



Estudios de susceptibilidad de cepas de *Leishmania aethiopica* frente a alcaloides de *Galipea longiflora* (Evanta)

Susceptibility studies on *Leishmania aethiopica* strains against total alkaloids from *Galipea longiflora* (Evanta)

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Resumen

El Instituto de Investigaciones Fármaco Bioquímicas (IIFB), de la Facultad de Ciencias Farmacéuticas y Bioquímicas, de la UMSA, desarrolla trabajos sobre la actividad leishmanicida, de los alcaloides totales (CAT) obtenidos de la corteza de la especie medicinal amazónica conocida como Evanta (*Galipea longiflora*) por los Pueblos Tacana, Tsimane y Mosekene.

Como parte de las actividades del Proyecto UMSA-ASDI "Biomoléculas de interés medicinal e industrial (antiparasitarios)" hemos podido contar con la estadía, en el IIFB, de un investigador del Armauer Hansen Research Institute (AHRI) de Etiopía, lo que nos ha permitido desarrollar evaluaciones de CAT, Miltefocina y Anfotericina B, frente a cepas de *Leishmania aethiopica*, agente causante de las diversas formas de *Leishmaniasis* cutánea en Etiopía.

Abstract

The Instituto de Investigaciones Fármaco Bioquímicas (IIFB), at the Faculty of Pharmaceutical and Biochemical Sciences, from UMSA, carry out work related to the leishmanicidal activity of the total alkaloids (CAT) obtained from the bark of the Amazonian medicinal species known as Evanta (*Galipea longiflora*) by the Tacana, Tsimane and Mosekene people.

As part of the activities developed by the UMSA-ASDI Project "Biomolecules of medicinal and industrial interest (antiparasitic)" we had a visit, in our laboratories at IIFB, of a researcher from The Armauer Hansen Research Institute (AHRI) from Ethiopia, during his stay we were able to carry out evaluations of CAT, Miltefocine and Amphotericin B, against strains of *L. aethiopica*, causative agent of the different manifestations of cutaneous leishmaniasis in Ethiopia.

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Un total de seis cepas, de *L. aethiopi-*ca, fueron adaptadas a condiciones *in vitro* y mostraron un comportamiento homogéneo frente a CAT, cinco de estas cepas mostraron un valor promedio de $IC_{50} = 8,68 \pm 1,56$ mg/mL, valor algo inferior a los calculados para nuestras cepas de referencia, *L. amazonensis* y *L. braziliensis* con $IC_{50} = 11,73 \pm 4,32$ mg/mL y $IC_{50} = 12,28 \pm 2,95$ mg/mL, respectivamente. Excepto por una cepa de *L. aethiopi-*ca que mostro valores consistentemente más elevados que el resto con $IC_{50} = 14,37 \pm 3,58$ mg/mL.

Como consecuencia de esta interacción científica, la Universidad Mayor de San Andrés (UMSA) ha firmado un Memorandum de Entendimiento para el desarrollo de investigaciones conjuntas, con el Armauer Hansen Research Institute (AHRI), dependiente del Ministerio de Salud de Etiopía y explorar la posibilidad de que nuestra experiencia de validación clínica con Evanta en el tratamiento de leishmaniasis cutánea, en Bolivia, podría ser replicada en Etiopía, donde se reportan entre 20,000 a 30,000 nuevos casos de Leishmaniasis por año.

PALABRAS CLAVE

Bolivia, Etiopía, *L. aethiopi-*ca, *L. amazonensis*, *L. braziliensis*, susceptibilidad Alcaloides, *Galipea longiflora*

A total of six strains of *L. aethiopi-*ca, were adapted to *in vitro* a conditions, at IIFB; and did show homogenous behavior against CAT. Five of the strains, showed an average calculated value for $IC_{50} = 8.68 \pm 1.56$ mg/mL, a value somewhat lower to the calculated for the reference strains *L. amazonensis* and *L. braziliensis* with $IC_{50} = 11.73 \pm 4.32$ mg/mL and $IC_{50} = 12.28 \pm 2.95$ mg/mL, respectively. Except for one strain that showed values somewhat higher, to the other strains, consistently through our studies, with $IC_{50} = 14.37 \pm 3.58$ mg/mL.

