https://doi.org/10.31351/vol34iss1pp266-274

Antileishmanial Evaluation and Phytochemical Screening of Iraqi *Sanchezia speciosa* Petroleum Ether Fraction *in vitro*

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Received 1/6/2024, Accepted 19/6/2024, Published 29/3/2024

Abstract

Leishmaniasis is a significant parasite issue. Vaccine inaccessibility and the absence of a safe and effective therapy that is not resistant to the illness hinders the total eradication of this disease and remains a challenge for the control of the disease; medications with harmful side effects or insufficient efficacy have resulted in disease recurrence. The path towards discovering alternative medications derived from natural resources with shown antileishmanial activity has been paved using these criteria. Consequently, two *Leishmania* parasites were examined for their antileishmanial activity, and an Iraqi plant was chosen. The anti-leishmania fraction activity was obtained from the aerial portions of *Sanchezia speciosa*, which has been assessed using in vitro methods. Maceration of the aerial portions of *Sanchezia speciosa* with petroleum ether was used to create the extract, which included active non-polar compound components. To ensure accuracy, an MTT assay was utilized and the extract was prepared as six distinct concentrations with 1% DMSO. the medium with parasite serving as negative controls and Pentostam (Sodium stibogluconate) as positive controls. *Leishmania donovani* and *Leishmania tropica* were both tested for their ability to be effectively inhibited by the fraction of plant that was extracted. Based on the extract of the plant at many doses tested, the results showed superior antileishmanial activity of macerated petroleum ether fraction with IC50 value (0.092 mg/ml) against *Leishmania tropica* and IC50 value (0.074 mg/ml) against *Leishmania donovani* when combined with the antileishmanial activity of the official treatment medication (Pentostam®); the chemical examination of the extracts revealed the presence of terpenes and steroids by qualitative phytochemical tests. The findings of this study indicate that the presence of certain secondary metabolites, such as terpene and steroid compounds in the materials utilized to make up this fraction likely explains this high potential antileishmanial action against *Leishmania tropica* and *Leishmania donovani*. Ongoing efforts are being made to find a successful approach for treating leishmaniasis with minimal side effects, and there is a significant focus on herbal medications.

Keywords: *Leishmania tropica, Leishmania donovani,* maceration, Pentostam, *Sanchezia speciosa*

Introduction

The *Leishmania* genus of protozoan parasites is responsible for a variety of disorders including leishmaniasis. [(1)] The parasite is spread by the bite of a female sandfly of the Phlebotomine species, leading to a range of illnesses with symptoms that vary from ulcers that heal on their own to life-threatening infections that damage the liver and spleen. The severity of the infection depends on the specific species of the parasite. [(2)] Leishmaniasis may be classified into many forms, including cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis. [(3)] This illness is a prominent endemic parasitic infection that is prevalent in around 98 countries, particularly in poorer nations. It poses a danger to over 1.7 billion individuals globally. [(4,5)] Leishmaniasis first-line medications, including Glucantime, Pantostam, and Pentacarinat, are ineffective when taken orally and need lengthy injections for therapy. Amphotericin B and pentamidine, which are classified as second-line medicines, exhibit high levels of toxicity. Moreover, the vaccination techniques have not yet been included in clinical trials, while chemotherapy remains the exclusive temporary therapeutic approach. Hence, there is a pressing need to develop innovative and highly efficient pharmaceuticals derived from plants or their extracts, which are anticipated to serve as a valuable reservoir of new substances. with enhanced functionalities to modify or add to the existing ones. [(6,7).] *Sanchezia speciosa*, a member of the Acanthaceae family, is a robust and upright shrub that is frequently referred to as Fire Fingers in the English language.[(8)] This species is often cultivated

for decorative purposes and is typically found in damp and shaded regions, as well as on several Pacific islands such as Hawaii, Fiji, and New Caledonia. [9] This herb has traditionally been used for the treatment of gastritis. [10] also used topically for treating wounds. Leaf extracts of *S. speciosa* include a variety of chemicals, including alkaloids, glycosides, flavonoids, triterpenoids, carbohydrates, steroids, phenolic compounds, saponins, and tannins. [11]

