Pharmacogn J. 2022; 14(6) Suppl: 973-977
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Antiparasitic effect of *Psidium guajava* on promastigotes and axenic amastigotes of *Leishmania*

Jesús Rojas-Jaimes[1,2,*], Marco Mesía-Guevara[1], Maria Rojas-Puell[1], Luis Castañeda- Pelaez[2]

ABSTRACT

Background: Leishmaniasis is a stigmatic and mutilating disease due to pathogenic species of the genus Leishmania which, depending on the species and the individual's immune status, may vary clinically from a cutaneous, mucosal, and visceral form, and for which there is no suitable treatment without significant side effects. **Objectives:** To measure the effect of ethanolic and aqueous extracts of *Psidium guajava* against axenic promastigotes and amastigotes of *Leishmania spp.* **Methods:** The method of [3-(3,4 -dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] was used to study the antiparasitic effects of ethanolic (100mg/mL) and aqueous (100mg/mL) extracts of *Psidium guajava* on axenic amastigotes cultures (8.1 x10^3 parasite/mL) and promastigotes (12 x 10^4 parasite/mL) obtained from a patient with cutaneous leishmaniasis, and the percentage of parasite death was evaluated in comparison with Glucantime (300mg/mL) and untreated parasite cultures. **Results:** Regarding parasite death in promastigotes, the ethanolic and aqueous extracts had a percentage of 22.58% and -45.16%, respectively, with no significant difference between treatments (N=3) (p= 0.058). In contrast, the ethanolic and aqueous extracts had an antiparasitic percentage of 91.67% and -70.83%, respectively, with a significant difference between treatments (N=3) (p<0.05). **Conclusions:** Our study showed high and significant effectiveness in parasite death (91.67%) of Leishmania axenic amastigotes of the ethanolic extract (100mg/mL) of *Psidium guajava*, being this result promising and the basis for in vivo studies, using the ethanolic extraction of *P. guajava*.
Key words: *Leishmania, Psidium guajava*, Promastigote, Amastigote.

Jesús Rojas-Jaimes[1,2,*], Marco Mesía-Guevara[1], Maria Rojas-Puell[1], Luis Castañeda- Pelaez[2]

[1]Facultad de Ciencias de la Salud, Universidad Científica del Sur, Lima, PERU.

[2]Facultad de Ciencias de la Salud, Universidad Privada del Norte, Lima, PERU.

Correspondence

Jesús Rojas-Jaimes

Facultad de Ciencias de la Salud, Universidad Científica del Sur, Universidad Privada del Norte, Lima, PERU.

E-mail: jesus.rojas.jaimes@gmail.com

History

- Submission Date: 13-10-2022;
- Review completed: 28-11-2022;
- Accepted Date: 04-12-2022.

DOI : 10.5530/pj.2022.14.198

Article Available online

http://www.phcogj.com/v14/i6

INTRODUCTION

Leishmaniasis is a disease that almost always has a chronic course, and is caused by the protozoan of the genus *Leishmania* being a difficult disease to treat because it is not easy to diagnose, the use of medication with considerable adverse effects. Treatments have serious drawbacks regarding resistance, stability, safety, and low tolerance, in addition to the traces that remain in the patient who has suffered from this disease and the co-infections that occur in the course of the disease, affecting their self-esteem.[1]

The classification of tegumentary leishmaniasis in Peru is: Andean cutaneous leishmaniasis, known as "uta"; Andean cutaneous-mucosal leishmaniasis; forest cutaneous leishmaniasis; and forest cutaneous-mucosal leishmaniasis, known as "espundia".[2]

"UTA" in Peru is produced by the agent *Leishmania peruviana*.[3,4] *Leishmania braziliensis*, which is resistant to antimonials, causes destructive damage to the skin and mucosa.[3,4] Treatment with antimonials and glucantime has side effects, which makes it difficult for the patient to adhere to the scheme since it has nephrotoxic and hepatotoxic consequences, as well as aplastic anemia.[3]