As a consequence of our scientific interaction, the Universidad Mayor de San Andrés (UMSA) has signed a Memorandum of Understanding for the development of joint research with the Armauer Hansen Research Institute (AHRI) that belongs to the Ministry of Health in Ethiopia, and explore the possibilities to replicate the Bolivian clinical validation experience of Evanta in the treatment of cutaneous leishmaniasis, in Ethiopia where the annual incidence is estimated to be between 20, 000 to 30, 0000.

KEY WORDS

Bolivia, Ethiopia, *L. aethiopi-*ca, *L. amazonensis*, *L. braziliensis*, susceptibility Alkaloids *Galipea longiflora*

INTRODUCTION

Leishmaniasis is part of the group of forgotten or neglected tropical diseases and one of the most important vector-borne diseases in humans. This disease could be caused by various species of *Leishmania* parasites, most of which are zoonotic. Different parasite species are associated with different clinical forms of the disease. Many species of *Leishmania* cause skin ulcers and nodules, leading to cutaneous leishmaniasis (CL) which may be local (LCL) or diffuse (DCL). Some species of these organisms can also affect mucous membranes and may cause injury and disfigure the nose, causing mucocutaneous leishmaniasis (MCL). Other species damage internal organs and cause visceral leishmaniasis (VL), the latter is fatal if not treated in time (Blackwell JM et al. 2009). *Leishmaniasis* predominantly affects poor people in Africa, Latin America and Asia. Globally estimated It is estimated to be endemic in 98 countries with annual incidence of 0.9 to 1.6 million cases per year

There are about 20 species of the *Leishmania* cause leishmaniasis in human (Table 1). These species belong to the Trypanosomatidae family that includes at least eight different Genera. The different clinical manifestations associated with

different *Leishmania* species. . Currently, the genus *Leishmania* is divided into two groups: the sub-Genus *Leishmania* and sub-Genus *Viannia* (Ardaya 2015).

In the Old World, leishmaniasis could be grouped into two classes, cutaneous (CL), associated with the *L. major*, *L. tropica* and *L. aethiopica* complexes, and visceral (VL), involves up to 4 different species all associated with *L. donovani* complex (Ayele & Ali 1984). VL is endemic in many tropical and subtropical areas of the world, is the most severe form of leishmaniasis, almost always fatal if left untreated (Lyons et al. 2003). It is estimated that between 200,000 and 400,000 new cases of VL occur in the world each year (WHO, 2013) and of these, East Africa has the second highest number of cases, after the Indian subcontinent (Alvar et al. 2007 and Alvar et al 2012). In the New World, CL infections (LCL and DCL) are associated with *L. mexicana* and *L. braziliensis* complexes. MCL, is most often associated with *L. braziliensis* and *L. panamensis*, although other species may be involved. The DCL occurs with some species of the parasite and due to host immune factors, while VL is associated with *L. chagasi*.

In Bolivia, an estimated 0.8 millones of individuals are at high risk of becoming infected with leishmaniasis, the disease is spread in 7 of the 9 departments (except Oruro and Potosi). It is estimated that there are approximately 2,500 new cases per year based on published data, *L. braziliensis* species (CL and MCL) and *L. amazonensis* (CL) have a clear dominance in national epidemiological indices. *L. lainsoni* (CL and MCL) and *L. chagasi* (VL) are presented in rare and few reports (Rojas et al. 2009; Martinez et al. 2002 and Mollinedo et al. 2000). In Bolivia, cases of leishmaniasis tend to increase every year, as well as other vector transmitted diseases like Dengue, Malaria, Chagas Disease (Garcia et al. 2009 and INLASA 2012).