Figure1. Iraqi *Sanchezia speciosa* plant

Research indicates that *Sanchezia speciosa* has a number of useful medicinal characteristics, including the ability to kill insects, as well as antifungal, antibacterial, [12] antioxidant, anticancer, [13], and anti-inflammatory activities. [14] Among these, terpenes found in *Sanchezia speciosa* have drawn attention for their pharmacological activities, including their ability to prevent and treat cardiovascular diseases, act as an antitumor, anti-inflammatory, antibacterial, antiviral, and antimalarial, neuroprotection, antioxidation, antiaging, immunoregulation [15] with its antileishmanial effects [16] .Terpenes are grouped according to their unit count: hemi terpenes, monoterpenes, iridoids, sesquiterpenes, diterpenes, ses terpenes, triterpenes, tetraterpenes, polyterpenes, and irregular terpenes. Irregular terpenes and polyterpenes, which contain eight or more isoprene units, are further types [17] Phytosterols, in particular, have demonstrated potential for anti-leishmanial activity. The proliferation of *Leishmania* parasites is inhibited by bioactive compounds such as β-sitosterol and stigmasterol, which are present in a variety of plants. These steroids disrupt the viability of parasites by interacting with cellular membranes and enzymes in a chemical manner. They may provide a complementary approach to traditional anti-leishmanial therapies by reducing parasitic burden and inflammation pharmacologically.[18] Plant steroids demonstrate additional pharmacological properties, including the enhancement of immune responses and anti-cancer properties, in addition to their anti-leishmanial activity.[19]

Material and methods

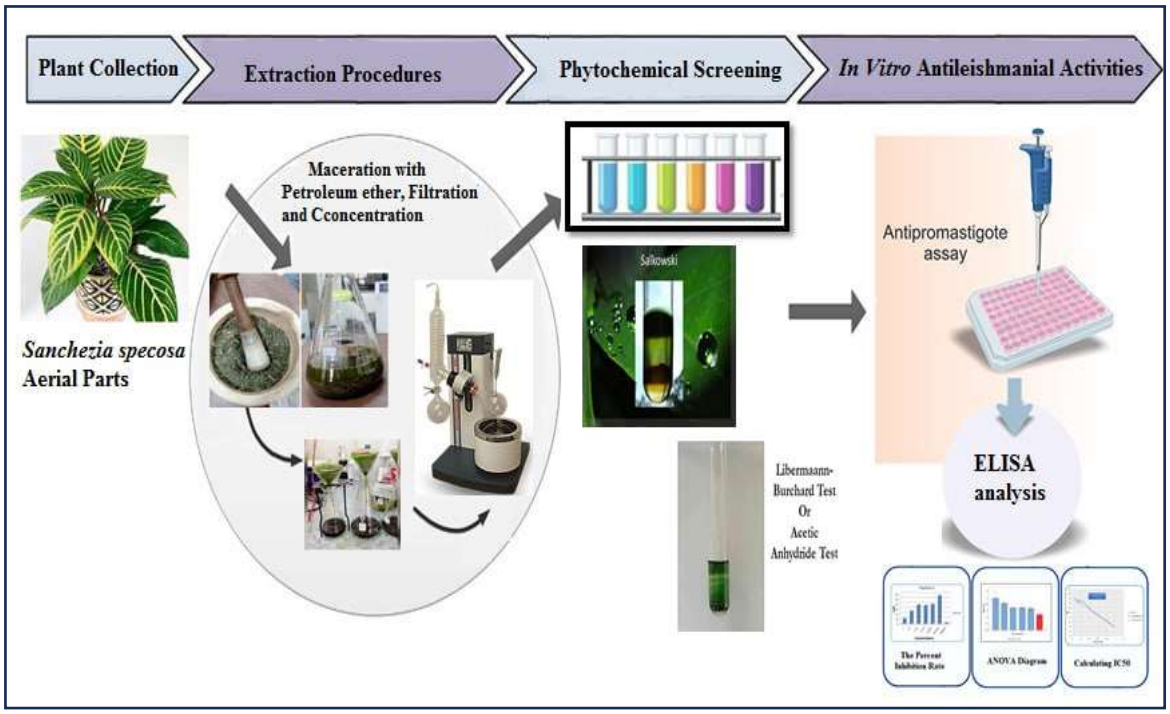
Collection and Authentication of Plant Materials

Fresh aerial parts were taken from the local botanical park in Baghdad. Subsequently, the Department of Biology at Baghdad University, the College recognized the newly obtained aerial parts, and the verification process was conducted. In plant extract preparation the maceration extraction process is used to extract various phytoconstituents, (steroidal and terpenes phytoconstituents). The aerial portions (leaves and stems) of *Sanchezia speciosa* were cleansed to eliminate any extraneous

substances, and then dried in a shady area with ambient air. The dried plant was then ground into a powder using an electric blender. The resulting powder was then stored in a tightly sealed container, and prepared for extraction.

