Research Article

Investigation of the In Vitro Therapeutic Potential of *Strigosella africana* Against *Leishmania tropica*

Suad S. Shahatha[1], Abdulwahab Mahmood Abdulazeez[2], Ayad J. Dhulkefl[3], Mustafa M. Fahad[1]*

[1]Center of Desert Studies, University of Anbar, Ramadi 31001, Iraq; [2]College of Pharmacy, University of Al Maarif, Ramadi 31001, Iraq; [3]Department of Occupational Safety and Occupational Medicine Techniques, College of Health and Medical Techniques, Northern Technical University, Kirkuk, Iraq.

Abstract | *Leishmania tropica* is a zoonotic protozoan parasite capable of infecting both humans and animals, with dogs identified as primary reservoir hosts. This study evaluated the in vitro effects of the alcoholic extract of *Strigosella africana* on the growth and development of *L. tropica* promastigotes. The extract exhibited a clear inhibitory effect on promastigote proliferation, reducing the number of generations and prolonging generation time over a 24–96 hour incubation period. Dose-dependent suppression was observed across concentrations ranging from 0.5 to 5.5 mg/ml, with higher concentrations producing a more pronounced decline in parasite count. Notably, the 5.5 mg/ml concentration completely inhibited promastigote growth by 72 hours. The half-maximal lethal dose (LD_{50}) was achieved at 2.5 mg/ml, reducing parasite density to 14.27×10^6 cells/cm^3 compared to 28.54×10^6 cells/cm^3 in the control group. Moreover, the number of generations decreased significantly, reaching 4.21 after 96 hours of treatment, compared to 8.43 in the untreated control. These results demonstrate that the alcoholic extract of *S. africana* possesses potent antiparasitic activity against *L. tropica* promastigotes in vitro. The findings support the potential use of bioactive plant-derived compounds as natural alternatives for developing safer and more effective therapies for parasitic infections. This study highlights the promise of *S. africana* in contributing to plant-based interventions for managing parasitic diseases in animals.

Keywords | *Strigosella africana*, Alcoholic extract, *Leishmania tropica*, Promastigote, Antiparasitic activity

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***Correspondence** | Mustafa M. Fahad, Center of Desert Studies, University of Anbar, Ramadi 31001, Iraq; **Email:** mustafa.moh@uoanbar.edu.iq
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INTRODUCTION

Leishmania is a genus of single-celled, flagellated blood parasites, and more than 30 species parasitize different vertebrate hosts, particularly mammals, of which 20 species infect humans, causing a range of pathological symptoms (Albuquerque-Wendt *et al.*, 2025). This genus belongs to the Trypanosomatidae family and the Zoomastigophora class, which obligately parasitizes within phagocytic cells in the retinal lining of vertebrate hosts (Palacios Cano and Zamora Herrera, 2024). The most common *Leishmania* species is *L. tropica*, which causes skin lesions that start as papules and develop into large ulcers, potentially resulting in permanent scars or severe disFigurement (Peç and da Silva, 2024). This parasite causes cutaneous leishmaniasis, which is known by various local names in endemic regions, such as Delhi boil, Aleppo boil, Baghdad boil, and Oriental boil (Ahmed *et al.*, 2023). The disease is endemic to over 88 countries, with an estimated 350 million people at risk (Shahatha, 2019).

The bite of an infected female sandfly transmits the parasite. Transmission may also occur through contact with crushed insects on the skin (Sultan *et al*., 2023). The parasite exists in two main stages: the amastigote stage, which is found inside the phagocytic cells of the vertebrate host and has a small, circular, or oval shape measuring 2–4 microns in length (Gonzalez-Garcia *et al*., 2025), and the promastigote stage, which is found in the midgut of the vector or culture media, which is characterized by its spindle shape and is equipped with a fine flagellum at its anterior end, which is why it is called the promastigote stage, and its length ranges from 10 to 15 µm (Pescher *et al*., 2025). Recently, research has shifted towards the use of medicinal herbs and plants to treat various diseases, including parasitic diseases. These offer safe therapeutic alternatives to harmful chemical treatments because of the abundance of beneficial compounds, such as volatile oils, alkaloids, flavonoids, glycosides, phenols, resins, and saponins which target pathogens and facilitate their elimination (Shahatha *et al*., 2022).