Table 1. Taxonomic Classification of Pathogenic Parasites of *Leishmania*

FAMILY	Genera	Sub-Genus	Complex	Species	Clinical Classification
TRYPANOSOMATIDAE	LEISHMANIA	LEISHMANIA	<i>L. donovani</i>	<i>L. donovani</i>	Visceral Leishmaniasis VL
				<i>L. chagasi</i>	
				<i>L. infantum</i>	
				<i>L. archibaldi</i>	
			<i>L. mexicana</i>	<i>L. mexicana</i>	Cutaneous Leishmaniasis CL
				<i>L. amazonensis</i>	
				<i>L. garhansi</i>	
				<i>L. pifanoi</i>	
		<i>L. tropica</i>	<i>L. tropica</i>		
			<i>L. Killicki</i>		
		<i>L. major</i>	<i>L. major</i>		
		<i>L. aethiopica</i>	<i>L. aethiopica</i>		
		VIANNIA	<i>L. braziliensis</i>	<i>L. braziliensis</i>	Mucocutaneous Leishmaniasis MCL
				<i>L. peruviana</i>	
<i>L. lainsoni</i>	<i>L. lainsoni</i>				
<i>L. guyanensis</i>	<i>L. guyanensis</i>				
		<i>L. panamensis</i>			

Adapted from Bañuls et al 2007

Although no official data is available, it is estimated that the population at high risk of contracting some form of leishmaniasis in Ethiopia, is about 30 million inhabitants and the total number of cases diagnosed as positive, each year, is estimated at around 20,000 to 30,000, with 25% of cases of VL (*L. donovani*) and 75% of cases of CL (*L. aethiopica*), the latter comes in three different clinical forms (LCL, MCL and DCL). LCL injuries by *L. aethiopica*, do not form open sores, as with strains in the New World, (*L. mexicana* complex). The injuries caused by *L. aethiopica* are closed (nodules) and is often at the site of vector inoculation. Although, occasionally, severe and persistent LCL, MCL and DCL cause disfiguring and often require prolonged treatment schemes with very low success rate. In the case of DCL, definitive cure is almost never achieved, and relapse is common (Hailu et al. 2006; Lemma et al. 1969; Bryceson 1969 and Sarojini 1984).

In the area of Pharmaceutical Chemistry at the IIFB (AQF-IIFB), at the Faculty of Pharmaceutical and Biochemical Sciences, UMSA, within the "Project UMSA-SIDA Infections Diseases: Evanta in the Treatment of cutaneous leishmaniasis", we have produced Evanta based treatments (creams and syrups) (Tacana 1999 and Beatriz 2006), and the effectiveness assessed in a clinical trial (Phase II, treatment of CL), in a comparative treatment versus Glucantime. The study has been carried out in the Hospital of Palos Blancos, from June 2007 to December 2012, with patients diagnosed with CL caused by *L. amazonensis* and *L. braziliensis*. A total of 60 individuals (patients with primary infection, over 18 and under 50 years old, as inclusion criteria), were treated, the results of our studies of comparative efficacy for Evanta (29 patients, creams and syrups) are bordering the 68% cure of patients and over 90% cure of patients treated with Glucantime (31 patients, intramuscular) (Magariños 2013).

Based on the information gathered in the last 10 years, from field and clinic experiences developed in Bolivia, and adding research results from our laboratories, at IIFB, using Evanta (CAT), we can suggest that our experiences of validation of traditional medicines, for the treatment of cutaneous leishmaniasis, could be replicated in Ethiopia (OMS, 2013), where there are reported up to ten times more new cases of leishmaniasis per year, compared to the estimated 2,500 cases per year, in Bolivia.

MATERIALS Y METHODS

Leishmania aethiopica strains from AHRI

The *Leishmania aethiopica*, strains were donated by AHRI, these were obtained from patients ulcers, from a total of ten strains received, six were able to adapt to *in vitro* cultures at IIFB and were used in the present study.