Extraction method (maceration)

The maceration extraction technique was used to carry out the phytochemical extraction. The extraction process included extracting 100 grams of dried powder from the aerial parts of the *Sanchezia speciosa* plant. The powder was then introduced in a glass container with a tight stopper. Next, it was extracted using 500 milliliters of petroleum ether with a boiling point range of 40-60 C°. Continuous shaking was used to carry out the extraction procedures. 1gm of petroleum ether extract was obtained by filtering the mixture using filter paper, collecting it, and then condensing it using a rotary evaporator under a vacuum at 40 C^0. Then, it was transported to drying trays.



(20)(21)

Figure 2. General scheme of Iraqi *Sanchezia speciose* extraction, phytochemical identification

Preliminary Identifying phytoconstituents

Phytoconstituent compounds isolated from the petroleum ether fraction of *Sanchezia speciosa* were identified using chemical assays.

Salkowski analysis

A mixture of 3 milliliters of concentrated sulfuric acid, 2 milliliters of chloroform, and a little amount of plant hexane extract was prepared. A rusty-brown hue was produced via oxidation. (22)

Liebermann-Burchard evaluation procedure:

Dissolve a little amount of the plant's hexane extract in 5 milliliters of chloroform, then remove the water from the chloroform layer using anhydrous Sodium sulfate. Afterward, this mixture was then mixed with 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. The substance undergoes oxidation, resulting in a bluish-green color shift, which indicates the presence of the steroidal nucleus. (23,24)

Antileishmanial activity

Preparation of inoculum

Leishmania samples were acquired by several verified patients gathered from Baghdad hospitals and sent for the Research Biological Technology Center belonging to the College of Science – University of Al-Nahrain. The plant extract's effect was tested by growing the organism on "Roswell-Park-Institute-Park-Memorial" (RPMI) medium in addition to serum12% calf fetal, a temperature of 25°C for five days until the parasite concentration reached an average of 5*1٠٦ parasites/ml as determined by hemocytometer counting. (25,26)

Preparation of plant extract concentration

The petroleum ether fraction was tested for its anti-parasitic effectiveness against both *L.donovani* and *L.tropica*, and the results were compared to those of the frequently used antileishmanial drug, (Pentostam). Dimethyl Sulfoxide (DMSO) was used as a solubilizer for

extract. The DMSO added not more than 20 µL. D.W was then added to reach the desired concentration of 1000µg/ml. This solution was used to create subsequent dilutions. The concentrations were (1000, 500, 250, 125, 62.5, 31.25) µg/ml. [27]

Preparation of positive control

The pentavalent antimonial, namely the sodium stibogluconate injection at a concentration of 100 mg/ml, from GlaxoSmithKline in the UK, served as the positive control. This medicine is considered a classic in the field. Sodium stibogluconate (100 mg/ml) was progressively diluted to achieve a 10000 µg/ml concentration. After that, 6 µl from this solution was added to each well which contained RPMI (1ml) with *L.donovani* and *L.tropica* inoculum. The control + wells were used to evaluate any differences with fraction concentrations. [28]

Assessment of sample fractions for leishmaniasis

In order to evaluate the activity as antileishmanial, a Plate with a flat bottom with wells was used. The plate consisted of 32 wells containing *L.donovani* and another 32 wells with *L.tropica*, as seen in Figure. 3, Each well was supplemented with the *leishmania* culture, subsequently, 10µL of the previously prepared fraction concentrations were added to 64 wells, Additionally, eight wells were designated as negative controls, where no additional ingredients were introduced to the *leishmania* culture, A sodium stibogluconate positive control was added to 8 wells. The remaining wells were filled with 50% DMSO, which acted as a blank. The plate was placed in an incubator at a temperature of 25 ± 1C° for a period of twenty-four hours. subsequent to this period, a 100µL solution of MTT dye (3-(4,5-dimethyl thiazo-2-yl)-2,5-diphenyltetrazolium bromide) was introduced into every well. The plate incubated for an additional four hours at 25C° in order to measure metabolic rate. Following this, A solubilizing agent, DMSO, was applied to each well. This chemical allows a scanning procedure to occur by focusing on dye "MTT" with purple color present in living stuff. [29,30]

