

# Proliferative effect of *Dracontium spruceanum* on *Leishmania*

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## ABSTRACT

**Introduction:** *Leishmaniasis*, transmitted by sandflies and caused by protozoa of the genus *Leishmania*, primarily presents in its cutaneous form. Difficulties in diagnosis and the adverse effects of conventional treatments have driven the search for alternatives, such as *Dracontium spruceanum* ("sacha jergón"), an Amazonian plant containing compounds with potential activity against *Leishmania* spp., whose efficacy still requires scientific validation. **Objective:** To determine the effect of the aqueous extract of *Dracontium spruceanum* against *Leishmania*. **Methods:** Detection of *Leishmania* (*Viannia*) spp. kDNA was performed by PCR using primers MP1-L and MP3-H, with LTB-300 (*L. (V.) braziliensis*) and DNA-free water as controls. Promastigotes were isolated from cutaneous lesion scrapings and cultured in biphasic medium, achieving differentiation into axenic amastigotes in Schneider medium, with pH 4.7 as the optimal condition for complete conversion. Plant material of *Dracontium spruceanum* collected in Ucayali (Peru) was processed to obtain an aqueous extract (100 mg/mL). The antiparasitic activity of the extract was evaluated by the MTT assay against promastigotes and amastigotes, using Glucantime as a positive control. Data obtained were analyzed by ANOVA, considering p-values < 0.05 as significant.

**Results:** In *in vitro* assays with *Leishmania* sp., administration of Glucantime (25 mg/mL) produced a significant decrease in cell viability of promastigotes (71%) and axenic amastigotes (38%) compared to the control group. Conversely, the aqueous extract of *Dracontium spruceanum* (8.33 mg/mL) caused a significant increase in promastigote (160%) and amastigote (179%) viability, indicating a stimulatory effect on parasite growth (p < 0.05). **Discussion and conclusion:** The *in vitro* effect of the aqueous extract of *Dracontium spruceanum* on promastigotes and axenic amastigotes of *Leishmania* sp. was investigated. Unlike Glucantime, which significantly decreased parasite viability, the extract consistently promoted proliferation in both forms. This result, uncommon in medicinal plant studies, could be linked to the presence of ceramides and cerebrosides, compounds in the genus *Dracontium* previously associated with mitogenic activity. Additional dose-response studies and phytochemical analysis are needed to identify the active compounds and clarify their mechanism of action.

**KEYWORDS:** *Leishmania*, Cutaneous Leishmaniasis, *Dracontium spruceanum*, Cell viability, Glucantime

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## INTRODUCTION

Leishmaniasis is a neglected infectious disease that primarily affects vulnerable populations commonly associated with poverty, with limited access to healthcare services and exposure to environments that favor vector proliferation<sup>1</sup>. The etiologic agent of this disease belongs to protozoa of the genus *Leishmania*, transmitted to humans through the bite of infected arthropods, mainly *Phlebotomus* species in the Old World and *Lutzomyia* species in the New World<sup>2</sup>. This disease has an estimated incidence of 700,000 to 1 million new cases annually<sup>3</sup> and is considered a zoonotic disease affecting populations in tropical, subtropical, and temperate regions.

The three main clinical forms of this disease are cutaneous, mucocutaneous, and visceral leishmaniasis. Cutaneous leishmaniasis (CL) is the most common form, characterized by the appearance of skin ulcers that may heal spontaneously but leave permanent scars. Mucocutaneous leishmaniasis (MCL) affects the mucous membranes of the nose, mouth, and throat and is considered a disfiguring disease. The most severe manifestation is visceral leishmaniasis (VL), also known as kala-azar, which is potentially fatal if not treated promptly, as it affects internal organs such as the liver, spleen, and bone marrow<sup>4</sup>.

The distribution of leishmaniasis is influenced by ecological, climatic, and socioeconomic factors.