Strigosella africana, locally known as khazima, belongs to the family *Brassicaceae* (*Cruciferae*). With highly branched stems at the base, this annual herb grows between 15 and 40 cm in height. Plants prefer moist areas and small irrigation ditches, regardless of the soil type. They are commonly found in tree-lined irrigation ditches, swamps near agricultural fields, meadows, and farming oases. Numerous active compounds are found in plants, including volatile oils, flavones, alkaloids, sterols, terpenes, and saponins (Acsad, 2008). The alcoholic extract of the plant has been shown to treat various conditions, including eczema and cutaneous leishmaniasis (Othman and Hamad, 2022). Moreover, it has antimicrobial properties and limits the growth of parasites and fungi (Noor *et al*., 2025). Several studies have investigated the effects of medicinal plants on *L. tropica*. For example, Shahatha and Saleh (Shahatha and Saleh, 2018) used an extract of *Rhanterium epapposum* to inhibit the proliferation of *L. tropica* in vitro. In addition, Balikci *et al*. (Balikci *et al*., 2021) studied the impact of *Pelargonium sidoides* on the promastigote forms of the same parasite in vitro. Furthermore, some researchers have conducted studies to determine the effect of *Carrichtera annua* plant extract on *L. donovani* activity in vitro (Shahatha *et al*., 2024).

In light of the widespread prevalence of *Leishmania tropica* and its severe pathological consequences, this study was conducted to explore the potential of *Strigosella africana* alcoholic extract as an effective alternative to conventional chemical treatments, which are often costly and associated with serious side effects. The research aimed to evaluate the extract's in vitro inhibitory effect on *L. tropica* promastigotes, with the goal of identifying a natural therapeutic option to reduce parasite burden and mitigate the associated health complications.

MATERIALS AND METHODS

Preparing an Alcoholic Plant Extract of S. Africana

A *S. africana* plant, as shown in Figure 1, was obtained from the Zankoura area, northwest of the Ramadi/Anbar province, and was identified at the University of Anbar's Herbarium. The plants were then cleaned and dried. Following the Harborne method (Harborne, 1984), 50 g of dried plant material was mixed with 500 mL of 70% ethanol (10:1 ratio) to prepare an alcoholic extract. The mixture was stirred using a medium-speed magnetic stirrer and was maintained at 40–45°C for 72 h. Subsequently, a Buchner funnel was used to filter it, and the resulting filtrate was collected.

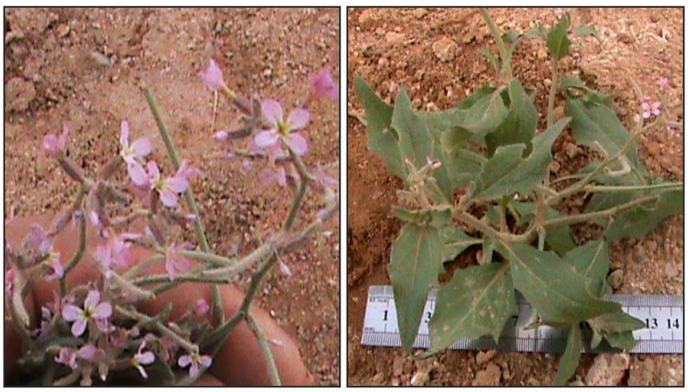

Figure 1: The Khazima plant (*Strigosella africana*).

The solvent was eliminated by evaporation at a moderate speed and 45°C in a rotating evaporator. Chlorophyll pigments were removed using filter sheets, after which the solution was re-evaporated to eliminate residual water; the extract underwent pasteurization at 62°C for ten minutes to obtain a concentrated form, after receiving the standard concentration of the extract, the required experimental concentrations (0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 mg/mL) were made.