Strain No	AHRI Code	Clinical Form	Place of lesion
1	396/04	LCL	Right Cheek
2	799/04	LCL	Left cheek
3	1116/03	LCL	Left cheek
5	1475/03	LCL	Left eye lid
7	1502/03	LCL	Nose
8	1540/03	LCL	Nose

Reference *Leishmania* strains at IIFB

Leishmania braziliensis (M2904 C192 RJA) and *Leishmania amazonensis* (Clon 1 NHOM-BR-76-LTB-012). *Leishmania lainsoni* (INL 125-11)

Drugs preparation.

The drugs used in the study were Amphotericin-B (ANFOTERICIN-CRITÁLIA), Miltefosine (IMPA VIDO) and Total Alkaloids from *Galipea longiflora* (CAT-IIFB). Amphotericin-B was diluted in Schneider medium (1mg/mL) and dilutions with concentrations ranging from 0.62 to 0.01µg/mL were prepared. Miltefosine and CAT were diluted with DMSO (10mg/mL), and dilutions with concentrations ranging from 100 to 1.5µg/mL were prepared. Final concentration of DMSO was kept below 1%.

Leishmanicidal test

The activity was measured on *in vitro* cultures of the *Leishmania* parasite in promastigote forms, (modified from Salamanca et al 2008), cultivated at 26°C in Schneider medium (pH 6.8) supplemented with inactivated (56°C x 30min) calf bovine serum (10%). Parasites in logarithmic phase of growth, at a concentration of 1×10^6 parasites/mL, were distributed on a 96 micro well plates and the different concentration of the drugs were added. The micro well plates were incubated for 72hrs at 26°C. After incubation, a solution of XTT (1mg/mL) in PBS (pH 7.0 at 37°C) with PMS (Sigma-Aldrich, 0.06mg/mL), was added (50µL/well), and incubated again for 4hrs at 26°C. DMSO (1%) and Anphotericine B (0.5 mg/mL) were used as reference drugs during the evaluations, were done by sextuplicate. Optical density of each well was obtained with a Synergy HT microplate reader with I 200-450nm. The IC₅₀ values were calculated using The Gen5 program (Biotek).

RESULTS

Because *Leishmaniasis* is a neglected disease, lack of attention also happens with research and development of new diagnostic tools, drugs or vaccines, because there is no market interested in social groups suffering from the disease (WHO). The misuse and mismanagement of drugs can lead to the emergence of resistance forms (Gil et al. 2007) such is the case with Glucantime, which is well documented in countries like India (Osorio et al 2005), Brazil (Nascimento-Zauli et al. 2010) and Bolivia (10% of treatment failure, Bermudez et al. 2006) and such behavior has been observed within patients with visceral L. coinfecting with HIV (Koert et al 2011). While in a clinical trial using Miltefosine, in Palos Blancos, Bolivia, only 50% of patients with MCL were cured (Soto et al. 2007).

The significant adverse effects reported, such as myalgia, arthralgia, anorexia, nausea and headache and the high costs, in the case of antimonials, stimulate that many patients do not conclude the treatment, favoring the reactivation of the injury, mucous damage and the emergence of drug resistance

(Clem A, 2010 and Bhandari et al. 2012). The disadvantages associated with the available drugs and the variability of response to those by *Leishmaniasis* species, have evidenced the urgent need to develop alternative treatments and monitor treatment efficacy and emerging resistance to drugs in use

The medicinal plant *Galipea longiflora* (Evanta) is used in the treatment of cutaneous leishmaniasis by different Amazonian ethnic groups, in Bolivia, and we evaluated the response of six *Leishmania* aethiopic strains, being the species responsible of the different forms of cutaneous leishmaniasis in Ethiopia, and compared with the response observed for the reference strains at IIFB, when the different strains were exposed to the total alkaloids (CAT) of Evanta. The results are shown on Table 2.