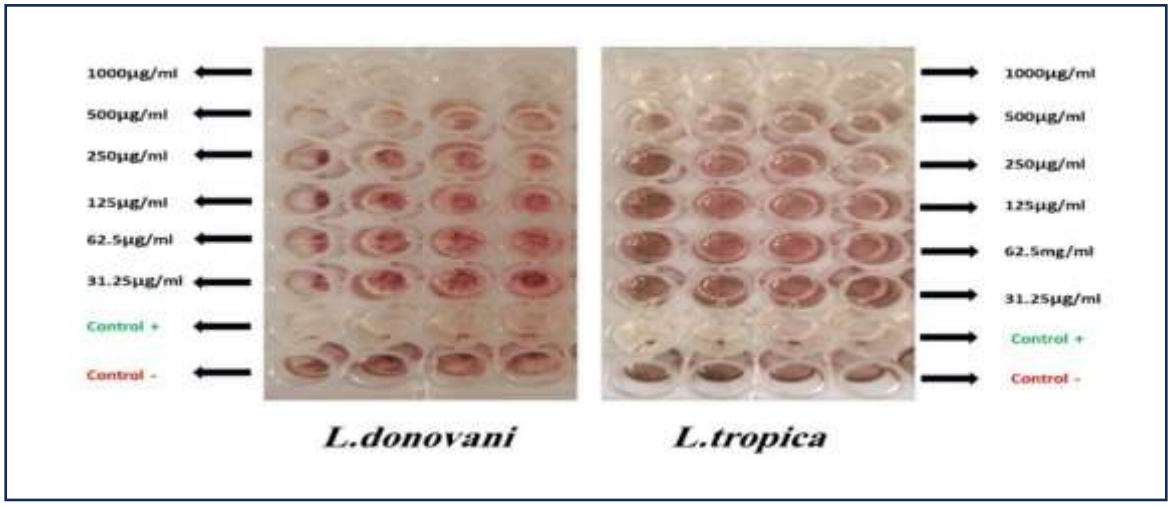


Figure 3. Plates containing wells that are prepared for ELISA scanning

Scanning

Each well's optical density is measured at 490 nm by the ELISA spectrophotometer. Intensity of purple dye seen is directly proportional to the amount of living matter present as seen in Figure 3. Consequently, a higher ELISA reading absorbance indicates a greater accuracy in quantifying the living matter.

Analysis of statistics

The significance of the findings was confirmed by comparing the means after computing the inhibition rate % for each of the six concentration gradients, by IBM software's one-way ANOVA test, we may use the Least statistically Significant Differences (LSD) and p-value to ascertain whether the inhibition rate differs substantially from that of sodium stibogluconate. [1]

Calculation of IC50

The half maximal inhibitory concentration (IC50) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. In this study, the IC50 values of the petroleum ether fraction against *L.donovani* and *L.tropica* were determined using dose-response curves generated in Microsoft Excel [30]. First, the percentage inhibition rates at various concentrations of the petroleum ether fraction were calculated using the formula: **% Inhibition Rate=(OD Control-OD Test/OD control) × 100.**

The inhibition rates were then plotted against the concentrations using Excel's scatter chart feature. To

determine the IC50 values, a logarithmic trendline was added to the scatter chart [31]. The logarithmic trendline equation, which is in the form of y = a ln(x) + b, was used to calculate the concentration at which 50% inhibition occurred.

