



ISSN: 0067-2904

Evaluating the *in vitro* anti-Leishmanial activity of essential oil extracted from *Cymbopogon Citratus* against *Leishmania Donovanii*

Mahmmoud Shakir Abdullah, Amjed Qays Ibrahim Alqaisi*

Department of Biology, College of Science University of Baghdad, Iraq

Received: 13/6/2021

Accepted: 19/8/2021

Published: 30\4\2022

Abstract:

Leishmania is one of the protozoan parasites that are transferred to human by infected sand flies and gives rise to a range of diseases entitled as Leishmaniasis. More than 20 known species of *Leishmania* can infect humans and cause various clinical symptoms. Three most known clinical manifestations are Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL) and Visceral Leishmaniasis (VL) (kala-azar or black fever). The difference in the clinical form dependent on several factors: species of *Leishmania*, type of vector that transmits the *Leishmania*, and the immune status of an infected individual. The current drugs which are used as anti-leishmanial treatment are characterized by enormous side effects, including their toxicity to human, long term treatment, liver problems and huge cost. Therefore, there is a necessity to find an alternative treatment marked as low cost, more specific against parasite's stages, and metabolic pathways, and non-toxicity to human. Plants are considered one of the important sources for the remedy of the tropical diseases caused by bacteria, viruses, and parasites. *Cymbopogon Citratus* (lemon grass) is a herbal medicine used as anti-inflammatory, antibacterial, antifungal, anti-malarial, anti-protozoal and for gastrointestinal problems remedy. In order to detect the effects of *C. Citratus* against Leishmaniasis, in this study, serial dilution for the essential oil of *C.citratus* (1000, 500, 250, 125, 62.5 and 31.25) µg/ml were used against *L. donovani* Promastigotes. Viability was also evaluated at 24, 48 and 72 hours post-treatment. The results revealed that high concentrations (1000, 500 and 250) µg/ml were more effective than other concentrations during all time intervals, and IC₅₀ values were 640, 492 and 442 µg/ml at 24, 48 and 72 hours respectively. In conclusion, this current study is one of a persistent search to find new treatments characterized by its high activity and low adverse effects to treat protozoa parasites for instance, *Leishmania*, and displays the effectiveness of the essential oils as a promising alternative

Keywords: Medicinal plant, *Cymbopogon Citratus*, Leishmaniasis, *Leishmania Donovanii*, Drug side effect

تقييم النشاط المضاد لطفيلي الليشمانيا مختبريا للزيت الطيارالمستخلص من نبات حشيشة الليمون

Leishmania Donovanii ضد طفيلي *Cymbopogon Citratus*

محمد شاكر عبد الله ، امجد قيس ابراهيم القيسي*

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

*Email: amjeddurham@gmail.com

الخلاصة:

يعد طفيلي الليشمانيا واحد من الطفيليات الابتدائية التي تنتقل إلى الإنسان عن طريق ذباب الرمل المصاب وتسبب مرض يسمى داء الليشمانيات. هناك أكثر من 20 نوعًا معروفًا من الليشمانيا يمكن أن تصيب البشر و تسبب أعراضًا سريرية مختلفة ؛ أكثر ثلاثة مظاهر سريرية معروفة هي داء الليشمانيات الجلدي (CL) وداء الليشمانيات الجلدي المخاطي (MCL) وداء الليشمانيات الحشوي أو ما يسمى (بالكالازار أو الحمى السوداء). يعتمد الاختلاف في الشكل السريري على عدة عوامل: أنواع الليشمانيا ، ونوع الناقل الذي ينقل الليشمانيا ، والحالة المناعية للشخص المصاب. تتميز الأدوية الحالية التي تستخدم كعلاج مضاد لهذا الطفيلي بآثار جانبية كبيرة منها سميتها للإنسان ، كونها علاج طويل الأمد ، تسبب مشاكل في الكبد إضافة إلى تكلفتها الباهظة. لذلك ، هناك ضرورة لإيجاد علاج بديل يتميز بتكلفة منخفضة ، وأكثر تخصصًا ضد أطوار الطفيلي المختلفة ، ومسارات الأيض الحياتي داخل الطفيلي ، وغير سام للإنسان. تعتبر النباتات من المصادر المهمة لعلاج أمراض المناطق المدارية التي تسببها البكتيريا والفيروسات والطفيليات. يعد نبات حشيشة الليمون *Cymbopogon Citratus* دواء عشبي يستخدم كمضاد للالتهابات ، ومضاد للبكتيريا ، ومضاد للفطريات ، ومضاد للملاريا ، ومضاد للطفيليات الابتدائية ، والاضطرابات المعدية المعوية. لغرض التحري عن تأثير نبات حشيشة الليمون ضد داء الليشمانيات ، تم في هذه الدراسة استخدام سلسلة من التخافيف للزيت الطيار لهذه النبتة وهي (1000 ، 500 ، 250 ، 125 ، 62.5 ، و 31.25) مايكروغرام / مل ضد الطور الامامي السوط لطفيلي الليشمانيا وبمدد زمنية مختلفة هي 24 و 48 و 72 ساعة. أوضحت النتائج أن التراكيز العالية (1000 ، 500 و 250) مايكرو غرام / مل كانت أكثر فاعلية من التراكيز الأخرى في جميع الفترات الزمنية ، وأن قيم IC_{50} كانت 640 ، 492 ، و 442 مايكرو غرام / مل عند 24 ، 48 و 72 ساعة على التوالي. كاستنتاج اعتمادًا على نتائج هذه الدراسة والتي تعد واحدة من الدراسات المستمرة لإيجاد علاجات جديدة تتميز بنشاطها العالي وتأثيراتها الضارة المنخفضة لعلاج طفيليات البروتوزوا على سبيل المثال ، الليشمانيا ، وامكانية استخدام الزيوت الطيارة كبديل واعد للعقارات الكيماوية المستخدمة حاليا.