Table 2. Susceptibility test of *Leishmania* strains against Amphotericin B, Miltefosine and CAT

Strain/Isolate	IC ₅₀ (Mean ± SD)		
	CAT	Amphotericin B	Miltefosine
<i>L. aethiopic</i> 1	8.23±2.16	0.08±0.01	2.43±1.1
<i>L. aethiopic</i> 2	8.68±1.59	0.19±0.20	2.57±1.41
<i>L. aethiopic</i> 3	6.9±2.16	0.44±0.12	1.57±0.06
<i>L. aethiopic</i> 5	14.37±3.58	0.22±0.11	3.7±2.39
<i>L. aethiopic</i> 7	8.33±0.90	0.11±0.11	1.6±0.05
<i>L. aethiopic</i> 8	11.27±0.97	0.27±0.04	1.67±0.06
<i>L. braziliensis</i> (M2904)	11.73±4.32	0.39±0.06	7.5±1.48
<i>L. amazonensis</i> (Lma)	12.28±2.95	0.33±0.04	6.7±0.6
<i>L. lainsoni</i> (INL 125-11)	9.70±1.41	0.22±0.02	3.5±0.35

DISCUSSIONS

In Bolivia leishmaniasis infection are caused mainly by *L. braziliensis* (CL and MCL) and *L. amazonensis* (CL) both species have a clear dominance in national epidemiological indices. While *L. lainsoni* (CL and MCL) and *L. chagasi* (VL) are presented in rare and few reports (Rojas E et al. 2009).

From the six strains of *L. aethiopic* included in the present studies, five showed homogeneous behavior against all drugs, with an average calculated value for IC₅₀=8.68 ± 1.56µg/mL, when exposed to CAT, a value somewhat inferior to the calculated for the reference strains *L. amazonensis*, and *L. braziliensis* with IC₅₀=11.73 ± 4.32µg/mL and IC₅₀=12.28 ± 2.95µg/mL, respectively, but closer to the *L. lainsoni*, IC₅₀=9.7 ± 1.45 µg/mL. (except *L. aethiopic* 5, with values somewhat superior to the other strains consistently through our studies: IC₅₀ = 14.37 ± 3.58µg/mL against CAT and Miltefosine IC₅₀=3.7 ± 2.39µg/mL).

All six *L. aethiopic* showed homogeneous behavior against Amphotericin B (average IC₅₀=0.22±0.02µg/mL) somewhat lower values when compared with the reference strains *L. braziliensis* (IC₅₀=0.39±0.06µg/mL) and *L. amazonensis* (IC₅₀=0.33±0.04µg/mL), a similar trend is observed when compared all *L. aethiopic* strains against Miltefosine (IC₅₀=2.26±0.76µg/mL) with *L. brazi-*

liensis and *L. amazonensis* ($IC_{50}=7.5\pm 1.48\mu\text{g/mL}$, and $IC_{50}=6.7\pm 0.6\mu\text{g/mL}$, respectively), Interesting to notice that susceptibility values for the *L. aethiopica* strains are much closer to the values calculated for the less common *L. lainsoni* strain in Bolivia ($IC_{50}=0.22\pm 0.02\mu\text{g/mL}$ and $IC_{50}=3.5\pm 0.35\mu\text{g/mL}$, respectively).

The Instituto de Investigaciones Fármaco Bioquímicas (IIFB) belonging to the Faculty of Biochemical and Pharmaceutical Sciences at UMSA, has been working in the analytical (Herrera et al. 2008 and Limachi 2013) chemical (Llanos et al. 2009; Ticona 2005 and 2008; Espinoza 2012) biological (Salamanca 2008 and Herrera 2008) and pharmaceutical formulations (Rodríguez 2006) assessments of the total alkaloids (CAT) produced by Evanta (*Galipea longiflora*) since 2000 (Gimenez et al. 2005). Moreover, in collaboration with researchers from Brazil, through the CYTED Ibero-american research network, CAT gastro-protective et al. 2009) and anti-nociceptive (Campos-Buzzi et al 2010) effects were assessed, and results on *G. longiflora* shows significant inhibition of ulcer formation by decreasing gastric secretion and increase gastric mucus content. Therefore, the IIFB, has produced Evanta based treatments which were used in a clinical study on patients diagnosed with LC, caused by *L. amazonensis* and *L. braziliensis*, in Palos Blancos Hospital (2007-12), with efficacy bordering the 68% cure of patients.