Identification of the Plant's Active Ingredients Chemically

Glycoside identification: One milligram of the dried plant extract was combined with ten millilitres of distilled water, filtered, and then a few drops of Fehling's reagent were added. Glycosides were present when red hues appeared.

Tannins identification: Five milliliters of the plant extract were mixed using several drops of lead acid (1%); tannins are available when a white, gelatinous deposit forms.

Volatile oils identification: The presence of volatile oils is shown by the formation of a grey hue when drops of the extract are applied to paper filters until saturated and exposed to UV light.

Flavonoids identification: The desiccated extract (10 g) was solubilized in 50 mL of 95% ethyl alcohol, followed by filtration of the solution. Subsequently, a solution was prepared by combining ten milliliters of 50% ethyl alcohol with ten milliliters of KOH at 50%, and equivalent volumes of the two solutions were mixed. Flavonoids can be identified by their yellow hue.

Alkaloids identification: Plant extracts (10 g) were heated with 50 mL of purified water that had been acidified with 4% HCl, and the mixture was then filtered. After cooling, half a milliliter of this mixture was examined.

Phenol identification: One milliliter of the dry plant extract was combined with one milliliter of a 1% $FeCl_3$ solution. Phenols were indicated when blue or olive hues appeared.

Terpene identification: The existence of terpenes is shown by the formation of a brown precipitate after one gram of the dried extract was diluted in two milliliters of chloroform, and concentrated $H_2SO_4$and anhydrous acetic acid were added.

Saponin identification: When 5 mL of the plant extract was combined with 3 mL of a 1% mercuric chloride ($HgCl_2$) solution, a white precipitate formed, demonstrating the presence of saponins. The identification methods used were based on the procedures described by Han *et al.* (2024).

Development and Calculation of *L. tropica* Promastigotes

The original stock culture of *L. tropica* (strain MHOM/IQ/1992/MREC3) containing promastigote stages, was acquired from the University of Nahrain's Medical Research Center, Medicine College. It was enzymatically characterized using the isoenzyme method (Aljeboori and Evans, 1980). The parasites were cultured in Tobie's medium (Tobie *et al.*, 1950). To cultivate *Leishmania* parasites, 0.1 cm^3 of Locke's solution containing live promastigotes (four days old) was added to glass bottles containing 1.9 cm^3 of liquid medium. The initial cell density was 2 × 10^5 cells/cm^3. The cultures were incubated at 26°C for four days. A Neubauer chamber was used to count promastigotes in each culture. The parasites were fixed while counting by adding 0.1 mL of 10% formalin (0.9 mL to a culture). Normal saline was used as a diluent to promote dense growth. Promastigotes were counted using a light microscope at 40× magnification, yielding a count of promastigote stages per 1 cm^3 of culture (Previti *et al.*, 2024).

Impact of Extract Concentrations on the Leishmania Promastigote

Alcoholic extract concentrations of *S. africana* (0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 mg/mL) were prepared and added to sterile glass bottles containing Tryptone-Soya broth (Tobie's medium). Each solution was inoculated with 2 × 10^5 cells/cm^3 of the *Leishmania* culture.

A control group without extract was also included, where 1.9 cm^3 of sterile Tobie's medium (Tryptone-Soya broth) was placed in sterile glass bottles and inoculated with 0.1 cm^3 of Locke's solution containing 2 × 10^5 *L. tropica* promastigotes per cm^3, without any addition of the plant extract. This setup mirrored the conditions of the treated groups but excluded the *S. africana* extract. The bottles were incubated at 26°C for four days, and promastigote counts were taken at 24, 48, 72, and 96 hours.

And all bottles were incubated at 26°C for four days. Parasite counts were assessed at different growth intervals (24, 48, 72, and 96 h), and the generation number and time were estimated following the method of Benjamin and Garman (Benjamin and Garman, 1993). Each experiment was performed in triplicate (n = 3) to ensure statistical reliability.

