Journal of Medical Genetics and Clinical Biology
Homepage : https://journal.antispublisher.id/index.php/JMGCB
Email : admin@antispublisher.com

e-ISSN : 3032-1085
JMGCB, Vol. 2, No. 2, February 2025
Page 89-95

The Effect of *psidium guajava* Leaves Extracts and Their Effective Compound Against *Leishmania infantum*

Sahar A. Taha
Department of Biology, College of Science, University of Kirkuk, Iraq

DOI : https://doi.org/10.61796/jmgcb.v2i2.1180

Sections Info

Article history:
Submitted: December 27, 2024
Final Revised: December 27, 2024
Accepted: December 28, 2024
Published: December 28, 2024

Keywords:
Psidium guajava L. extract
HPLC
Leishmania infantum
Promastigote
Phenolic compound
Leishmaniasis

ABSTRACT

Objective: *This study aims to evaluate the anti-leishmanial activity of aqueous and alcoholic extracts of Psidium guajava L. against Leishmania infantum. Additionally, it involves screening the phenolic and flavonoid compounds in the extracts using High-Performance Liquid Chromatography (HPLC).* ***Methods:*** *Standardized methods were employed to prepare aqueous and alcoholic extracts of Psidium guajava L. In vitro assays tested the inhibitory effect of these extracts on the growth of Leishmania infantum promastigotes, with the IC50 values determined. HPLC analysis identified flavonoids and phenolic compounds in the alcoholic extract. Data analysis explored the correlation between compound concentration and anti-leishmanial activity.* ***Results: :*** *The aqueous and alcoholic extracts exhibited significant inhibitory effects on Leishmania infantum promastigote growth. The IC50 value for the aqueous extract was 0.25 mg/mL, while the alcoholic extract showed a slightly higher potency with an IC50 of 0.23 mg/mL. HPLC analysis confirmed the presence of phenolic and flavonoid compounds in the alcoholic extract, which are likely responsible for the observed anti-leishmanial activity.* ***Novelty:*** *This study demonstrates the anti-leishmanial potential of Psidium guajava L., highlighting its aqueous and alcoholic extracts as promising candidates for further investigation. The identification of specific phenolic and flavonoid compounds provides insights into the bioactive components contributing to the inhibitory effects.*

INTRODUCTION

Leishmaniasis is a zoonotic disease that affect both human and animals which is caused by different species (more than 20 SP.) of Leishmania and cause various clinical symptoms [1]. It divided in human into three form (visceral, cutaneous, mucocutaneous) depending on the tissue affected [2]. It is classified by world Health organization as one of the six most tropical uncontrolled disease. It is endemic in 98 developing countries, each year 500000 cases of the visceral leishmaniasis are reported and 50000 individuals are died [3]. This protozoan parasite is belong to Family Trypanosomatidae, Sub family *Leishmaniinae*, order Trypanosomatida. It reveal in to two morphological form in life cycle. The non-flagellated (amastigote) found in macrophages of the human and other mammalian host and flagellated, elongated (promastigote) found in the midgut of the vector [4]. The vector of leishmaniasis is a female sandfly which becomes infected when it feeds on the bloods of an infected host [5]. The Leishmania parasites develop in the sandflies midgut and transform in to the infective promastigote form. when the sandfly inject the new host the promastigote transmit to the host and the infection occurs [6], [7].

Pentavalent antimoniales is the drug thatused for the treatment of cutaneous and visceral leishmaniasis [8], [9], but frequently is failed to eradicate the Leishmania

parasite[10], [11] because the Toxicity and lower efficacy and resistance [12] all these reason beside absence of vaccine against Leishmania parasite led researchers to find alternative treatment for chemical compounds [13], [14]. The use of medicinal plant is safer than chemical compound. The natural plant compounds such as (flavonoids, phenolic compound) play a critical role for defense against Leishmaniasis [15], [16].

Psidium guajava has edible Fruit and belongs to the family Myrtaceae [17], [18]. It is richness in Secondary metabolites and active compound so it is used as antimicrobial, antibiotic, anti- allergic, anti-inflammatory [19].

RESEARCH METHOD

A. Parasite Growth

The promastigote form of *Leishmania infantum* was obtained in postgraduate parasitology laboratory in college of science, University of Kirkuk.it was grown at 26C in NNN as describe by [10].