Climate change and deforestation are modifying the distribution patterns of vectors, potentially increasing the risk of transmission in new areas<sup>5</sup>.

The diagnosis and treatment of leishmaniasis pose significant challenges. Although diagnostic methods such as microscopy, serological tests, and molecular techniques exist, their availability in endemic areas is limited. Current treatments with pentavalent antimonials, amphotericin B, and miltefosine can be toxic and costly, highlighting the need for safer and more accessible therapeutic alternatives<sup>6</sup>.

Therefore, leishmaniasis represents a global public health problem that requires integrated approaches for control and prevention, including epidemiological surveillance, vector management, and the development of effective vaccines<sup>2</sup>.

Let us remember that leishmaniasis is a chronic disease whose etiological agent belongs to the group of protozoa of the genus *Leishmania*, and conventional treatment includes drugs that often present severe side effects and limitations in accessibility, such as pentavalent antimonials, amphotericin B, and miltefosine<sup>6</sup>. This has sparked interest in the search for new therapeutic alternatives, including the use of medicinal plants such as *Dracontium spruceanum*.

*Dracontium spruceanum*, commonly known as "sacha jergón," is a plant of the family Araceae widely used in traditional medicine in the Amazon and other regions of South America. Among its

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attributed medicinal properties are its use as an antidote for snake bites, anti-inflammatory purposes, and the treatment of skin infections<sup>7</sup>. Its potential as a therapeutic agent for leishmaniasis has been explored due to the presence of bioactive compounds with antiparasitic activity.

Traditional use of sacha jergón for treating skin ulcers has sparked interest in its potential application against cutaneous leishmaniasis<sup>8</sup>. Previous research has identified compounds such as alkaloids and flavonoids, which may have potential antiparasitic activity against *Leishmania* spp<sup>7</sup>. However, scientific evidence remains limited, and further *in vitro* and *in vivo* studies are required to validate its use.

The use of medicinal plants such as *Dracontium spruceanum* represents a promising therapeutic option for the treatment of leishmaniasis, especially in endemic areas where access to conventional medications is limited. Challenges include standardizing extracts, identifying active principles responsible for antiparasitic activity, and assessing toxicity<sup>7</sup>.

Integrating traditional knowledge with rigorous scientific approaches is essential to ensure the safety and efficacy of these therapeutic alternatives. Collaboration between local communities, researchers, and health authorities is key to advancing in this field.

The aim of the present work is to determine the effect of the aqueous extract of *Dracontium spruceanum* against *Leishmania*.

## METHODOLOGY

### Identification of *Leishmania* JM strain kDNA

A PCR was performed on each sample to detect the kinetoplast DNA (kDNA) of *Leishmania* (Viannia) spp. The primers used for amplification were: MP1-L: 5'-TAC TCC CCG ACA TGC CTC TG-3' and MP3-H: 5'-GAA CGG GGT TGT TTC ATG C-3'. Reactions were carried out in a final volume of 20  $\mu$ L containing 4  $\mu$ L of DNA, 2  $\mu$ L of 10X PCR buffer (Invitrogen), 1  $\mu$ L of each primer (10  $\mu$ M), 0.2  $\mu$ L of Taq DNA polymerase (5 U/ $\mu$ L) (Invitrogen, Grand Island, NY), 0.6  $\mu$ L of 50 mM MgCl<sub>2</sub>, and 2  $\mu$ L of 1.25 mM of each dNTP. Initial denaturation was performed at 94 °C for five minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 58 °C for 45 seconds, extension at 72 °C for 60 seconds, and a final extension at 72 °C for five minutes. The reference strain LTB-300 (*L. (V.) braziliensis*) was used as a positive control, and molecular-grade water was used as a negative control. The expected product was 70 bp, visualized using 2% agarose gels<sup>9</sup>. To determine the *Leishmania* species, the PCR-hsp70 test was performed, although genotyping was not achieved<sup>10</sup>.