CONCLUSIONS

Our *in vitro* studies developed at UMSA by researchers from IIFB and AHRI, using strains of *L. aethiopica* and different local leishmania spp strains circulating in Bolivia, against total alkaloids of Evanta (CAT), have shown that *L. aethiopica* parasites (media $IC_{50}=6.93\pm 1.89$) and the Bolivian strains (media $IC_{50}=11.24\pm 2.89$) are sensitive to Evanta alkaloids, suggesting that our experience in clinical validation, previously carried out in Bolivia, could be replicated in Ethiopia, where an estimated of 20,000 to 30,000 new cases of leishmaniasis are reported each year.

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REFERENCES

- Alvar J, Bashaye S, Argaw D, Cruz I, Aparicio P, et al. (2007) Kala-azar outbreak in Libo Kemkem, Ethiopia: epidemiologic and parasitologic assessment. *Am J Trop Med Hyg* 77: 275–282
- Ardaya Daza Cecilia Lorena (2015) ESTUDIO MOLECULAR DE ESPECIES DE *Leishmania* spp." "Tesis para optar al título de Magister Scientiarum en Ciencias Biológicas y Biomédicas, UMSA, Bolivia.
- Ayele T, Ali A (1984) The distribution of visceral leishmaniasis in Ethiopia. *Am J Trop Med Hyg* 33: 548–552.
- Desjeux P (2004) *Leishmaniasis*: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 27: 305–18.