Results and Discussion

Extraction and fractionation

The extraction process depends on many factors, including the plant material's composition, the solvent's pH, temperature, solvent-to-sample ratio, and the solvent itself. The utilization of the ultimate products is an additional determinant. The selection of the solvent is influenced by the type of plant, the specific section of the plant that requires extraction, the characteristics of the bioactive components, and the availability of the solvent. Polar solvents such as water, methanol, and ethanol are often used for extracting polar compounds, while nonpolar solvents like hexane, dichloromethane, and petroleum ether are utilized for extracting nonpolar compounds. [32]

In this study, cold maceration technique was used to extract steroidal compounds and terpenoids from the Iraqi *Sanchezia speciosa*. Non-polar solvents may be used to extract steroids and terpenoids, and their presence was identified using specific chemical reagents such as Salkowski and Liberman-Burchard, respectively.

Assessment of antileishmanial activity

Plant therapy has become a cornerstone of a scientific research, and the use of herbal medicine is highly growing, [33] so *Sanchezia speciosa* is considered one of them. The results of the ELISA assay for Optical Density (OD) were used to determine the proportion of organisms that have been eradicated from the fraction with its six concentrations, employing: **% Inhibition Rate = (OD Control-OD Test/OD control) × 100.** [29]

The percentage inhibition rate was determined for each concentration and compared to the calculations of the positive and negative controls. The inhibition rate of *L. donovani* was computed, and the mean for the petroleum ether fraction at six different concentrations was determined. The results showed that the inhibition rate was greater in concentrations Don1, Don2, and Don3, indicating strong anti-leishmaniasis activity. This activity was compared to the inhibition rate of the positive control, which was 67.23%, as shown in Table 1.

Table 1. Averaging the percentage of IR across all *L.donovani* concentration gradients

Sample name	Concentration µg/ml	%IR ± SD
Don1	1000	75.59172 ± 0.015927
Don2	500	72.92899 ± 0.080085
Don3	250	70.04438 ± 0.108509
Don4	125	56.28698 ± 0.10963
Don5	62.5	48.9645 ± 0.096723
Don6	31.25	36.42751 ± 0.041065

The inhibition rate percentage (IR) for *L.donovani* as a function of concentrations, along in a comparison with the positive control to be better inhibition rate than the positive control for concentrations of (1000,500,250) µg/ml as shown in Figure 4.

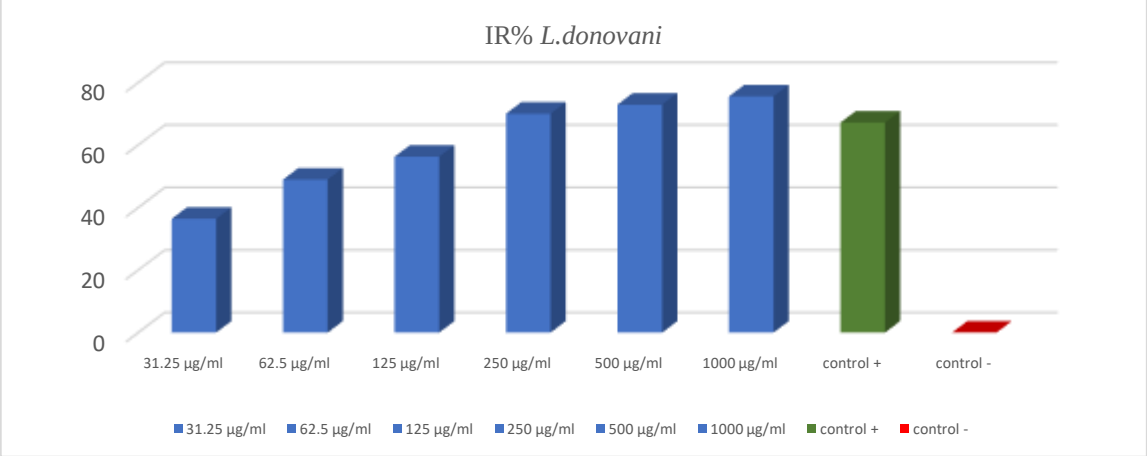


Figure 4. % IR of *L.donovani* against concentrations of petroleum ether fraction

The IC50 value of the petroleum ether extract utilized was determined to be 74 µg/ml for L.donovani, using a logarithmic equation.