Introduction

The Leishmaniasis are a category of neglected tropical diseases resulting from the genus of *Leishmania* parasite [1]. All genus of this parasite (*Leishmania*) are obligate intracellular that infect macrophage cells of the mammalian hosts [2]. *Leishmania* is transferred to humans through bite of a female of sand fly vectors, *Phlebotomus* (with old-world Leishmaniasis), and *Lutzomyia* (with new world Leishmaniasis) [3].

More than 20 known species of *Leishmania* can infect humans and can cause various clinical symptoms, including three common clinical implications for example, Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), Visceral Leishmaniasis (VL) (kala-azar or black fever). The difference in the clinical form is dependent on several factors like the species of *Leishmania*, type of vector that transmits the *Leishmania*, in addition to the immune status of infected individuals [4,5,6].

The most endemic *Leishmania* species found in Iraq are VL and CL. *L. tropica* and *L. major* are the causative agents the CL [7]. They spread in all country except three provinces in the north of country. The VL in Iraq is caused by *L. donovani* and *L. Infantum*. VL which represents the most serious form of Leishmaniasis spreads widely among children under five years (90 %). Most cases appear in central and some southeastern provinces [8,9,10].

Currently, no certified vaccine against Leishmaniasis is being used in humans. As a result, due to some difficulties relating to the vector control and other factors, all of which are contributed together to increase in the cases that lead to the failure of controlling the spread of the disease [11].

The first medicines that were used for leishmaniasis treatment are antimonial which include Pentostam (Sodium Stibogluconate) and Glucantime (Meglumine antimoniate). However,

various adverse effects as a result of using these drugs, includes their toxicity in humans, prolonged treatment, increased parasite resistance, leukopenia, liver problems, cardio toxicity and cardiac arrhythmia [12]. Furthermore, alternative drugs to antimonials, like amphotericin B, miltefosine, pentamidine and paromomycin also showed side effects including toxicity, high cost and drug resistance. All of these reasons mentioned above lead to drug resistance and therapeutic failure, making it necessary to develop alternative drugs characterized by its lower cost, toxicity, and highest efficacy, and its availability in low-income countries [13].

Natural products from medicinal plants are considered one of the strategies to produce drugs. Estimates have showed that about 25% of contemporary medicines are adapted from medicinal plants instantly or indirectly. Numerous studies have shown that a large variety of plants in many regions in the world contain components that act as anti-protozoan. Various essential oils have demonstrated inhibition activity against certain human parasites such as *Leishmania* [14].

Cymbopogon citratus (DC) is a medicinal plant that belongs to Poaceae family which is extensively used in Southeast Asia, South American countries, and Africa. *C. citratus* is also as anti-inflammatory, anti-pyretic, diuretic, antispasmodic and gastrointestinal disturbances remedy in herbal medicine field. Additionally, a number of studies have revealed the effects of this plant by using it as antibacterial, antifungal, antimalarial and antiprotozoal [15,16,17,18].