There are drugs used as first-line treatment for leishmaniasis, called antimonials. However, this treatment may cause side effects inherent to the medication that sometimes make people abandon the treatment.[5-7] However, it is still necessary to find new molecules with biological activity that can work as a treatment for such infection, and plant extracts and metabolites are a promising option. The therapeutic use of medicinal plants supports pharmaceutical research to combat various pathogens. In this sense, *P. guajava* is a tree belonging to the Myrtaceae family, which has its origin in Latin America, has been used as a food and therapeutic agent, the root, bark, leaves, fruits, flowers and seeds are used for medicinal purposes, particularly in infusions and decoctions for use topical and oral using it in the treatment of diseases caused by the activity of pathogenic and/or opportunistic microorganisms, describing its use as an antidiarrheal, against Gram +, Gram negative bacteria and *Candida albicans*.[8-12] A previous study reported in *P. guajava* phenolic components, tannins, flavonoids, triterpenoids, saponins, glycosides.[13] Additionally, *P guajava* is a commercial plant for its fruit.[14]

Previous studies have concluded that to extrapolate *in vitro* assays of substances against leishmaniasis, samples should be used in which the parasite is in a similar condition to when it infects humans, as "amastigote" form.[6,7] Promastigotes, which have a different function and form than the parasite found in mammals, can be used as a comparison group. In addition, working with intracellular amastigotes results in complex and costly procedures, and their application is often limited.[7]

Through *in vitro* techniques, it has been possible to develop axenic amastigotes with free-living characteristics in the form of artificial cultures, which can have functions and forms similar to those of the parasites found in human cells. For this reason, this is useful to identify new substances against leishmaniasis.[7] Therefore, this study aims to measure

Cite this article: Rojas-Jaimes J, Mesía-Guevara M, Rojas-Puell M, Castañeda-Pelaez L. Antiparasitic effect of *Psidium guajava* on promastigotes and axenic amastigotes of *Leishmania*. Pharmacogn J. 2022;14(6)Suppl: 973-977.

the effect of ethanolic and aqueous extracts of *Psidium guajava* against axenic promastigotes and amastigotes of *Leishmania spp*.

MATERIAL AND METHODS

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 µL in 96 microplate wells at a concentration of 2.34 x 10^6/mL in 300 µL 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 - 5.7 - 6.2) at 35 ºC, 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 µL 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 µL/ number of parasites observed in 10 µL * 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 µL of culture medium with parasites (axenic amastigotes at evaluated pHs) was taken and added to 300 µL 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/µL on day 7, up to 300/µL 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.

Obtaining botanical material

Two kilograms of fresh leaves of *Psidium guajava* 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. Subsequently, a sample of the plant with leaves and fruit was taken to the natural history museum for taxonomic classification as *Psidium guajava*.

Obtaining ethanolic and aqueous extracts of *P. guajava*

The leaves were separated from the stems and fruits, later they were left to dry in the open air and once dry they were cleaned with a paper towel and the leaves were crushed manually. Finally, it was weighed, obtaining a total of 450 grams. After selecting the leaves, 450 grams of material were obtained and separated into two equal parts of 225 grams for maceration. For the ethanolic extract, 2L of 70° alcohols were added in a glass container and externally wrapped with a black bag. Then, the leaves were left to macerate for 7 days and the container was stirred daily for 10 minutes. 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 macerate was successively filtered 9 times using rough filter paper with a porosity of 12 µm. 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 µL for the control group and at a ratio of 20 µL/100µL of the drug concerning the Schneider medium with an initial inoculum for promastigotes of 12 x 10^4 parasite/mL and axenic amastigotes of 8.1 x10^3 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 *P. guajava* extracts, ethanolic (100mg/mL) at a final concentration of 8.33 mg/mL, and aqueous (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 10µl 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 µl 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. To take the final readings, the readings of the pure extracts of *P. guajava* and the Schneider medium were subtracted from the readings of the treatments.

Statistical analysis

A one-way analysis of variance (ANOVA) with a significance level of 95%, $p<0.05$, was performed for a comparison between the drugs used and the control groups for both axenic amastigotes and promastigotes. The Bonferroni algorithm was used to determine the specific difference between treatments, using SPSS version 28.

Cell viability was calculated using the formula: (OD treatment /OD negative control) x 100.

RESULTS

Leishmania promastigotes obtained in biphasic medium in 14 days, *in vitro*, at 25°C, in an RPMI medium at pH 7 and *Leishmania* axenic amastigotes in Schneider medium at pH 4.7 at 35 ºC on the 56th day are shown. (figure 1).

The results of the analysis of variance (ANOVA) between treatments on axenic amastigotes showed a significant difference ($p < 0.05$), while for promastigotes no statistically significant difference was found between treatments ($p = 0.58$) (table 1, table 2).