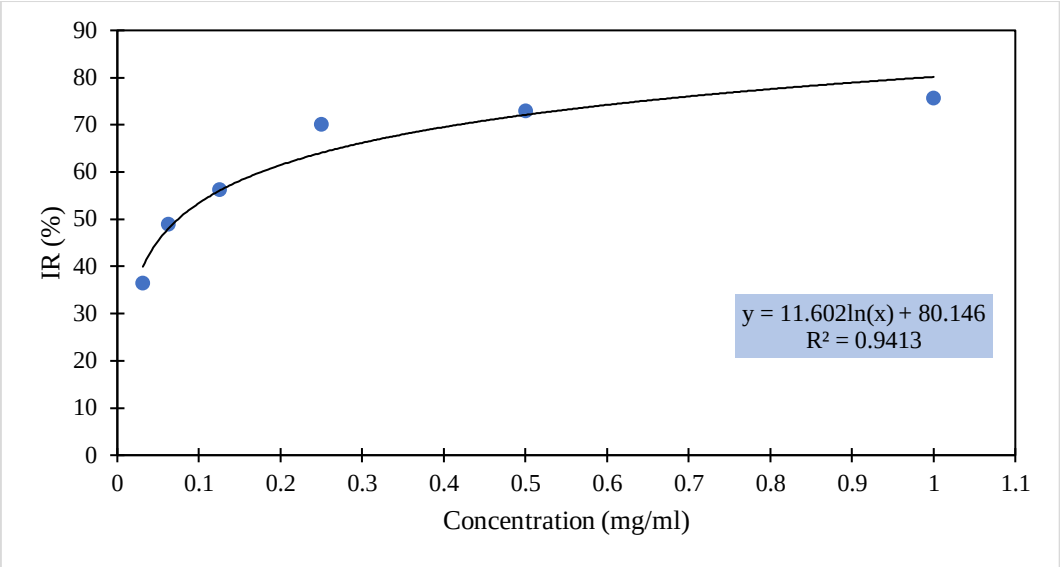


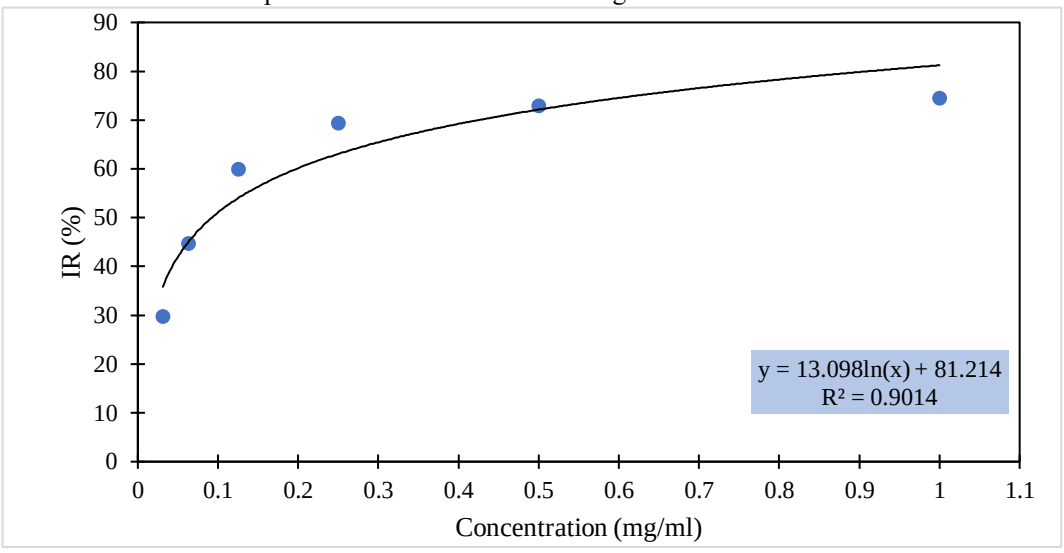
Figure 5. IC50 for petroleum ether fraction against *L.donovani*

The inhibition rate of *L.tropica* was computed, and the mean for the petroleum ether fraction at six different concentrations was determined. The results showed that the inhibition rate was particularly high in (Tro1, Tro2, and Tro3) for anti-leishmaniasis activity, compared to the positive control inhibition rate of 67.23% as indicated in Table 2.