B. Preparation of Plant Extracts

The Leaves of plant Guava (*Psidium guajava* L.) were cleaned with water and dried at room temperature then kept in free moisture conditions. Alcoholic extraction of plant was prepared according to the method of [13]. (20gm) of dry plant was taken material and use the Saxholate extraction device using (400ml) of ethyl alcohol at a concentration of 70% for (24hours). After that the extract was dried in an electric oven at a temperature of 40c.The original stock solution was prepared by taking (2gm) of the dry extract and dissolving it in a small amount of ethyl alcohol, then diluting it with distilled water by adding (100ml) of It. The concentration of original solution became (20mg/ml. Then (0.5, 1,1.5 mg/ml) concentration were prepared from it. The aqueous extraction of plant was prepared depending on [18]. (10gm) from dried plant leaves was taken. then added to (200 ml) of distilled water.then it was mixed for 15 minutes with a magnetic mixer, then kept the solutionin a laboratory at room temperature, after(24 hours) the solution of extraction was filtration through (0.22) micron filter paper. the filtrate was centrifuged at a speed of 3000 rpm (1) for 10 minutes to precipitate the suspended plant parts and to obtain a clear solution. the clear solution was isolated and dried in an electric oven at a temperature o f40c and kept it at 4c in the refrigerator until use.

C. *Leishmaniain in fantum* Parasite Vitality Test

The parasite vitality was tested using 0.4%Erythrocin-B dye according to the method of [15] as follow: Equal volumes of (100µl) of parasite suspention and 0.4%dye solution diluted with erythrocin-B (phosphate buffer) were mixed and cooled to 4c for 5 min. Then a drop of the mixture was examined at least 100cell were counted in order to estimate the percentage of cell viability using a haemocytometer, (pigmented parasite refer to dead cell, and non-pigmented parasite refer to live cell. The calculation was made by making 5 replicates for each sample and for each concentration.

D. HPLC Analysis

The (High performance liquid chromatography) this analysis was carried out for determination of some phenolic and flavonoid compound in psidium guajava L.in leaf ethanolic extract70%. The method was done by depending on (Mardu etal;2012) using device SYKAM liquid high performance the phase has been used the methanol: distilled water: acetic acid (75:13:2) the separation column was C18-ODS (25cm*4.6mm) to separate the detected compound and reagent was used UV-360nm while the velocity of flow of the carrier phase was 1ml/min.

RESULTS AND DISCUSSION

As indicated in Table 1 the number of *L.infantum*decrease gradually by using 0.5 to 1.5 mg/ml concentration of aqueous extract of*psidiumguajava* and at 0.5,1,1.5 mg/ml after 96 hours inhibited the parasite growth by(64,75,84) % respectively. The (IC_{50}) inhibitory concentration of 50% of L. infantum was found to be (0.25) mg/ml for aqueous extract, thus it becomes clear the toxic inhibitory effect of the aqueous extract on the number of promastigote. Then alcoholic extract as indicated in Table 2 reveal anti Leishmanial activity and reduced the number of promastigote of *L.infuntum* at 96 hours by (65,77,85) %. The IC_{50} of alcoholic extraction was found to be (0.23) mg/ml which cause the loss of 50% of parasite promastigote. This result is agree with the result of [20]. The growth of promastigote is usually estimated bydetermining its number and the number of generation and generation time. All their it were calculated, Table3 reveal it. The NNN medium was used for the growth of promastigote. The number of promastigote increased with the increase of growth period, At 96 hours the limited growth was observed, the logarithmic phase of growth was 96 hours. This result is agree with the results of [21]. Treatment of Leishmaniasis is limited to the use of a limited number of drugs, which may have side effectsincluding nerve inflammation andliver and kidney complication, especially in elderly people. Therefore, there is an urgent need to find alternatives to chemotherapy, and recent studies have turned to alternative medicine treatment. It has become imperative for researchers to find modern way to develop this type of treatment by dealing with medical plant extracts and using the active compounds isolated from them in treatment aftere ensuring that there are no side effects. The current study deal with testing the sensitivity of the promastigote to plant extracts prepared from Pisidium guajava leaves. And detecting some of the active compound in them and their effects on the parasite. The Posidium guajava plant has high biological activity against some pathogens such as Bacteria, Fung, and use as antioxidants and anticancer [22]. The result of The Qualitative diagnosis of phenolic and flavonoids compoundsusing HPLC for *Pisidium guajava* leaves alcoholic extract as showed in Table 4.it contain (Gallic acid, Catechin, Ferulic acid, Caffeic acid, Rutin, Quererecetin, kaempferol, Chlorogenic acid) which may contribute to the inhibitory effect on growth of promastigote Leishmania infuntumand may be attributed to the luteolin. this result were agree with [20] which

peoved that the luteoline has an inhibitory effect against the growth of Leishmaniasis [23].

Table 1. Effect of different concentration of aqueous extract of *Psidium guajava L.* on the number of promastigotes of *Leishmania infantum* at different growth intervals.