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS, 2012). A one-way analysis of variance (ANOVA) was performed to determine significant differences among treatment groups. Means were compared using Duncan's multiple range test at a significance level of $p < 0.05$. Results were expressed as mean ± standard deviation (SD), based on triplicate measurements ($n = 3$). Furthermore, the median inhibitory concentration (LD_{50}) of *S. africana* alcoholic extract against *L. tropica* promastigotes was determined using nonlinear regression analysis performed in IBM SPSS Statistics, version 25.0 (IBM Corp, 2017), based on the dose–response curve generated from parasite counts at various extract concentrations.

RESULTS AND DISCUSSION

This is the first study to utilize *S. africana* against the *L. tropica* parasite in vitro. This lays the groundwork for investigating the potential for therapy using plants and herbs against parasitic infections due to their content of bioactive compounds that can inhibit or eliminate pathogenic parasites.

This study revealed a clear inhibitory effect of the *S. africana* alcoholic extract on *L. tropica* promastigote growth at varying concentrations, in contrast to the control (untreated) group, as illustrated in Figure 2, where promastigotes appeared abundant, slender, and exhibited active motility under the microscope, indicating normal growth and viability.

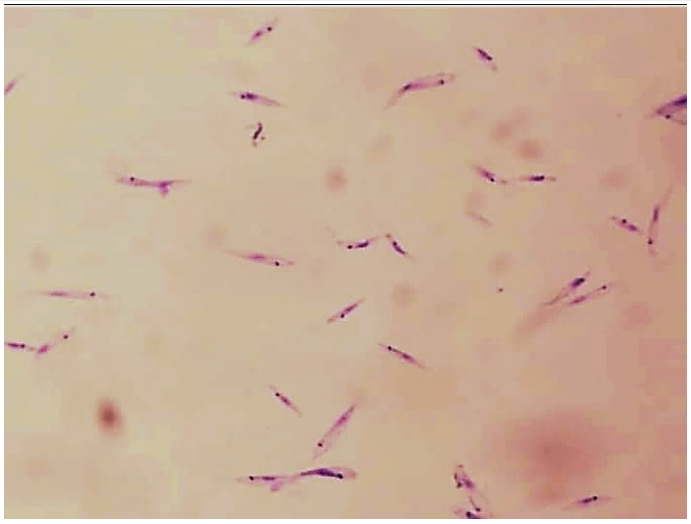

Figure 2: *L. tropica* promastigote, untreated with extract concentrations (control group), observed under 40x magnification.

Table 1: Impact of various concentrations of alcohol extract of *S. Africana* on the number of *L. tropica* ($\times 10^6$) at different growth times (number of promastigotes utilised in culture 2×10^5 cells/cm^3).

Concentration (mg/ml)	24 h (AV ± SD)	GR %	48 h (AV ± SD)	GR %	72 h (AV ± SD)	GR %	96 h (AV ± SD)	GR %
Control (0)	1.24 ± 0.12^a	100	7.8 ± 0.98^a	100	18.01 ± 0.15^a	100	28.54 ± 0.04^a	100
0.5	1.04 ± 0.22^b	82.2	6.5 ± 0.23^b	81.0	17.03 ±0.33^b	80.5	23.66 ± 0.58^b	82.1
1.5	0.89 ± 0.02^c	68.5	5.6 ± 0.91^c	63.0	13.79 ± 0.18^c	58.9	20.80 ± 1.03^c	55.2
2.5	0.62 ± 0.35^c	54.2	3.5 ± 0.16^d	52.9	8.18 ± 0.14^d	49.0	14.27 ± 0.09^d	50.1
3.5	0.51 ± 0.09^d	41.3	2.5 ± 0.78^e	38.2	5.34 ± 0.39^e	25.3	7.08 ± 0.78^e	24.5
4.5	0.38 ± 0.11^e	31.0	1.9 ± 0.10^f	18.6	2.91 ± 0.02^f	14.1	0.00 ± 0.00^f	0.0
5.5	0.20 ± 0.43^f	21.5	1.1 ± 0.22^g	12.6	0.00 ± 0.00^g	0.0	0.00 ± 0.00^f	0.0

* Values are means ± standard deviations (n=3). Different superscript letters within columns indicate significant differences ($p < 0.05$). AV: Average; SD: Standard Deviation; GR%: Growth Rate Percentage.