DISCUSSION

Our study on axenic amastigotes showed that there was a significant difference ($p< 0.05$) between all treatments, emphasizing the significant reduction of parasite viability (91.67%) in the treatment of the ethanolic extract of *P. guajava* using the drug Glucantime as a positive control (62.5%), and this difference was significant.

In the case of treatments against promastigotes, there was no significant difference between ethanolic and aqueous extracts concerning the drug Glucantime used as a positive control. These differences between axenic amastigotes and promastigotes are due to the different physiology of the parasite in its different phases as has been demonstrated in a study

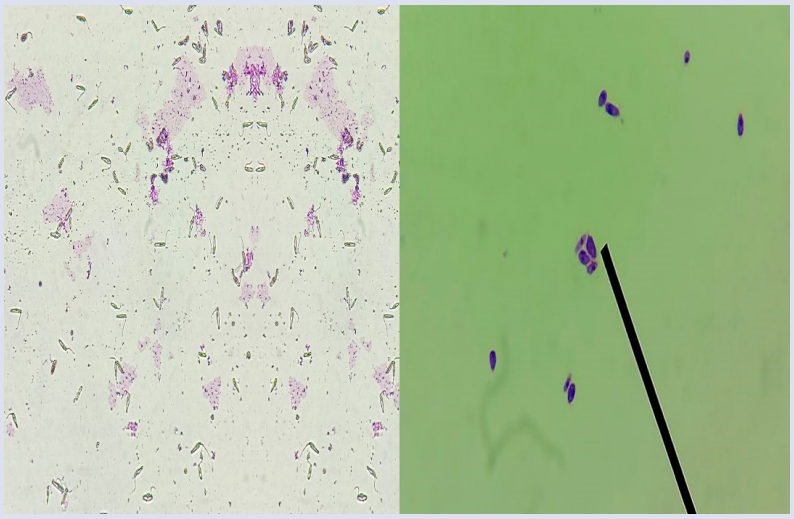

Figure 1: From left to right, the *Leishmania* promastigotes are observed, followed by the figure on the right belonging to the *Leishmania* axenic amastigotes.

Table 1: Descriptive statistics and statistical significance between treatments against *Leishmania* axenic amastigotes.

	N	Median	Standard deviation	Cell viability-Cell death	(I) Treatments		Sig.
Negative control (8,1 x10^3 parasite/mL)	3	0.2387	0.03500	100% (0%)	Negative control (culture)	Positive control	0.001
Positive control (Glucantime 300mg/mL)	3	0.0943	0.01595	37.5% (62.5%)		P.g. A	0.000
Aqueous *P. guajava* (100mg/mL)	3	0.4143	0.02754	170.83% (-70.83%)		P.g. E	0.000
Ethanolic *P. guajava* (100mg/mL)	3	0.0187	0.01629	8.33% (91.67%)	Positive control (Glucantime)	Negative control	0.001
						P.g. A	0.000
						P.g. E	0.036
					Aqueous *Psidium guajava*	Negative control	0.000
						Positive control	0.000
						P.g. E	0.000
					Ethanolic *Psidium guajava*	Negative control	0.000
						Positive control	0.036
						P.g. A	0.000

Unimodal Analysis of Variance (ANOVA), $p < 0.05$, there was a significant difference between all treatments (N=3).

*P.g E= Ethanolic extract of *Psidium guajava*

* P.g A= Aqueous extract of *Psidium guajava*

Table 2: Descriptive statistics and statistical significance between treatments against *Leishmania* promastigotes.

	N	Median	Standard deviation	Cell viability-Cell death	(I) Treatments		Sig.
Negative control (12 x 10^4 parasite/mL)	3	0.3127	0.02159	100 % (0%)	Negative control	Positive control	1.000
Positive control (Glucantime 300mg/mL)	3	0.2153	0.12848	70.97 (29.03%)		P.g.A	0.658
Aqueous *P. guajava*. (100mg/mL)	3	0.4443	0.09336	145.16 (-45.16%)		P.g.E	1.000
Ethanolic *P. guajava* (100mg/mL)	3	0.2490	0.08022	77.42 (22.58%)	Positive control	Negative control	1.000
						P.g.A	0.084
						P.g.E	1.000
					Aqueous *Psidium guajava*	Negative control	0.658
						Positive control	0.084
						P.g.E	0.170
					Ethanolic *Psidium guajava*	Negative control	1.000
						Positive control	1.000
						P.g.A	0.170

Unimodal Analysis of Variance (ANOVA), p= 0.058, there was no significant difference between treatments (N=3).