	24hr			48hr			72hr			96hr		
	Number of promastigotes	G%	I%	Number of promastigotes	G%	I%	Number of promastigotes	G%	I%	Number of promastigotes	G%	I%
Control	5 x10^5	100	---	1.9 x10^6	100	---	5.2 x10^6	100	---	1.2 x10^7	100	----
0.5 mg/ml	3.6 x10^5	72	28	1.3 x10^6	68	32	2.4 x10^6	46	54	4.4 x10^6	36	64
1 mg/ml	3x10^5	60	40	8x10^5	47	53	1.5x10^6	30	70	3 x10^6	25	75
1.5 mg/ml	2 x10^5	40	60	4.1x10^5	21	79	9.2x10^5	17	83	2 x10^6	16	84

G%= Percentage of growth: I% Percentage of inhibition.

Table 2. Effect of different concentration of alcoholic extract of Psidium guajava L. on the number of promastigotes of Leishmania infantum at different growth intervals.

	24hr			48hr			72hr			96hr		
	Number of promastigotes	G%	I%	Number of promastigotes	G%	I%	Number of promastigotes	G%	I%	Number of promastigotes	G%	I%
Control	5 x10^5	100	---	1.9 x10^6	100	---	5.2 x10^6	100	---	1.2 x10^7	100	---
0.5mg/ml	3.8 x10^5	76	24	1 x10^6	52	48	2 x10^6	38	62	4.2 x10^6	35	65
1 mg/ml	3.2 x10^5	64	36	7,3 x10^5	38	62	1.3 x10^6	25	75	2.8 x10^6	23	77
1.5 mg/ml	2.6 x10^5	52	48	4.4 x10^5	23	77	9 x10^6	17	83	1.8 x10^6	15	85

G%= Percentage of growth: I% Percentage of inhibition.

Table 3. Number of promastigote, number of generation and generation time (hours) growing in (NNN) medium at different time periods.

Time(hours)	Number of promastigote	Generation number	Generation time(hours)
24	5×10^5	2.318	10.353
48	1. 9 $\times10^6$	4.245	11.307
72	5.2$\times$.10^6	5.7	12.631
96	1.2 $\times$ 10^7	6.9	13.913

Table 4. HPLC analysis data of some phenolic and flavonoids compound and their concentration and Retention time(min).

Compound	Retention time (min)	Concentration(mg/ml)
Gallic acid	7.04	4.71
Catechin	9.875	5.12
Ferulic acid	12.67	3.55
Chlorogenic acid	10.60	2,93
Caffeic acid	11.26	4.37
Rutin	13.03	6.37
Querecetin	13.43	8.94
Kaempferol	15.74	1.89

CONCLUSION

Fundamental Finding : The aqueous and alcoholic extracts of the plant demonstrated significant inhibitory effects on the growth of *Leishmania infantum*. The identified phenolic and flavonoid compounds are believed to contribute to this bioactivity, highlighting the plant's potential as a source of antiparasitic agents. **Implication :** These findings suggest that plant-derived extracts could serve as a promising basis for developing new treatments against *Leishmania infantum*. Their natural composition offers an alternative to synthetic drugs, potentially reducing side effects and addressing drug resistance concerns. **Limitation :** This study is limited by its in vitro nature, which does not account for the complexity of biological interactions in living organisms. Additionally, the specific mechanisms of action of the phenolic and flavonoid compounds remain unclear, requiring further biochemical exploration. **Future Research :** Future investigations should focus on elucidating the precise mechanisms of action of the active compounds, conducting in vivo studies to evaluate their safety and efficacy, and exploring their potential for formulation into effective therapeutic agents.

REFERENCES

[1] M. S. Abdullah and A. Q. Alqaisi, "Evaluating the in vitro anti-Leishmanial activity of essential oil extracted from *Cymbopogon Citratus* against *Leishmania Donovani*," *Iraqi Journal of Science*, vol. 63, no. 4, pp. 1453–1463, Apr. 2022.

[2] J. Saki, E. Biranvand, and R. Arjmand, "The in vitro anti-Leishmania Effect of *Zingiber officinale* Extract on Promastigotes and Amastigotes of *Leishmania major* and *Leishmania tropica*," *In Vitro*, vol. 46, no. 2, pp. 91–96, Jun. 2022.

[3] G. A. Gutiérrez-Rebolledo, S. Drier-Jonas, and M. A. Jiménez-Arellanes, "Natural compounds and extracts from Mexican medicinal plants with anti-leishmaniasis activity: An update," *Asian Pacific Journal of Tropical Medicine*, vol. 10, no. 12, pp. 1105–1110, Dec. 2017.

[4] R. Mansour et al., "The effect of *Vitis vinifera* L. leaves extract on *Leishmania infantum*," *Iranian Journal of Pharmaceutical Research: IJPR*, vol. 12, no. 3, pp. 349–354, 2013.