As shown in Table 1, promastigote counts in the control group increased significantly over time, reaching (1.24, 7.87, 18.01, and 28.54) × 10^5 cells/cm^3 at 24, 48, 72, and 96 h, respectively. In contrast, treatment with various extract concentrations (0.5, 1.5, 2.5, 2.0, 3.5, 4.5, and 5.5 mg/ml) inhibited the growth of promastigotes. A marked reduction in parasite numbers was observed as the extract concentration increased across all time intervals, as the maximum concentration of 5.5 mg/ml entirely stopped parasite growth 72 h after treatment, as demonstrated in Figure 3, including morphological changes and a significant reduction in parasite numbers.

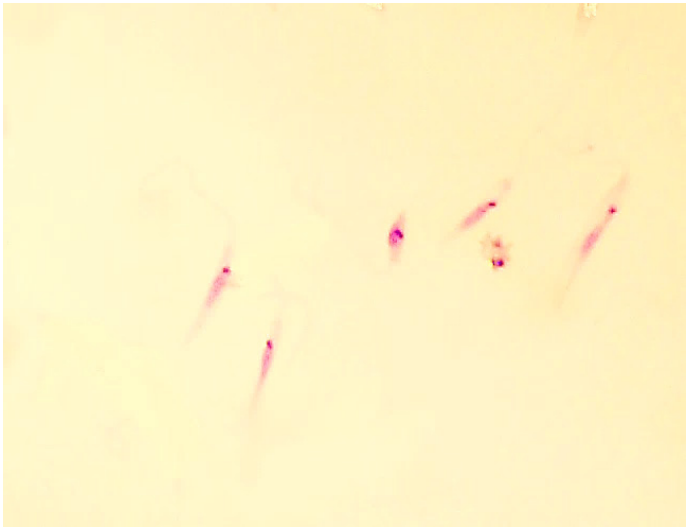

Figure 3: *L. tropica* promastigote treated with 5.5 mg/mL of *S. africana* extract, observed under 40x magnification.

There were noticeable variations ($P< 0.05$) in the growth rates of the promastigotes after treatment compared with the untreated group. After 96 h, 50% inhibition was observed at a 2.5 mg/mL concentration (LD < > < B-B->). The growth percentage also declined at higher doses. At 24 hours, the reduction ranged from 82.2% to 21.5% for 0.5 and 5.5 mg/mL, respectively. At 48 h, the decrease was from 81% to 12.6%, and by 72 h, the growth was reduced from 80.5% to 0%. At 96 h, parasite growth decreased from 82.1% to 0% at the highest concentration, showing significant differences from the untreated control, where no inhibition occurred. A standard antileishmanial drug (e.g., Amphotericin B) was not included as a positive control; future comparative studies are recommended to validate the relative efficacy of the extract.

This aligns with the results of Akya *et al.* (2020) and Badirzadeh *et al.* (2020). The pronounced inhibitory effect of the alcoholic extract of *S. africana* on *L. tropica* promastigote growth is attributed to its rich composition of bioactive substances, including sterols, saponins, volatile oils, alkaloids, flavonoids, and terpenes, which directly interfere with vital cellular functions, including enzyme synthesis and mitochondrial activity, ultimately resulting in cytotoxicity and parasite destruction (Mousa *et al.*, 2024). It may also lead to inactivation of the enzyme dihydrofolate reductase (DHFR), which is responsible for building thymine nucleotides through the de novo pathway, forming the primary sources of DNA, RNA, protein, and phospholipids. In addition, it converts dihydrofolate to tetrahydrofolate, as inhibition of this enzyme leads to inhibition of tetrahydrofolate, thus causing cell poisoning and parasite

death (Salih *et al.*, 2019). Furthermore, the extract also appears to suppress acetylcholinesterase enzyme activity, which governs essential physiological processes in the parasite and regulates ion permeability of the cell membrane, ultimately resulting in its death (Ben Miri, 2025). The proposed mechanism, involving inhibiting enzymes such as dihydrofolate reductase (DHFR) and acetylcholinesterase, is hypothetical. These mechanistic interpretations are based on prior literature related to similar phytochemicals, and no direct biochemical assays were performed in the present study to confirm these effects.

