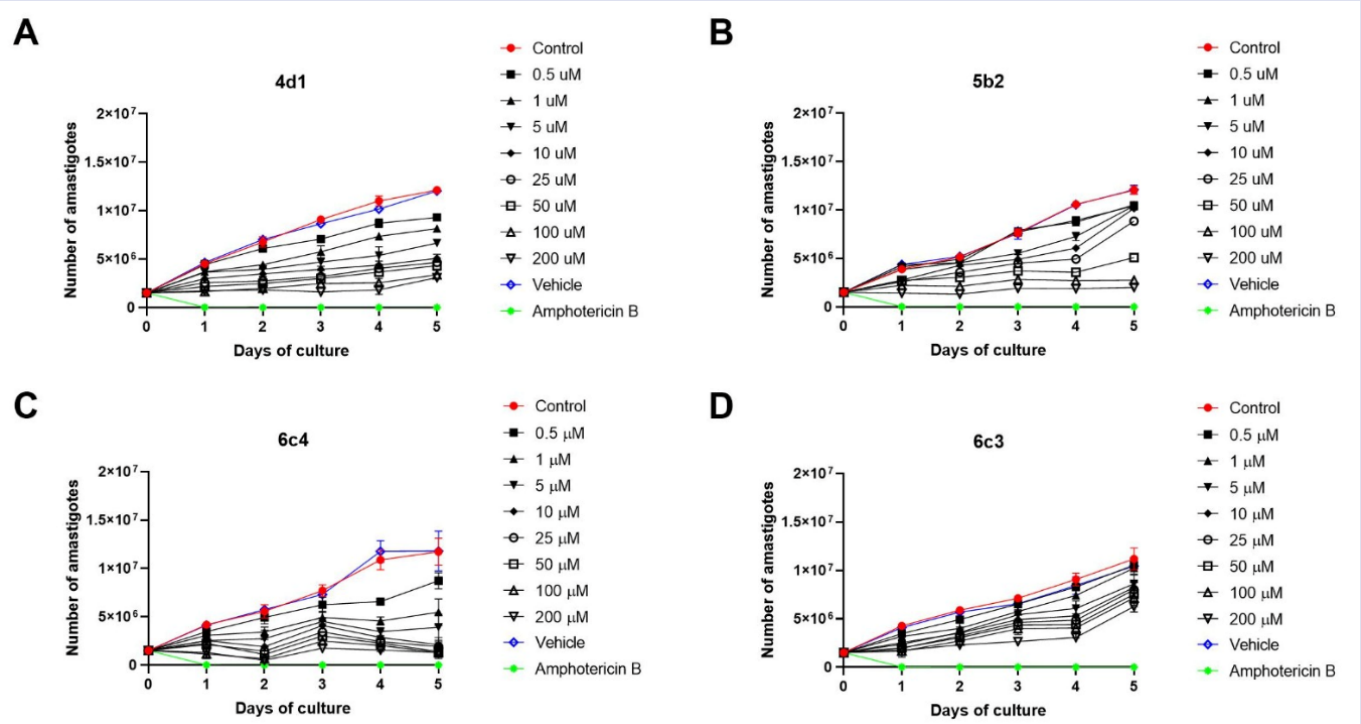


**Figure 4**. Leishmanicidal activity of A) Ehw-4d1, B) Ehw-5b2, C) Ehw-6c4 and D) Ehw-6c3, over the growth of axenic amastigotes of *Leishmania mexicana*. The amastigotes were incubated at different concentrations. The viability was determined by daily counting. Data are presented as the mean (±) of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

## DISCUSSION

### Leishmanicidal activity of isolated compounds

Pure compounds isolated compounds isolated from *E. hirta* (Figure 3) 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*. Previous investigations reported an inhibitory effect of **1** against the rCPB2.8 proteinase ($IC_{50}$= 18.03 µM) from *L. mexicana*. In another study, it was demonstrated an $IC_{50}$= 10 µM of **1** over amastigotes of *L. amazonensis*. Similar to the preceding findings, that revealed the bioactivity of **1**, both of them with $IC_{50}$= 4.3 µM.[24 - 27] 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.[28] By its part, in our assay, **2** showed an $IC_{50}$= 12.3 µM ± 0.57, meanwhile, another research group reported an $IC_{50}$= 1.3 µM in *L. donovani* amastigotes; on the contrary it was also isolated three flavonol arabinosidoes with myricetin skeleton but none of them showed bioactivity with *L. mexicana*.[29,30] 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. Certain investigations revealed that the hydroxy group in the C-4′ position plays the biggest role in the activity of myricetin against lipid peroxide radical $CH_3OO\cdot$.[31] 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;[32] 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.[33] 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, it was previously publish the the bioactivity of **3**, reporting an $IC_{50}$= 30.49 µM against amastigotes of *L. brazilensis*.[35] 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**.[36] According to some revealed data, it was 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).[37] 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.

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## CONFLICTS OF INTEREST

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

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