According to Machado *et al.*, (2012) [19], the essential oil of *C.citratus* has indicated the cytotoxicity against *Leishmania* parasite.

Material and method

Plant collection:

C.citratus plants were obtained from the herbal center at the University of Baghdad / College of Science / Department of Biology. Plants' aerial parts (leaves) were then dried and divided into small pieces in order to extract the essential oil. The extraction process used for essential oil extraction, stated in the method described in the European Pharmacopoeia (Council of Europe, 1997) [20], depended on the hydro distillation for 3 hours by Clevenger apparatus.

Parasite isolation:

L.donovani isolate (MHOM/IQ/2005/MRU15) was obtained from the Laboratory of Parasitology for graduate studies, University of Baghdad / College of Science / Department of Biology.

Leishmania donovani culture:

Novy-Mac Neal-Nicolle (NNN) medium was used to stimulate the *Leishmania donovani* promastigotes, which were then transferred to RPMI-1640 media accompanied by 10% FBS (gibco®) and 1% Antibiotic (penicillin & streptomycin), and incubated at 26°C with high sterilization conditions to obtain the logarithm phase. Neubauer chamber was used to count the parasite. Finally, the promastigotes in the logarithm phase were concentrated to 2×10^6 for the purpose of the cytotoxicity assay.

Dilution and preparation of concentrations from the crude essential oil extraction of C. citratus:

After crude oils extraction using Clevenger apparatus, the stock was prepared in concentration 10000 µg/ml from essential oil. Desired concentrations (1000, 500, 250, 125, 62.5, and 31.25) µg/ml were then prepared from the stock solution by dissolving these concentrations in 1% Dimethylsulfoxide (DMSO) stored at room temperature away from the light [21].

Anti-leishmanial assay or 3-(4, 5- dimethyl-thiazolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT assay):

One of the techniques used to measure cellular vitality or cytotoxicity potential of drugs is MTT assay or Colorimetric assay. The MTT assay mechanism depends on converting water-soluble yellow tetrazolium MTT [3-(4, 5- dimethyl-thiazolyl-2)-2, 5-diphenyl tetrazolium

bromide] (1 mg / ml) dye that uptakes viable cells into water-insoluble blue formazan. The formazan dye formed under the effect of the mitochondrial enzyme succinate-dehydrogenase within the viable cells. The amount of dye formed is proportional to the number of the viable cells. Produced formazan is dissolved in dimethylsulfoxide (DMSO) to give a purple color with a characteristic absorption at 620 nm. Although [22] mentioned that the range of wavelength ranges from 565 – 630 nm, and 570 is the best wavelength. However, 620 nm is also within the range, and the reason for the use 620 nm in this study depended on the instrument in our lab as it was set up to measure the absorption at 620 nm only. The intensity of the purple color is directly proportional to the number of viable cells [22,23].

The experiment was designed to estimate the effect of serial dilutions for the crude essential oil of *C.citratus* 1000, 500,250,125, 62.5 and 31.2) µg/ml on *L.donovani* promastigotes proliferation by using sterile 96 well plates by following the protocol.

Final volume (50 µl) of the desired crude essential oil concentrations dissolved in 1% DMSO, were added in each well. *L.donovani* promastigotes at $2 \times 10^6 \text{ ml}^{-1}$ in 50 µl of RPMI-1640 media supplemented with 10% FBS, and 1% Antibiotic (penicillin& streptomycin) were added to each well to get the final volume of 100 µl. In addition to the treated promastigotes, the control group used in this experiment were untreated promastigotes. Treated and untreated promastigotes were incubated at 26 c for 24, 48 and 72 hours. By the end of each period time, 10µl of MTT dye was added to each well according to the manufacturer's (iNtRON®). Then the plates were incubated for 4 hours at 26c before reading the results by the ELISA reader Avusturya.

The result was calculated by ELISA reader in microtiter-plate., percentage of the viability was calculated from this equation:

$$\% \text{ Viability} = \frac{\text{Mean absorbance of test sample at 620 nm}}{\text{Mean absorbance of control at 620 nm}} \times 100$$

Thereafter, the inhibitory concentration (IC₅₀) was determined for each time, by using SPSS software.