Effect of S. Africana Extract on *L. tropica* Promastigote Generation Number

These findings confirmed the effect of *S. africana's* alcoholic extract of *S. africana* on the promastigote generation number, showing a reduction proportional to the increase in concentration. Notable variances ($P<0.05$) were noted in the number of promastigote generations between the treated and control groups during different incubation periods.

Complete inhibition of generation was observed after 72 h of treatment with 5.5 mg/mL, similarly, 4.5 mg/mL inhibited generation entirely after 96 h. Within 24 hours, the number of generations decreased from 2.62 to 0.38 when using the concentration from 0.5 to 5.5 mg/ml, compared to 2.98 in the control. After 48 h, the number dropped from 4.49 to 0.98 compared to 4.92 in the control group. At 96 hours, 2.5 mg/mL resulted in 4.21 generations, representing the (LD_{50}) calculated using nonlinear regression analysis with IBM SPSS Statistics version 25.0 (IBM Corp, 2017), compared to 8.43 in the control group; these generation differences are detailed in Table 2.

Table 2: Impact of various concentrations of alcohol extract of *S. Africana* on the generation number of *L. tropica* promastigote ($x10^6$) at different times (number of promastigotes utilised in culture 2×10^5 cells/cm^3).

Concentration (mg/ml)	24 h (AV ± SD)	48 h (AV ± SD)	72 h (AV ± SD)	96 h (AV ± SD)
Control (0)	2.98±0.31^a	4.92±0.53^a	7.97±0.63^a	8.43±0.20^a
0.5	2.62±0.21^b	4.49±0.01^b	6.64±0.03^b	7.85±0.65^b
1.5	2.30±0.03^b	4.31±0.12^b	5.71±0.73^c	6.32±0.09^c
2.5	1.45±0.04^c	3.78±0.03^c	3.85±0.06^d	4.21±0.80^d
3.5	1.25±0.10^c	3.11±0.22^d	3.20±0.23^e	2.58±0.24^e
4.5	1.08±0.12^d	2.14±0.93^e	2.23±0.12^f	0.00±0.00^f
5.5	0.38±0.05^e	0.98±0.44^f	0.00±0.00^g	0.00±0.00^f

* Values are means ± standard deviations (n=3). Different superscript letters within columns indicate significant differences ($p < 0.05$).

These results align with those of (Özpinar *et al.*, 2024), which showed similar effects of various plant extracts on the generation rate of promastigotes in vitro. The inhibitory effect of *S. africana* alcoholic extract on the generation number is attributed to its significant suppression of parasite growth, likely due to disrupted reproductive mechanisms, inhibition of genetic material production, and impaired formation of new generations. The bioactive constituents of the extract create suboptimal conditions for parasite development and reproduction (Dalimi *et al.*, 2024).

Effect of S. africana Extract on *L. tropica* Promastigote Generation Time

Table 3 shows the impact of the *S. Africana* on the parasite's generation time. An increase in the extract concentration was associated with a prolonged generation time. The results demonstrated an apparent influence of extract concentration on promastigote generation time, resulting in extended generation periods due to growth inhibition compared to the control group.