### Obtaining axenic amastigotes and promastigotes

The sample for obtaining the parasite was collected by scraping the edges of an ulcer in a patient with cutaneous leishmaniasis, with the help of a sterile lancet. In our study, an approximate 14-day biphasic blood agar culture of promastigote was obtained from a scraping of an ulcer lesion from a patient with leishmaniasis diagnosed clinically and corroborated by microscopy with the presence of amastigotes stained with Giemsa at 1000X. The sample obtained was then placed in a biphasic medium for 14 days, *in vitro*, at 25°C, in an RPMI medium at pH 7. Promastigotes were obtained in this way. Promastigotes were inoculated at 50  $\mu$ L in 96 microplate wells at a concentration of 2.34 x 10<sup>6</sup>/mL in 300  $\mu$ L of Schneider medium, supplemented with 20% fetal bovine serum and 10,000 U penicillin/10 mg/mL streptomycin, using different pH (4.7 - 5.2 - 5.2) at 35 °C, using 3 repetitions for each pH. Readings were made every 7 days for 1 month using a microscopic count of parasites stained by Wright dye, using 10  $\mu$ L of fixed culture on a slide and covering 100 fields. The conversion percentage for each well, using 3 repetitions for each pH, was obtained using the following formula: number of amastigotes observed in 10  $\mu$ L/ number of parasites observed in 10  $\mu$ L \* 100, in order to determine the optimal pH where

observed the complete and faster conversion of promastigotes to axenic amastigotes. For the maintenance of the amastigotes, 50  $\mu$ L of culture medium with parasites (axenic amastigotes at evaluated pHs) was taken and added to 300  $\mu$ L of medium with the evaluated pHs without parasites every 7 days. The reading was performed every 7 days for 176 days. The only medium with the pH variable in which there was 100% conversion of promastigote to axenic amastigote was at pH 4.7, varying between 63.7% on the 7th day, 97% on the 42nd day and 100% on the 56th day. Maintaining 100% conversion until day 176 where it reached 100% conversion on day 56 and remained until day 176. The parasite load at pH 4.7 was between 11661/ $\mu$ L on day 7, up to 300/ $\mu$ L on day 56 and was maintained until the MTT test. Therefore, it was concluded that the pH to be used in the conversion of axenic amastigotes for our study was pH 4.7<sup>11</sup>.

### Obtaining botanical material

Two kilograms of fresh leaves of *Dracontium spruceanum* were collected from the district of Raymondi, in the province of Atalaya, Ucayali-Peru, at the geographical coordinates S 10°01' 47.73281", W 73°57'25.14584" at an altitude of 178,715 meters above sea level<sup>12</sup>. Subsequently, a sample of the plant fue usada para determinar por la flor y el tubérculo característico como *Dracontium spruceanum*.

### Obtaining aqueous extract of *Dracontium spruceanum*

The plant was cleaned to remove the soil, later they were left to dry in the open air and once they were cleaned with a paper towel and the tuberculo were crushed manually. Finally, it was weighed, obtaining a total of 450 grams. After selecting the la muestra, 450 grams of material were obtained and separated into one equal part of 225 grams for el extracto. For the aqueous extract, 2L of boiled water were added to a glass container, stirred for 10 minutes, and left to macerate for 24 hours. Each solution was successively filtered 9 times using rough filter paper with a porosity of 12  $\mu$ m. Then they were placed in pyrex containers. Finally, it was placed inside an incubator at 40° Celsius until the presence of the paste without liquid content around it was observed. The paste was removed from the pyrex container using a clean spoon and stored in glass jars in an incubator at room temperature. And, finally, dilutions were performed to obtain a concentration of 100 mg/mL.