Statistical Analysis:

The Statistical Analysis System- SAS (2012) [24] program was used in this study to observe the effects of difference factors in this study. Least significant difference – LSD test (Analysis of Variation-ANOVA) was applied to test the significance of relationship between means. Furthermore, Chi-square test was utilized to show the significant correlation between percentage at probability $P \leq 0.01$ and 0.05) in this study. Also IC₅₀ values were calculated by SPSS software and excel 2010.

Results:

Anti-leishmanial activity of C.citratus crude essential oil against L.donovani promastigotes within 24, 48, 72hours.

The results in general revealed the significant inhibitory effects of *C.citratus* crude essential oil on *L. donovani* promastigote proliferation during different time intervals 24, 48 and 72 hours), particularly in high concentration. The results showed that the highest concentrations (1000, 500, 250 µg/ml) had the highest inhibitory effect against parasites. However, 125 µg/ml concentration showed effects only after 72 hours. In addition, no inhibitory effects at the lower concentrations 62.5 and 31.25 µg/ml were shown in all intervals.(Figures 1, 2 and 3) (Tables 1, 2 and 3).

The results demonstrated that the most effective concentrations of the crude extract oil of *C. citratus* in a 24-hour post-treatment were 1000, 500 and 250 µg/ml, and the viability of promastigotes were significant 46%, 51% and 54% respectively, with no inhibitory effect at concentrations 125, 62.5 and 31.25 µg/ml (Table 1) (Figure 1) with IC₅₀ 640 µg/ml.

Table 1: The cytotoxicity effect of *C.citratus* on the viability of *L.donovani* promastigotes at 24-hour post-treatment

Concentrations of <i>C.citratus</i> ($\mu\text{g/ml}$)	Mean \pm SD	Viability percentage (%)
1000	0.0887 \pm 0.0055 b	46 %
500	0.1003 \pm 0.0069 b	51 %
250	0.1065 \pm 0.0233 b	54 %
125	Null	100 %
62.5	Null	100 %
31.25	Null	100 %
Control	0.0715 \pm 0.0044 a	100 %
LSD value	0.0208 *	Chi-Square (χ^2) = 9.892 **
Means having the different letters in same column differed significantly. * (P \leq 0.05), ** (P \leq 0.01).		

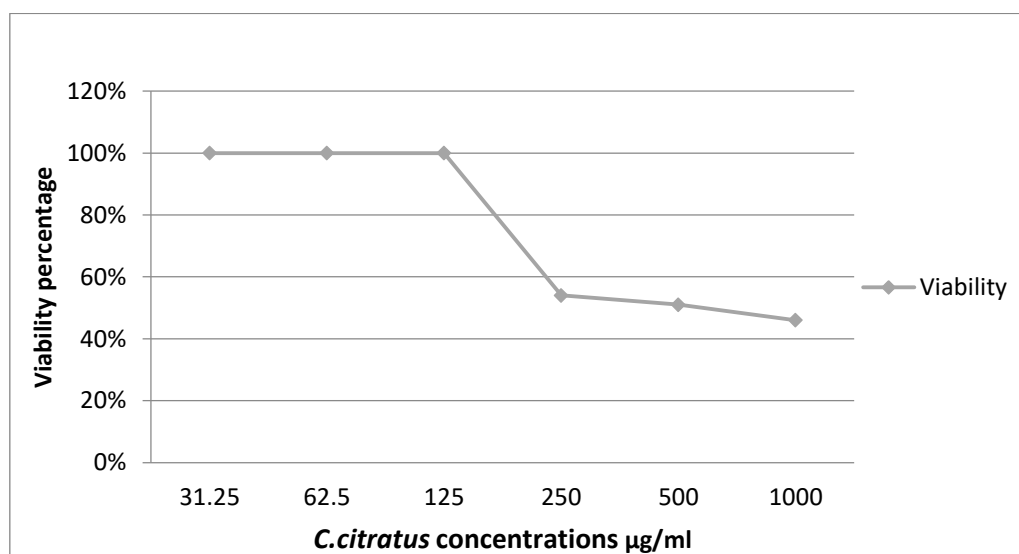


Figure 1- Inhibitory effect of *C.citratus* concentrations against *L.donovani* promastigotes, 24-hour post-treatment

However, the results of a 48-hour promastigotes post-treatment with *C.citratus* crude extract oil revealed that the most potent concentrations were 250, 1000 and 500 $\mu\text{g/ml}$ with no significant differences, and the viability of promastigotes were 37%, 41% and 43% respectively. No inhibitory effect was recorded at concentrations 125, 62.5 and 31.25 $\mu\text{g/ml}$ (Table 2) (Figure 2) with IC_{50} 492 $\mu\text{g/ml}$.