Table 3: Impact of concentrations of alcohol extract of *S. Africana* on the generation time (hours) of the *L. tropica* promastigote ($x10^6$) at different times (number of promastigotes utilised in culture 2×10^5 cells/cm^3)

Concentration (mg/ml)	24 h (AV ± SD)	48 h (AV ± SD)	72 h (AV ± SD)	96 h (AV ± SD)
Control(0)	9.50±0.11^a	13.60±0.18^a	15.16±0.05^a	17.35±0.18^a
0.5	10.21±0.60^b	14.12±0.31^b	15.84±0.03^b	17.46±0.11^b
1.5	14.80±0.03^c	16.81±0.72^c	19.31±0.50^c	21.03±0.15^c
2.5	16.77±0.71^d	20.08±0.03^d	23.15±0.06^d	25.64±0.80^d
3.5	19.85±0.09^e	25.21±0.12^e	30.10±0.13^e	32.10±0.34^e
4.5	20.01±0.72^f	29.55±0.33^f	33.27±0.62^f	0.00±0.00^f
5.5	28.10±0.55^g	34.20±0.05^g	0.00±0.00^g	0.00±0.00^f

* Values are means ± standard deviations (n=3). Different superscript letters within columns indicate significant differences ($p < 0.05$).

Concentrations ranging from 0.5 to 5.5 mg/ml increased the generation time from 10.21 hours to 28.10 hours, compared to 9.50 hours in the control. At 48 h, the generation time ranged from 14.12 to 34.20 hours, while the control remained at 13.60 hours. At 72 hours, generation time increased from 15.84 to 33.27 hours at concentrations from 0.5 to 4.5 mg/mL, compared to 15.16 hours in the control. At 96 hours, generation time ranged from 17.46 to 32.10 hours for concentrations between 0.5 and 3.5 mg/mL. These findings align with those of (Al-Doori, 2020), who demonstrated that fruit and leaf extracts from *Melia azedarach* and *Nerium oleander* extended the generation time of *L. tropica* by disrupting its physiology and development. Phytochemical analysis of *S. africana* identified bioactive substances, such as sterols, flavones, alkaloids, saponins,

terpenes, and volatile oils. These compounds completely inhibited promastigote development at the maximum tested concentrations. Yang *et al*. (2024) noted that various medicinal plants contain active ingredients that have antibiotic-like effects on parasites, bacteria, and fungi. However, only qualitative analysis was performed in this study; future work should include quantitative phytochemical characterization using advanced methods such as GC-MS or HPLC.

These results indicate that *S. africana* extracts are essential for the treatment of leishmaniasis. Our results are consistent with some of the findings of previous studies mentioned above and thus confirm the potential future use of medicinal plants as an alternative to anti-leishmaniasis treatments and other parasitic diseases (Aghaei *et al*., 2024). The current study did not include cytotoxicity testing of the extract on mammalian host cells; it is advisable to assess this using cell lines such as Vero or HepG2 in subsequent investigations.

CONCLUSIONS AND RECOMMENDATIONS

This study concludes that the alcoholic extract of *S. africana* is effective and highly efficient in treating *L. tropica* parasites and eliminating them through the significant inhibition of promastigote growth of these parasites in the culture media, resulting from numerous active chemicals that kill the parasites. Hence, it is vital to employ medicinal herbs and plants as safe therapeutic methods to eliminate pathogens and the serious health problems they cause.

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NOVELTY STATEMENTS

This study is the first to report the in vitro antileishmanial activity of Strigosella africana alcoholic extract against Leishmania tropica promastigotes. The research not only demonstrates a dose-dependent inhibitory effect on parasite growth but also quantifies the impact on generation number and duration, providing mechanistic insights. These findings highlight the potential of S. africana as a novel source of bioactive compounds for developing safer, plant-based therapies for parasitic infections in both humans and animals.

AUTHOR'S CONTRIBUTIONS

Suad S. Shahatha: Conceived and designed the study, performed the experimental work, and contributed to data analysis.
Abdulwahab Mahmood Abdulazeez: Assisted in methodology development, supervised laboratory procedures, and reviewed the manuscript.
Ayad J. Dhulkefl: Contributed to data interpretation, prepared figures and tables, and participated in manuscript editing.
Mustafa M. Fahad: Led the overall research project, analyzed and interpreted the results, drafted the manuscript, and performed the final critical revisions.
All authors read and approved the final manuscript.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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