Table 2. Results for *L. tropica* overall concentration gradients, measured in % IR

Sample name	Concentration µg/ml	IR% ± SD
Tro1	1000	74.50185 ± 0.034495
Tro2	500	72.87823 ± 0.019754
Tro3	250	69.33579 ± 0.011564
Tro4	125	59.9262 ± 0.004113
Tro5	62.5	44.76015 ± 0.035738
Tro6	31.25	29.7048 ± 0.100634

The Figure displays the inhibition rate percentage (IR) for *L.tropica* at different concentrations. It also compares the results with the positive control to be a better inhibition rate than the positive control for concentations of (1000, 500, 250) µg/ml utilized in Figure 6.



.**Figure 6. % IR of *L.tropica* against concentrations of petroleum ether fraction.**

The IC50 value for the petroleum ether extract utilized was determined to be 92 µg/ml for *L.tropica* using a logarithmic equation.

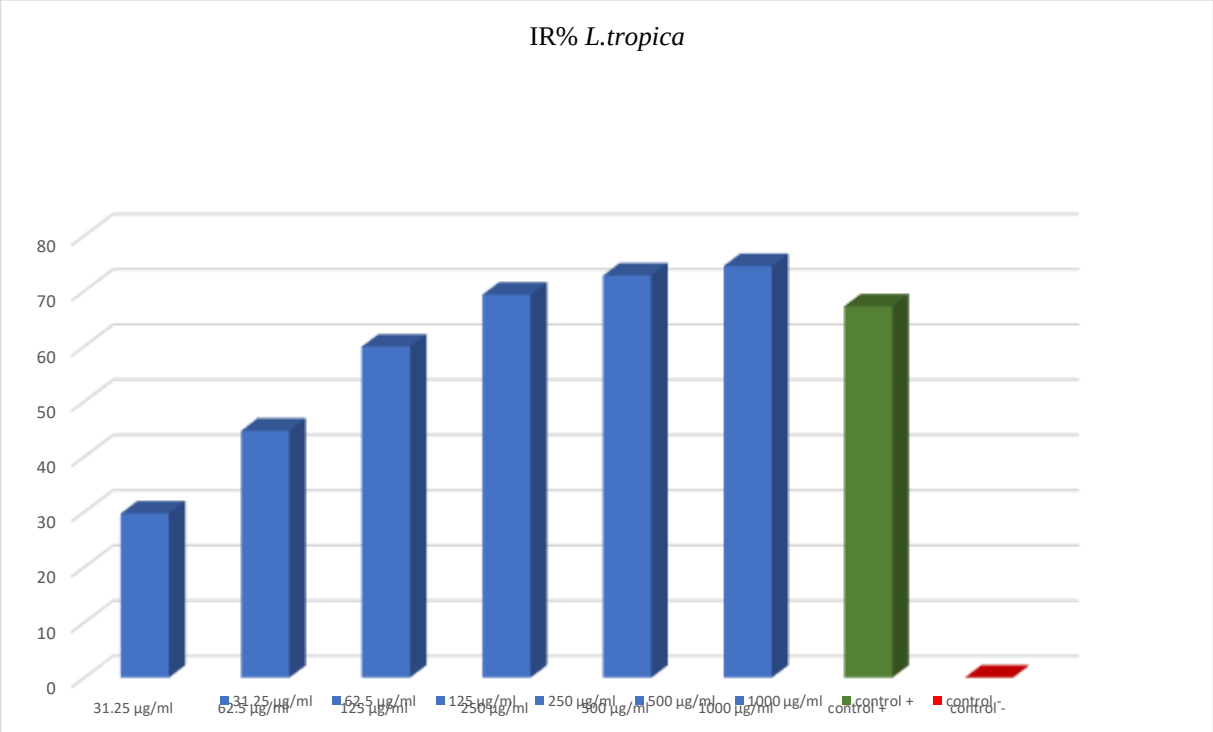


Figure 7. IC50 for petroleum ether fraction against *L.tropica*

Conclusion

This research identified the aerial section of the Iraqi *Sanchezia speciosa* plant as a herb with therapeutic uses that contains several Phytochemical compounds. These constituents have been shown to possess antileishmanial properties, similar to the effects of pentostam therapy. The fraction chosen for this experiment is based on the elevated levels of steroid and terpenoid compounds found in the petroleum ether fraction of the aerial part. The petroleum ether fraction exhibited higher activity than pentostam at concentrations of 1000 µg/ml, 500 µg/ml, and 250 µg/ml, in terms of its steroid and terpenoid content. The IC50 values for both types of *leishmania*, *donovani* and *tropica*, were (74) µg/ml and (92) µg/ml, respectively. This not only supports the traditional assertion about the plants, but also offers indications for additional investigation into the active components of these plants in order to produce antileishmanial medications that are both effective and safe.