* P.g E= Ethanolic extract of *Psidium guajava*

* P.g A= Aqueous extract of *Psidium guajava*

where the essential oil of *Matricaria chamomilla* was evaluated against *L. braziliensis* and *L. panamensis*.[15] An interesting phenomenon shown in aqueous extracts against axenic amastigotes and promastigotes was that the extracts stimulated cell proliferation at 170.83% and 145.16%, respectively.

Plants are important sources of active principles against *Leishmania* as shown by a study in Pakistan that reported that 23 plants were tested, using mostly leaves of the genus *Lamiaceae* and, out of these plants, only 11 were tested *in vitro* with good results regarding inhibition of the parasite.[16] Another study which also mostly used leaves indicated that among the plants most used by folk medicine to treat leishmaniasis were *Carica papaya (Caricaceae), Cedrela odorata (Meliaceae), Musa paradisiaca (Musaceae)*, and *Nicotiana tabacum (Solanaceae)*.[17] A study in Mexico showed that dichloromethane and dichloromethane/methanol extracts of *Schinus molle*, dichloromethane of *Lantana camara*, and aqueous extracts of *Prosopis laevigata* had an inhibitory effect on *Leishmania amazonensis*, although this study also tested aqueous, dichloromethane and dichloromethane/methanol extracts of the leaves and branches of *P. guajava* against *L. amazonensis*; and no active or specific effect was observed.[18] Another study on promastigotes and amastigotes of *L. amazonensis* showed that the hydroalcoholic extract of *P. guajava* leaves at 100 µg /mL reduced growth by 65.4% and 52%, respectively.[19] Another species of the Psidium genus, P. salutare, showed a limited inhibition effect on the growth of promastigotes of *L. amazonensis*.[20]

Specific metabolites such as kaempferol, a flavonol found in different plants such as *Brassica oleracea* and *Hamamelis virginiana,* have shown to be effective against axenic amastigotes and amastigotes in macrophages of *L. donovani*.[21] Previous studies report the use of *P. guajava* in traditional medicine against leishmaniasis in local communities where the plant grows.[22-26]

Another study on the metabolites of *P. guajava* leaves against the axenic amastigotes and promastigotes of *L. infantum* showed parasite activity mainly in the apolar fractions, and the crude ethanolic extract at 25 µg/mL against axenic amastigotes inhibited 50% of the parasites in the crude extract and up to 80% in the apolar fractions as triterpenoids including jacumaric acid and corosolic acid, while, at 25 µg/mL and 50 µg/mL in promastigotes, no activity was observed in either the polar or apolar fractions.[27] This study confirms this previous study in which the ethanolic extract had a significant inhibitory effect on axenic amastigotes with 91.67% inhibition compared to 22.58% inhibition on promastigotes.

Other studies in Brazil tested the use of aqueous and hydroethanolic extracts of *P. guajava* and *P. brownianum* against promastigotes of *L. braziliensis* and *L. infantum*. Only the hydroethanolic extracts at 1000 µg/mL of *P. brownianum* had antiparasitic effectiveness on *L. braziliensis* (58.46%) and *L. infantum* (37.16%).[28] In this study, it was confirmed that the ethanolic extract of *P. guajava* did not have a significant efficiency on cell death, which was 22.58%. Additionally, cytotoxicity of the ethanolic extract of *P. guajava* leaves was tested and its low toxicity on fibroblasts was demonstrated, indicating that only high concentrations of the extracts would be the most effective (1000 µg/mL) and that the ethanolic extract was more effective than its metabolic fractions, so the metabolites could synergize in their parasitic activity against the promastigotes of *L. brazilensis* and *L. infantum*.[29] Therefore, this study was conducted to test doses of 100 mg/mL to evaluate the antiparasitic activity. Additionally, previous studies have shown parasitic inhibition of 75% and 90% for *L. infantum* and *L. donovani* promastigotes, respectively,[29,30] although it is known that the promastigote model is not suitable for testing antiparasitic drugs because it is a model that is not found in mammalian infection. Other species of the genus *Psidium* such as the ethanolic extract of *P. brownianum* leaves and the essential oils of *P. myrsinites* leaves have been tested against *L. braziliensis* and *L. infantum* promastigotes with moderate antiparasitic effects, reaching up to 81.34% antiparasitic activity.[29,31] In contrast, in this study, the ethanolic extract (100mg/mL) of *P. guajava* leaves reached an antiparasitic effect of 22.58% in promastigotes, although in axenic amastigotes it reached 91.67% of parasitic death.