- Bañuls AL, Hide M, Prugnolle F. (2007) *Leishmania* and the leishmaniasis a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. *Adv Parasitol*; 64:1-109.
- Bermudez H, Rojas E, Garcia J, Desjeux P, Dujardin J.C, Boelaert M, Chappuis F. (2006). Generic sodium stibogluconate is as safe and effective as branded meglumine antimoniate, for the treatment of tegumentary leishmaniasis in Isiboro Secure Park, Bolivia. *Annals of Tropical Medicine and Parasitology*:100:591-600.
- Blackwell JM, Fakiola M, Ibrahim ME, Jamieson SE, Jeronimo SB, Miller EN, Mishra a, Mohamed HS, Peacock C, Raju M, Sundar s, Wilson ME. (2009) Genetics and visceral leishmaniasis of mice and man. *Parasite Immunol*: 31:254-66.
- Bryceson AD (1969). Diffuse cutaneous leishmaniasis in Ethiopia. I. The clinical and histological features of the disease. *Trans R Soc Trop Med Hyg* 63:708-737
- Campos-Buzzi F, M. Fracasso, BK Clasen, JC Ticona, A. Giménez and V. Cechinel-Filho. (2010) Evaluation of antinociceptive effects of *Galipea longiflora* alkaloid extract and major alkaloid 2-fenilquinoline. *Methods and Findings in Experimental and Clinical Pharmacology*, 32 (10): 707-711.
- Espinoza Cruz Boris Mayko (2012). "Estudio de Plantas Antiparasitarias de Farmacopeas Tradicionales de Bolivia "Galipea longiflora Krause y Piper hispidum Swartz" Tesis de Maestría en Ciencias Biológicas y Biomédicas, Mención BIOLOGÍA DE POBLACIONES, UMSA
- García AL, Parrado R, Rojas E, Delgado R, Dujardin JC, Reithinger R (2009). *Leishmaniasis* in Bolivia: Comprehensive review and current status. *Am J Trop Med Hyg* 80:704-711
- Gil Eric, Cunha Luiz, Goncalves Aurèlia, Souza Aparecido, Negron Valderrama (2007). Importancia de los Compuestos Inorgánicos en el Tratamiento de la *Leishmaniasis*. *Latin American Journal of Pharmacy*. 26 (3): 454-461.
- A. Giménez, G. Ruiz, J.A. Avila, et al. (2005) Estudios químicos, biológicos y farmacológicos de *Galipea longiflora*, Krause. *Revista Boliviana de Química*. Vol 22 No 1, 94-107.
- Herrera Vania, Juan Carlos Ticona, Enrique Udaeta, Rogelio Chuqui, Alberto Giménez (2008) "Validación del método analítico para la cuantificación de alcaloides quinolinicos del extracto de *Galipea longiflora* Krause" *BIOFARBO*; Vol 16, 47 - 53
- Herrera Choque Vania Cecilia (2008). Toxicidad de extractos de alcaloides totales de la *Galipea longiflora* Krause Kallunki (Evanta) en fase preclínica. Tesis de Maestría en Ciencias Biológicas y Biomédicas, Mención PARASITOLOGIA. UMSA
- INLASA (2012): Norma de vigilancia y control de leishmaniasis en Bolivia.
- Koert Ritmeijer, Rachel ter Horst, Solomon Chane, Endashaw Mengistu Aderie, Turid Piening, Simon M. Collin, Robert N. Davidson (2011). Limited Effectiveness of High-Dose Liposomal Amphotericin B (AmBisome) for Treatment of Visceral *Leishmaniasis* in an Ethiopian Population With High HIV Prevalence. *Clinical Infectious Diseases* 53:152-58.
- Lemma A, Foster WA, Gemetchu T, Preston PM, Bryceson A, Minter DM (1969). Studies on leishmaniasis in Ethiopia. I. Preliminary investigation into the epidemiology of cutaneous leishmaniasis in the highlands. *Ann Trop Med Parasitol*, 63:455-472
- Limachi Valdez Ivan (2013) "Análisis químico cuantitativo y el uso como antiparasitario del extracto etanólico de *Galipea longiflora* (Evanta)" Tesis de Maestría en Ciencias Biológicas y Biomédicas, Mención PRODUCTOS NATURALES. UMSA
- Llanos Medina Fabiola, Boris Espinoza Cruz, Efraín Salamanca Capusiri, Rogelio Chuqui, Ninoska Flores Quisbert, Alberto Giménez Turba (2009). "Extracción Acuosa de Corteza de *Galipea longiflora* y su actividad *Leishmaniacida*" *BIOFARBO*; Vol 17 (2), 32 - 38
- Magariños Walter (2013). MD Responsable Proyecto Enfermedades Infecciosas comunicación personal
- Lyons S, Veeken H, Long J (2003) Visceral leishmaniasis and HIV in Tigray, Ethiopia. *Trop Med Int Health* 8: 733-739
- Martinez E, Mollinedo S, Torrez M, Muñoz M, Bañuls AL, Le Pont F (2002). Co-infection by *Leishmania amazonensis* and *L. infantum* chagasi in a case of diffuse cutaneous leishmaniasis in Bolivia. *Trans R Soc Trop Med Hyg*; 96:529-532.
- Mollinedo S, Torrez M, Holguin E, Vargas F (2000). *Leishmaniasis* en Bolivia. www.galenored.com
- Nascimento-Zauli Rogèria, Miguel Danilo, Yokoyama-Yasuyama Jenicer, Pereira Ledice, Pelli de Oliveira Milton, Ribeiro-Dias Fátima, Dorta Mirian, Uliana Silvia (2010). In vitro sensitivity of *Leishmania* (Viannia) *braziliensis* and *Leishmania* (*Leishmania*) *amazonensis* Brazilian isolates to meglumine antimoniate and amphotericin B. *Tropical Medicine And International Health*. 15: 68-76.
- Osorio Edison, Robledo Sara, Arango Gabriel, Muskus Carlos (2005). *Leishmania*: papel de la glicoproteína P en la medición de la resistencia a medicamentos y estrategias de reversión. *Biomedica* 25:242-260.
- Rodríguez Olguín Beatriz Amparo (2006). "Estudio de Preformulación en una forma farmacéutica semisólida de uso tópico para el extracto orgánico y concentrado de alcaloides totales de la especie *Galipea longiflora* Krause "Evanta". Tesis de Maestría en Tecnología Farmacéutica y Control de Calidad de Medicamentos. FCFYB UMSA. Tutores: Alberto Giménez Turba y Francisco López Naranjo
- Rojas E, Parrado R, Delgado R, Reithinger R, García AL (2009). *Leishmaniasis* in Chaparé, Bolivia. *Emerging Infectious Diseases*.; 15 (4): 678-680