Acknowledgment

We are grateful to the faculty and Baghdad University's staff / Pharmacy college and the Department of Pharmacognosy and Medicinal Plants for their support in providing the essential facilities.

Conflicts of Interest

There is no conflict of interest regarding the publication of my manuscript.

Funding

The research has not received financial support from any Institution.

Ethics Statements

The manuscript did not include human and/or animal studies, so ethical approval is unnecessary for this research.

Author Contribution

The authors conceived and planned the experiments and carried out the sample preparation, extraction process, identification. The authors also wrote the manuscript.

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التقييم المضاد للشمانيا والفحص الكيميائي النباتي لجزء الأيثر البترولي للنبات العراقي سانشيزيا سبيشوزا في المختبر

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[٢]فرع العقاقير والنباتات الطبية، كلية الصيدلة، جامعة بغداد، بغداد، العراق

الخلاصة

داء الليشمانيا هو مشكلة مرضية طفيلية هامة. انعدام اللقاح وعدم وجود علاج امن وفعال لا يقاوم المرض هو المعوق للقضاء التام على هذا المرض ويظلان تحديا لمكافحة المرض؛ الأدوية ذات الآثار الجانبية الضارة أو الفعالية غير الكافية أدت إلى تكرار المرض بالنظر الى هذه المعايير تم تمهيد الطريق نحو اكتشاف الأدوية البديلة المشتقة من الموارد الطبيعية ذات النشاط المضاد للشمانيا ونتيجة لذلك، تم فحص اثنين من طفيليات الليشمانيا لنشاطهما المضاد للشمانيا، وقد تم اختيار النبات العراقي. فعالية الجزء المضاد لليشمانيا قد تم الحصول عليها من الأجزاء الهوائية من السانشيزيا سبيشوزا، والتي تم تقييمها باستخدام الطرق المختبرية. تنقيع الأجزاء الهوائية للسانشيزيا سبيشوزا بالأيثر البترولي قد تم لإنشاء المستخلص، والذي تضمن المكونات غير القطبية النشطة. لضمان الدقة، تم استخدام اختبار ام تي تي وتم تحضير المستخلص بستة تراكيز مميزة مع ١٪ دي ام اس او. الوسط الحاوي على الطفيلي يعمل كمعيار سلبي والبنتوستام (ستيبوكلوكونات الصوديوم) كمعيار إيجابي. اللشمانيا الأحشائية واللشمانيا المدارية كلاهما قد تم اختبارقابليتهما على التثبط الفعال بواسطة جزء النبات المستخلص. بالاعتماد على مستخلص النبات للجرعات المعتدده المستخدمة، أظهرت النتائج أن جزء الأيثر البترولي للأجزاء الهوائية المنقوعة له نشاطا فائقا مضادا للشمانيا بحساب الجرعة القاتلة لنصف الخلايا وكانت بقيمة (٠,٠٩٢ ملغم / مل) ضد الليشمانيا المدارية وقيمة تركيز النصف (٠,٠٧٤ملغم / مل) ضد الليشمانيا ألاحشائية عند مقارنتها للعلاج الرسمي (بنتوستام®)؛ الفحص الكيميائي للمستخلص دل على وجود التربين والستيرويدات عن طريق الاختبارات الكيميائية النباتية النوعية. نتائج هذه الدراسة اثبتت وجود مستقلبات ثانوية محددة مثل مركبات التربين والستيرويد المكونة لهذا الجزء لتفسر هذه الفعالية العالية المضاده للشمانيا ضد الليشمانيا المدارية والليشمانيا الاحشائية. جهود حثيثة تبذل لإيجاد نهج ناجح لعلاج داء الليشمانيا بأقل آثار جانبية، وهناك تركيز واضح على الأدوية العشبية.

الكلمات المفتاحية: اللشمانيا المدارية، اللشمانيا الأحشائية، التنقيع، بنتوستام، سانشيزيا سبيشوز