Table 2- Cytotoxicity effect of *C.citratus* on the viability of *L.donovani* promastigotes at 48 hour post-treatment

Concentrations of <i>C.citratus</i> ($\mu\text{g/ml}$)	Mean \pm SD of Absorbency	Viability percentage (%)
1000	0.0785 \pm 0.0019 a	41 %
500	0.0820 \pm 0.0041 a	43 %
250	0.0715 \pm 0.0044 a	37 %
125	Null	100 %
62.5	Null	100 %
31.25	Null	100 %
Control	0.1450 \pm 0.0967 a	41 %
LSD value	0.0747 NS	Chi-Square (χ^2) = 11.064 **
** (P \leq 0.01), NS: Non-Significant.		

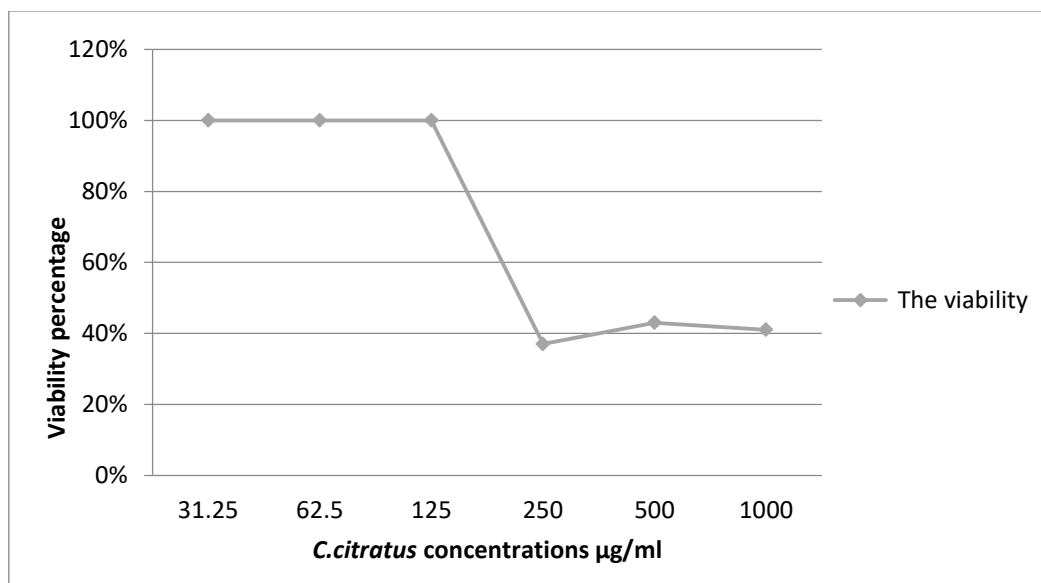


Figure 2- The inhibitory effect of *C.citratus* concentrations against *L.donovani* promastigotes, at 48-hour post treatment

Finally, the results of a 72-hour post-treatment identified that the most efficacious concentrations were 1000, 500, 250 and 125 µg/ml, and the viability were 27%, 29%, 30% and 42% respectively. Furthermore, no inhibitory effect reported at concentrations 62.5 and 31.25 µg/ml (Table 3) (Figure 3) with IC_{50} 442 µg/ml at 72 hours.

Table 3-Cytotoxicity effect of *C.citratus* on the viability of *L.donovani* promastigotes at 72-hour post-treatment

Concentrations of <i>C.citratus</i> (µg/ml)	Mean ± SD of Absorbency	Viability percentage (%)
1000	0.0660 ±0.0066 c	27 %
500	0.0700 ±0.0125 bc	29 %
250	0.0730 ±0.0092 bc	30 %
125	0.1030 ±0.0481 b	42 %
62.5	Null	100 %
31.25	Null	100 %
Control	0.2265 ±0.0152 a	100 %
LSD value	0.0359 *	Chi-Square (χ^2) = 13.754 **

Means having the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).