- Salamanca Capusiri Efrain, Grace Ruiz Pinnell, Juan Carlos Ticona Huallpara, Alberto Giménez Turba (2008) "Método colorimétrico – XTT: como evaluación de alto rendimiento de sustancias con actividad leishmanicida", BIOFARBO; Vol 16, 21 – 27
- Salamanca Capusiri Efrain (2008). Actividad antiparasitaria múltiple de los alcaloides totales de corteza de *Galipea longiflora* Krause Kallunki (Evanta) Tesis de Maestría en Ciencias Biológicas y Biomédicas, Mención PARASITOLOGÍA. UMSA.
- Sarojini PA, Humber DP, Yemane-Berhan T, Fekete E, Belehu A, Mock B, Warndorff JA (1984). Cutaneous leishmaniasis cases seen in two years at the All Africa Leprosy and Rehabilitation Training Centre Hospital. *Ethiop Med J* 22:7-11
- Soto J, Toledo J, Valda L, Balderrama M, Rea I, Parra R, Ardiles J, Soto P, Gomez A, Molleda F, Fuentelsaz C, Anders G, Sindermann H, Engel J, Berman J. (2007). Treatment of Bolivian Mucosal *Leishmaniasis* with Miltefosine. *Clinical Infectious Diseases*;44:350–356.
- "TACANA: ECUANASHA AQUÍ, ECUANASHA ID'RENE CUANA, ME SCHANAPA-QUE" (TACANA: Conozcan nuestros árboles, nuestras hierbas). (1999) Editorial Plural La Paz, Bolivia Editores. Responsables: G. Bourdy-IRD, A. Giménez-UMSA y Celin Quenevo-CIPTA Autores: Varios
- Ticona Huallpara Juan Carlos (2005) "Estudio comparativo de métodos de extracción de alcaloides quinolinicos de la *Angostura longiflora* K. Kallunki (Evanta) con actividad leishmanicida". Tesina de Licenciatura en Bioquímica, UMSA.
- Ticona Huallpara Juan Carlos (2008). Estudio químico de dos plantas bolivianas de la Etnia Tacana: *Galipea longiflora* y *Bowdichia virgilioides*" Tesis de Maestría en Ciencias Biológicas y Biomédicas, Mención BIOLOGÍA DE POBLACIONES. UMSA
- Tsegaw T, Gadisa E, Seid A, Abera A, Teshome A, et al. (2013) Identification of environmental parameters and risk mapping of visceral leishmaniasis in Ethiopia by using geographical information systems and a statistical approach. *Geospat Health* 7: 299–308
- Zanatta F, Gandolfi RB, Lemos M, Ticona JC, Gimenez A, Clasen BK, et al. (2009) Gastroprotective activity of alkaloid extract and 2-phenylquinoline obtained from the bark of *Galipea longiflora* Krause (Rutaceae). *Chem Biol Interact.* 180(2): 312-17.
- WHO (2013) *Leishmaniasis*, Fact sheet N°375: <http://www.who.int/mediacentre/factsheets/fs375/en/>.
- WHO (2010) Control of the leishmaniasis. WHO Tec Rep Ser 949: 1–186.
- WHO http://www.who.int/gho/neglected_diseases/leishmaniasis/en/.