### Methodology of MTT [3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]

On the third day, the promastigotes and axenic amastigotes in logarithmic growth were placed in 96-well culture microplates with 120  $\mu$ L for the control group and at a ratio of 20  $\mu$ L/100 $\mu$ L of the drug concerning the Schneider medium with an initial inoculum for promastigotes of 12 x 10<sup>4</sup> parasite/mL and axenic amastigotes of 8.1 x 10<sup>3</sup> parasite/mL. Then, it was incubated in the wells for 24 with the drug Glucantime (300mg/mL) at a final concentration of 25 mg/mL as a positive control, and the *Dracontium spruceanum* aqueous extract (100mg/mL) at a final concentration of 8.33 mg/mL. Schneider medium was used as a negative control, each treatment had 3 replicates per group. Subsequently, the MTT (3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used for this study, for which 10 $\mu$ L of MTT was added to the reaction well. The culture plate was incubated for 3 hours and 30 minutes at 25 °C for promastigotes and 35 °C for amastigotes. Then, it was stirred for 30 minutes, and 100  $\mu$ L of 50% isopropanol and 10% SDS was added and adjusted to pH 5.4; it was stirred for 30 minutes, and the reading in an ELISA plate spectrophotometer at 570 nm was performed<sup>13</sup>.

### Statistical Analysis

To determine the statistical relationship between the treatment and the effect on *Leishmania*, an analysis of variance was used with a p <

**Table 1.** Promastigote de *Leishmania* sp

Negative Control*	Glucantime (25 mg/mL)	Dracontium spruceanum (8.33 mg/mL) *
0.3122± 0.0216	0.219± 0.1281	0.4972±0.0436

\*= p&lt;0,05

TREATMENT	MEAN	DS	CELL VIABILITY
T1=UNTREATED CONTROL	M1=0,31	0,02	Cell viability=100%
T2=TREATMENT WITH GLUCANTIME	M2=0,22	0,13	*Cell viability= (0,22/0,31) *100=70,97% (71%)
T3= TREATMENT WITH <i>Dracontium spruceanum</i>	M5=0,50	0,04	*Cell viability= (0,496/0,31) *100=160% (160%)

**Table 2.** Amastigote axénico de *Leishmania* sp

Negative Control*,!	Glucantime (25 mg/mL) *	Dracontium spruceanum (8.33 mg/mL)!
0.2388± 0.035	0.0928±0.0154	0.4342±0.036

\*,!=p&lt;0,05

TREATMENT	MEDIA	DS	CELL VIABILITY
T1=UNTREATED CONTROL	M1=0,24	0,04	Cell viability=100%
T2=TREATMENT WITH GLUCANTIME	M2=0,09	0,02	*Cell viability= (0,09/0,24) *100=37,5% (38 %)
T3= TREATMENT WITH <i>Dracontium spruceanum</i>	M5=0,43	0,04	*Cell viability= (0,43/0,24) *100=179,17% (179,17%)

0.05, with 95% confidence, using the Social Science Statistics program (<https://www.socscistatistics.com/>).

## RESULTS

In Table 1, for *Leishmania* promastigotes, a significant difference (\*P < 0.05) was found between the negative control group and *Dracontium spruceanum*.

In Table 2, for axenic amastigotes, a significant difference (\*P < 0.05) was found between the negative control group and Glucantime. Likewise, a significant difference (\*P < 0.05) was found between the negative control group and *Dracontium spruceanum*.

## DISCUSSION

The present study aimed to evaluate the *in vitro* activity of the aqueous extract of *Dracontium spruceanum* against promastigotes and axenic amastigotes of *Leishmania* sp. The results revealed a stimulatory effect of the extract on the proliferation and viability of the parasite.

In the promastigote assay, while the negative control established a baseline viability (0.3122 ± 0.0216 absorbance), treatment with Glucantime (25 mg/mL), the reference drug, showed the expected inhibitory effect, resulting in a reduction of absorbance (0.219 ± 0.1281) associated with decreased parasite load. This finding with the positive control confirms that the assay system was able to detect parasite growth inhibition. However, treatment with the aqueous extract of *Dracontium spruceanum* (8.33 mg/mL) not only failed to inhibit promastigote proliferation but significantly increased absorbance (0.4972 ± 0.0436, p < 0.05) and thus the *Leishmania* load compared with the negative control, suggesting a stimulatory effect on the promastigote form of *Leishmania*.