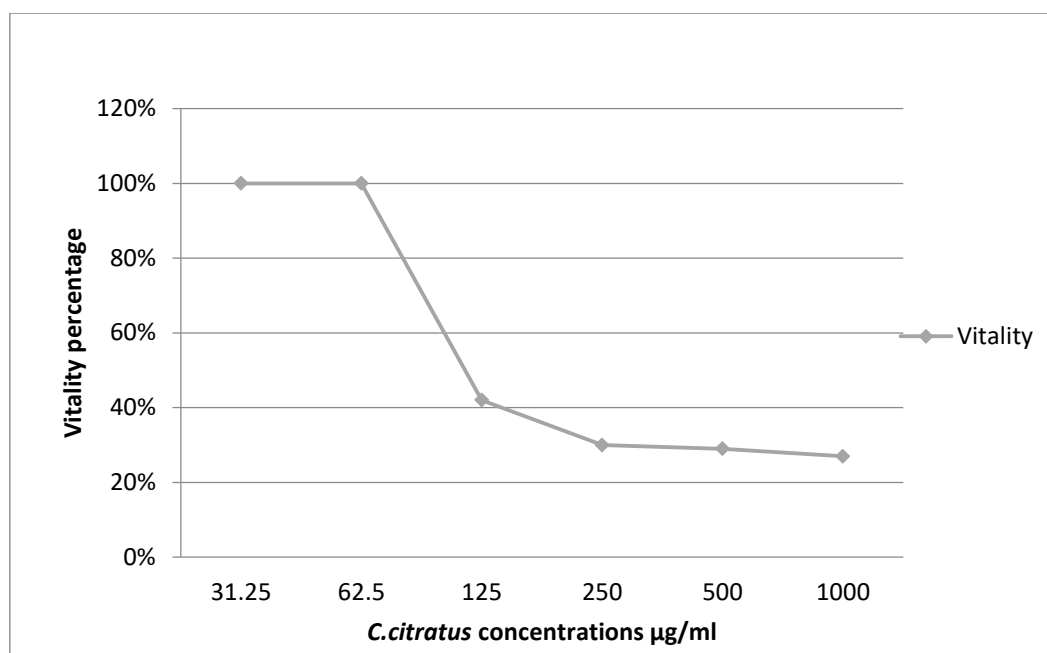


Figure 3-Inhibitory effect of *C. citratus* concentrations against *L. donovani* promastigotes, at 72 hour post-treatment

Discussion:

Pentamidine, Amphotericin-B, Glucantime and other drugs that are being used as anti-leishmanial treatment, are characterized by high cost, enormous side effects, and could lead to the emergence of drug-resistant parasites, ineffective and variable degree of efficacy. All these reasons has increased the efforts to find alternative therapies, strongly marked with inexpensive, safe and being more specific against the pathogen, easily administrated and effective [25].

Recently, the scientific researchers are focusing on medicinal plants, and their leaves that are use in folk medicine as anti-inflammation and anti-microorganisms [21].

These plants produce essential oils (EOs) as secondary metabolites with bioactive properties and contain a wide variety of chemical components characterized by antimicrobial potential and anti-parasites. Previous studies have shown promising results in plant-derived essential oils, crude extracts, and compounds with action against pathogens, including *Leishmania* spp. [26,27]. Moreover, many essential oils of plants have been tested on *Leishmania* in amastigote and promastigote form and epimastigote forms of *Trypanosoma cruzi*. The results have indicated the potency of these medicinal plants including *C. citratus* against *Leishmania* and *T. cruzi* [28]. Furthermore, one of the studies for plant-derived components that are used as an alternative treatment against Leishmaniasis, indicated that the *C. citratus* essential oil is more effective in inhibiting the *Leishmania* growth by inducing an apoptosis process [13].

C. citratus is a herb widely used in the folk medicine in Asia, African and South American countries for the treatment of gastrointestinal disturbances for its antispasmodic, anti-inflammatory, anti-pyretic, diuretic, antibacterial, antifungal, and antiprotozoa properties [18,19,30].

Crude essential oil of *C. citratus* has health restorative capacity, that belongs to its derivatives that work as the secondary metabolite products. In addition, it contains some active components such as citral. It also contains fats, proteins, carbohydrates, fiber, minerals, energy and several other bioactive compounds. Some studies on herbal plants, including *C. citratus*, demonstrated that this plant has an anti-leishmanial activity by stimulating a programmed cell death process by citrus which is considered a major constituent of *C. citratus* essential oil [31].