CONCLUSION

Our study showed that the ethanol extract of *P. guajava* (100mg/mL) had a high percentage of antiparasitic effect against *Leishmania* axenic amastigotes (91.67%) which is a better model compared to promastigotes not found in mammals. This antiparasitic percentage (91.67%) was statistically and significantly higher than the antiparasitic percentage of the control drug Glucantime (300mg/mL) (62.5%). Therefore, the antiparasitic effectiveness against *Leishmania* that causes cutaneous leishmaniasis is shown.

AUTHOR CONTRIBUTIONS

Data curation, JRJ, LCP, MRP; project administration, JRJ, MMG; supervision, JRJ; writing—original draft, JRJ, LCP, MRP.; writing—review and editing, JRJ. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research received no external funding.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Burstein Z. Leishmaniasis Tegumentaria en el Perú Clínica y Terapéutica. VIII Congreso Academia Nacional de Medicina. Arequipa. 2005.
2. Burstein Z, Guillén A. Leishmaniasis cutánea y mucocutánea (CIE-10 B55 1-B55-2)* en el Perú. Revista Peruana de Medicina Experimental Y Salud Pública. 2002;19(4):215-8.
3. Arevalo J, Ramirez l, Adaui V, Zimic M, Tulliano G, Miranda-Verástegui C. Influence of Leishmania (Viannia) species on the response to antimonial treatment in patients with American tegumentary leishmaniasis. J Infect Dis. 2007;195(12):1846-51.
4. Gomes C, Oliveira de Morais O, Roselino A, Aparecida de Paula N, Ataíde Ribeiro R. Complementary exams in the diagnosis of American tegumentary leishmaniasis. An Bras Dermatol. 2014;89(5):701-11.
5. Sifontes S. Descubrimiento racional de compuestos químicos para el tratamiento de la leishmaniosis y la enfermedad de Chagas. Universidad Complutense De Madrid Facultad De Farmacia Departamento De Parasitología. 2017.
6. Hodgkinson V, Herman R, Semprevivo L. Leishmania donovani: Correlation among assays of amastigote viability. Exp Parasitol. 1980;50(1):397-408.
7. Hodgkinson V, Herman R. In vivo Assay of Viability of Amastigotes of Leishmania donovani. J Parasitol. 1980;66(2):245-9.
8. Piña-Vázquez DM, Mayoral-Peña Z, Gómez-Sánchez M, Salazar-Olivo LA, Arellano-Carbajal F. Anthelmintic effect of Psidium guajava and Tagetes erecta on wild-type and Levamisole-resistant Caenorhabditis elegans strains. J Ethnopharmacol. 2017;202:92-6.
9. Gutiérrez RMP, Mitchell S, Solis RV. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2008;117(1):1-27.