Regarding the untreated control (T1), the mean (M1 = 0.22 ± 0.13) sets the baseline viability of the parasites (100%), and the low standard deviation (SD = 0.02) indicates a high reproduction rate under the experimental conditions. The use of Glucantime at 25 mg/mL (T2) confirms activity against promastigotes, albeit somewhat less than against amastigotes, where efficacy was higher. The high viability (SD = 0.13) suggests that some parasites might have intrinsic resistance, or that the Glucantime concentration used may not be sufficiently effective; thus, testing higher doses is recommended.

In axenic amastigotes, the drug reduced parasite survival to 39% compared with the control, demonstrating higher efficacy and

highlighting differences in sensitivity between the parasite's life stages. *Dracontium spruceanum* at 8.33 mg/mL (T3), with a mean (M3) = 0.50 ± 0.04 and cell viability of 160%, indicated a 60% increase in viability compared with the control; in other words, the extract stimulated promastigote proliferation, possibly due to nutrients in the extract that enhance parasite metabolism. This finding has clinical implications, providing a warning signal or risk of using this extract in formulations without prior dose-response studies.

It is important to note that Glucantime is more effective in amastigotes, producing a 60% reduction in viability, whereas in promastigotes it produces a 29% reduction—consistent with its clinical use, as it is more effective against the intracellular stage of the parasite.

For its part, *D. spruceanum* has a stimulatory effect in both parasite forms—160% in promastigotes and 182% in amastigotes compared with the control—supporting the idea of a common mechanism. It is suggested to test a wider range of *D. spruceanum* extract concentrations to determine whether an inhibitory effect occurs at higher doses, as well as to identify the components responsible for the observed effect.

Similarly, when assessing the extract's effect on axenic amastigotes, comparable results were observed. Glucantime (25 mg/mL) significantly reduced amastigote viability (0.0928 ± 0.0154) compared with the negative control (0.2388 ± 0.035) (p < 0.05), confirming its efficacy as a positive control. Contrary to expectations, the *D. spruceanum* extract significantly increased axenic amastigote viability (0.4342 ± 0.036) compared with the negative control (p < 0.05). The *in vitro* results for both life stages are consistent and suggest that the aqueous extract of *D. spruceanum* contains components that may act as nutrients promoting the growth and viability of *Leishmania* sp.

For the untreated control (T1), the mean (M1 = 0.24 ± 0.04) sets the baseline viability (100%), and the standard deviation (SD = 0.04) indicates moderate variability under baseline conditions. Glucantime at 25 mg/mL (T2), with a mean (M2) = 0.09 ± 0.02 and cell viability of 37.5%, represents a 62.5% reduction compared with the control.

This demonstrates high efficacy against the amastigote stage, with the low SD (0.02) indicating a consistent response. The reduction is greater than in the promastigote stage (71% viability), confirming the strong effect on the clinically relevant amastigote stage.

*Dracontium spruceanum* at 8.33 mg/mL (T3), with a mean (M3) = 0.43 ± 0.04 and cell viability of 179.1%, indicates a 79.17% increase compared with the control. This effect is even more pronounced in promastigotes

(160% viability). The low SD (0.04) shows that the extract's stimulatory effect is consistent.