[5] C. K. Glidden et al., "Phylogenetic and biogeographical traits predict unrecognized hosts of zoonotic leishmaniasis," *PLOS Neglected Tropical Diseases*, vol. 17, no. 5, p. e00107, May 2023.

[6] E. Berriatua et al., "Leishmaniases in the European Union and neighboring countries," *Emerging Infectious Diseases*, vol. 27, no. 6, pp. 1723–1730, Jun. 2021.

[7] M. Y. Ali, "A study of the prevalence of leishmaniasis in Kirkuk Governorate and evaluation of some immunological and biochemical indicators in leishmaniasis patients," *Web of Agriculture: Journal of Agriculture and Biological Sciences*, vol. 2, no. 5, pp. 122–134, May 2024.

[8] H. F. Hassan, A. A. Khalaf, and N. A. Ahmed, "Epidemiological survey of cutaneous leishmaniasis in Al-Hawija District, Kirkuk Province, Iraq," *HIV Nursing*, vol. 23, no. 1, pp. 703–710, 2023.

[9] A. A. Rahi, "Cutaneous leishmaniasis in Iraq: A clinicoepidemiological descriptive study," *Scholars Journal of Applied Medical Sciences*, vol. 1, no. 6, pp. 1021–1025, 2013.

[10] H. F. Hassan and S. A. Taha, "The use of *Cymbopogon citratus* (lemongrass) extracts as antileishmanial activity," *Biochemical & Cellular Archives*, vol. 20, no. 2, pp. 2733–2736, Oct. 2020.

[11] H. M. Taher, S. A. Mohammed, and A. R. Mahmood, "Epidemiological study of cutaneous leishmaniasis in Kirkuk Province, Iraq," *Plant Archives*, vol. 20, no. 2, pp. 2733–2736, 2020.

[12] A. Dayakar et al., "In vitro and in vivo evaluation of anti-leishmanial and immunomodulatory activity of Neem leaf extract in *Leishmania donovani* infection," *Experimental Parasitology*, vol. 153, pp. 45–54, Jun. 2015.

[13] H. Nigatu et al., "In vitro antileishmanial activity of some Ethiopian medicinal plants," *Journal of Experimental Pharmacology*, vol. 13, no. 1, pp. 15–22, Jan. 2021.

[14] E. Ghaderian et al., "Anti-leishmanial effects of *Eryngium planum* and *Ecbilium elaterium* methanolic extract against *Leishmania major*," *AMB Express*, vol. 14, no. 1, p. 3, Jan. 2024.

[15] S. Naseer et al., "The phytochemistry and medicinal value of *Psidium guajava* (guava)," *Clinical Phytoscience*, vol. 4, no. 1, pp. 1–8, Dec. 2018.

[16] G. A. Pereira et al., "Antimicrobial activity of *Psidium guajava* aqueous extract against sensitive and resistant bacterial strains," *Microorganisms*, vol. 11, no. 7, p. 1784, Jul. 2023.

[17] M. E. El-Sesy and B. N. Mahran, "The antibacterial and coagulant activity of *Psidium guajava* leaves extracts in purification of wastewater," *Biosciences Biotechnology Research Asia*, vol. 17, no. 1, pp. 191–203, Mar. 2020.

[18] A. O. Sule, A. O. Olalemi, and A. O. Ogundare, "Efficacy of leaf extracts of *Psidium guajava* (L.) on enteric bacterial isolates from faecally impacted groundwater," *Water Practice & Technology*, vol. 17, no. 1, pp. 167–174, Jan. 2022.

[19] N. S. Sampath Kumar et al., "Extraction of bioactive compounds from *Psidium guajava* leaves and its utilization in preparation of jellies," *AMB Express*, vol. 11, pp. 1–9, Dec. 2021.

[20] H. Zhang et al., "Progress in antileishmanial drugs: Mechanisms, challenges, and prospects," *PLOS Neglected Tropical Diseases*, vol. 19, no. 1, p. e0012735, Jan. 2025.

[21] C. A. Antwi et al., "In vitro activity and mode of action of phenolic compounds on *Leishmania donovani*," *PLOS Neglected Tropical Diseases*, vol. 13, no. 2, p. e0007206, Feb. 2019.

[22] M. G. Correa and J. S. Couto, "Anticancer properties of *Psidium guajava*—A mini-review," *Asian Pacific Journal of Cancer Prevention*, vol. 17, no. 9, pp. 4199–4204, Sep. 2016.

[23] K. Iqbal et al., "Luteoline as a potent anti-leishmanial agent against intracellular *Leishmania tropica* parasite," *Journal of Pharmaceutical Research*, vol. 16, no. 2, pp. 337–342, 2017.

*Sahar A. Taha (Corresponding Author)
Department of Biology, College of Science, University of Kirkuk, Iraq
Email: sahakram@uokirkuk.edu.iq