10. Dubost JM, Phakeovilay C, Her C, Bochaton A, Elliott E, Deharo E, *et al*. Hmong herbal medicine and herbalists in Lao PDR: pharmacopeia and knowledge transmission. J Ethnobiol Ethnomed. 2019;15(1):1-15.
11. Sajjekhan S, Jha PK, Dakappa SS. Pharmacognostic evaluation of Psidium guajava Linn. leaves (Mrytaceae). Med Aromat Plant Sci Biotechnol. 2011;5:156-9.
12. Growther L, Sukirtha K. Phytochemical analysis and antimicrobial properties of leaves and Psidium guajava bark extracts. Asian J Pharm. 2018;24(7):318-23.
13. Morais-Braga MFB, Carneiro JNP, Machado AJT, Sales DL, dos Santos ATL, Boligon AA, *et al*. Phenolic composition and medicinal usage of Psidiumguajava Linn.: antifungal activity or inhibition of virulence? Saudi J Biol Sci. 2015;24(2):302-13.
14. Franzon RC, Campos LDO, Proença CEB, Sousa-Silva JC. Araçás do Gênero Psidium: principais espécies, ocorrência, descrição e usos. Embrapa Cerrados. 2009.
15. Ríos YR, Otero AJ, Muñoz DL, Echeverry M, Robledo SM, Yepes MA. Actividad citotóxica y leishmanicida in vitro del aceite esencial de manzanilla (Matricaria chamomilla). Rev Colomb Cienc Quím Farm. 2008;37(2):200-11.
16. Tariq A, Adnan M, Amber R, Pan K, Mussarat S, Shinwari Z. Ethnomedicines and anti-parasitic activities of Pakistani medicinal plants against Plasmodia and Leishmania parasites. Ann Clin Microbiol Antimicrob. 2016;15(1):52.
17. Passero LFD, Brunelli ES, Sauini T, Amorim Pavani TF, Jesus JA, Rodrigues E. The Potential of Traditional Knowledge to Develop Effective Medicines for the Treatment of Leishmaniasis. Front Pharmacol. 2021;12:690432.
18. Delgado-Altamirano R, Monzote L, Piñón-Tápanes A, Vibrans H, Rivero-Cruz JF, Ibarra-Alvarado C, *et al*. In vitro antileishmanial activity of Mexican medicinal plants. Heliyon. 2017;3(9):e00394.
19. Luize PS, Tiuman TS, Morello LG, Maza PK, Ueda-Nakamura T, Filho BPD, *et al*. Effects of medicinal plant extracts on growth of Leishmania (L.) amazonensis and Trypanosoma cruzi. Rev Bras Ciênc Farm. 2005;41(7-8):85-94.
20. Kauffmann C, Ethur E, Buhl B, Scheibel T, Machado G, Cavalheiro M. Potential Antileishmanial Activity of Essential Oils of Native Species from Southern Brazil. Env Nat Resources Res. 2016;6(4):18-25.
21. Halder A, Das S, Bera T, Mukherjee A. Rapid synthesis for monodispersed gold nanoparticles in kaempferol and anti-leishmanial efficacy against wild and drug-resistant strains. RSC Adv. 2017;7(23):14159-67.
22. Andrade-Cetto A. Ethnobotanical study of the medicinal plants from Tlanchinol, Hidalgo, México. J Ethnopharmacol. 2009;122(1):163-71.
23. Alonso-Castro AJ, Maldonado-Miranda JJ, Zarate-Martinez A, delRosarioJacoboSalcedo M, Fernández-Galicia C, Figueroa-Zuñiga, *et al*. Medicinal plants used in the Huasteca Potosina, Mexico. J Ethnopharmacol. 2012;143(1):292-8.
24. Gachet MS, Lecaro JS, Kaiser M, Brun R, Navarrete H, Muñoz RA, *et al*. Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis. J Ethnopharmacol. 2010;128(1):184-97.
25. Semenya SS, Maroyi A. Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. J Ethnopharmacol. 2012;144(2):395-401.
26. Rishika D, Sharma R. An update of pharmacological activity of Psidiumguajava in the management of various disorders. Int J Pharm Sci Res. 2012;3(1):3577-84.
27. Phakeovilay C, Bourgeade-Delmas S, Perio P, Valentin A, Chassagne F, Deharo E, *et al*. Antileishmanial Compounds Isolated from Psidium Guajava L. Using a Metabolomic Approach. Molecules. 2019;24(24):4536.
28. Machado AJT. Antiparasitic effect of the Psidium guajava L. (guava) and Psidium brownianum MART. EX DC. (araçá-de-veado) extracts. Food Chem Toxicol. 2018;119:275-80.
29. Souza CES, Silva ARP, Gomez MCV, Rolóm M, Coronel C, Costa JGM, *et al*. Anti-trypanosoma, anti-leishmania and cytotoxic activities of natural products from Psidium brownianum Mart. ex DC. and Psidium guajava var. Pomifera analysed by LC–MS. Acta Trop. 2017;176:80-384.
30. Gawad SMA, Hetta MH, Ross SA, Badria FAER. Antiprotozoal and antimicrobial activity of selected medicinal plants growing in Upper Egypt, Beni-Suef Region. World J Pharm Sci. 2015;4(1):1720-40.
31. Vandesmet L, Almeida de Menezes S, Machado B, Vidal M, Ramon Bezerra dos C Correia M. Leishmanicidal and trypanocidal potential of the essential oil of Psidium myrsinites DC., Nat Prod Res. 2022;36(4):1038-42.

Cite this article: Rojas-Jaimes J, Mesía-Guevara M, Rojas-Puell M, Castañeda-Pelaez L. Antiparasitic effect of *Psidium guajava* on promastigotes and axenic amastigotes of *Leishmania*. Pharmacogn J. 2022;14(6)Suppl: 973-977.