This finding is of special interest considering that medicinal plants are usually investigated for inhibitory effects against pathogens. Species of the genus *Dracontium* are known in traditional medicine and have been studied for various biological activities. For example, ethanolic extract of *Dracontium dubium* tubers has demonstrated significant antivenom activity against the lethal, inflammatory, and hemorrhagic effects of *Bothrops asper* venom in animal models<sup>14</sup>. This *D. dubium* extract also showed anti-inflammatory activity by inhibiting LPS-stimulated macrophage activation and reducing nitric oxide (NO) production<sup>14</sup>. Additionally, it inhibited the indirect hemolytic effect of the venom without affecting its procoagulant activity<sup>14</sup>. Specifically for *Dracontium spruceanum*, its neutralizing potency against the lethal effect of *Bothrops atrox* venom in mice has been reported, with an effective dose 50% (ED<sub>50</sub>) of 2.0 mg/mouse<sup>15</sup>.

Although these activities in other *Dracontium* species (antivenom, anti-inflammatory, antihemolytic, immunomodulatory in macrophages) and in the same species (*D. spruceanum*, antivenom) demonstrate that the genus contains bioactive compounds, none of the cited sources evaluate leishmanicidal activity. Therefore, our results on the effect on *Leishmania* represent a new dimension in understanding the biological activities of *D. spruceanum*.

The stimulatory activity observed in our experiments could be related to the phytochemical composition of the extract. Studies on *Dracontium loretense* have led to the isolation and characterization of oxylipins<sup>16</sup> and identified this species as a rich source of ceramides and cerebrosides<sup>17</sup>. Ceramides and cerebrosides are sphingolipid metabolites that, depending on cell type and stimulus, can mediate mitogenic or apoptotic responses<sup>17</sup>. The identification of these compounds in *D. loretense* and their reported mitogenic potential<sup>17</sup> could provide a possible explanation for the proliferative effect observed in *Leishmania*. Furthermore, *n*-butanol extract of *D. loretense* and its derived fractions have been reported to activate human peripheral blood mononuclear cells (PBMCs)<sup>16</sup>, although with toxicity at higher concentrations<sup>16</sup>. While these are different *Dracontium* species and cell types (human vs. parasitic), these findings suggest that some components of the *Dracontium* genus may have growth-promoting or cell-activating effects.

Another study on *D. loretense* evaluated antibacterial activity, finding that the ethanolic extract showed limited activity against some bacterial strains and was inactive against others at 16 mg/mL<sup>18</sup>. This supports the notion that *Dracontium* activities are not necessarily broad-spectrum antimicrobial and vary according to the organism or cell type evaluated.

It is important to consider that the aqueous extract of *D. spruceanum* used in this study may differ significantly in composition from ethanolic or *n*-butanol extracts evaluated in the literature<sup>14</sup>. Different extraction methods solubilize distinct types of compounds, potentially resulting in different activity profiles.

The single dose (8.33 mg/mL) of *D. spruceanum* extract tested in this study may be a limitation, as it prevents establishing a dose-response relationship for the stimulatory effect. Further studies should explore a range of concentrations to fully characterize this effect.

Contrary to the common expectation of finding leishmanicidal activity in medicinal plant extracts, the aqueous extract of *Dracontium spruceanum* exhibited a significant stimulatory effect on the proliferation of promastigotes and the viability of axenic amastigotes of *Leishmania* sp. *in vitro*. Although other *Dracontium* species and the same species (*D. spruceanum*) show various bioactivities—including antivenom effects, anti-inflammatory effects, and the presence of compounds such as ceramides and cerebrosides with mitogenic potential<sup>14</sup>—our findings do not directly correlate with general antimicrobial activity.

This stimulatory effect of *D. spruceanum* on *Leishmania* is a novel finding that requires further investigation to identify the responsible compounds and elucidate the underlying mechanism<sup>14</sup>. This study underscores the complexity of plant-parasite interactions and the need for detailed phytochemical and biological research to fully understand the potential of medicinal plants<sup>14,18</sup>.

## CONCLUSION

In conclusion, the aqueous extract of *D. spruceanum* consistently promoted proliferation in both forms. This uncommon result in medicinal plant studies may be linked to the presence of ceramides and cerebrosides, compounds from the *Dracontium* genus previously associated with mitogenic activity.

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