The results of this study represent first ever study for the effects of essential oil extracted from *Cymbopogon citratus* on *L.donovani* in promastigote stage in Iraq. It also agreed with other studies found *C.citratus* essential oil's proven efficacy as anti-parasites by experimented it on all three evaluative stages of *Trypanosoma cruzi* (trypomastigote, epimastigote and amastigotes). The results have indicated anti-proliferative effect of essential oil on these stages [18]. Besides this, another study has revealed anti-parasites action of *C.citratus* essential oil on the parasites that infected fish [32]. Another study used the essential oil of *C.citratus* against Sarcoptes mite's parasite. This study demonstrated that this oil has mitocidal and ovicidal action against Sarcoptes mites [33].

A study by Machado *et al.*, 2010 [34] proved the activity of some essential oils, including essential oils from *C.citratus*, and its active compounds (myrcene, neral, and geranial) on the growth of *L.infantum* promastigotes.

Also, essential oils of some plants, including *C.citratus* essential oils were experimented *in-vitro* and *ex-vivo* on four types of *Leishmania* parasite., The results demonstrated the activity of *C.citratus* essential oils on the four type (*L. panamensis*, *L. braziliensis*, *L. major*, and *L. guyanensi*). *In-vitro* experiment, however, showed toxic effects against macrophages cell line, though it may not be active against the host cells [35].

Furthermore, anti-Leishmanial activity of *C.citratus* essential oils was studied on *L.chagasi* promastigotes, indicating the effects for the essential oils of *C.citratus* on the parasite viability. Several appearance morphological alterations, including swelling round form and ultra-structure such as aberrant multi-septation of the cell body, were also detected [36].

In another study, major compounds of *C.citratus* essential oil and citral were experimented on *Leishmania amazonensis* *in-vitro* and *ex-vivo*. The results showed the anti-proliferative activity of essential oil on promastigotes and axenic amastigotes, with IC_{50} 1.7 $\mu\text{g/ml}$ [14].

Another study about the anti-leishmanial activity of *C.citratus* by Machado *et al.*(2012) [19], revealed that the major components of *C.citratus* essential oils are myrcene and citral. This study also experimented *in-vitro* and *ex-vivo* on *L. infantum*, *L. tropica* and *L. major* for 24 and 48 hours, IC_{50} concentrations of active components of *C.citratus* essential oils ranging from 25 to 52 $\mu\text{g/ml}$ and IC_{50} of citral from 34 to 42 $\mu\text{g/ml}$. The study indicated that the major components of *C.citratus* essential oils showed an anti-leishmanial activity against three types of *Leishmania* in varying proportions, and the citral was more effective against *leishmania*.The study also refers to the anti-leishmanial effects of *C.citratus* essential oils which occurred by triggering a programmed cell death (apoptosis).

In this study, high concentration of the essential oil of *C. citratus* particularly 1000, 500 and 250 $\mu\text{g/ml}$, and in all intervals 24, 48 and 72 hours, showed the most effective concentrations than the others. Also, a logarithmic rise in the percentage inhibition increased with the concentration range and time period, achieving a highest inhibition approximately 27% and an IC_{50} value of about 442 $\mu\text{g/ml}$ in a 72 hours post-treatment could be due to the high concentration of active compounds that are found in the crude essential oil of *C. citratus* which increased with time period.

The effects of the crude essential oil in this study against *L. donovani* could be due to the fact that the crude essential oil is a network composition of active substances, and a higher concentration of these active compoundlike citral plays an important role in increasing the inhibition of *L. donovani* promastigotes. It agreed with Machado *et al.*, 2012 [19] who suggested that the citral, a major components that found in *C. citratus* crude essential oil, stimulated the anti-leishmanicidal impact in *L. infantum* by activating programmed cell death, in addition to its effect on the mitochondrial membrane, and cell cycle inhibition inside the parasite.

Moreover, a study by Halabi and Sheikh (2014) [37], revealed that the citral found in *C. citratus* has anti-proliferative effect on some cancer cells lines. This agreed with the results of this study where the inhibitory effect of crude essential oil against *L. donovani* also increased. In conclusion, the results of this study demonstrated the potential therapeutic impact of the essential oil of *C. citratus* against *L. donovani*.

References

- [1] E. G., Armitage, A. Q. Alqaisi, G. Joanna, P. Imanol, A. J. Mbekeani, Vanesa A.H., Ángeles L. G., Julio, M., Raquel, G., Paul, W. D., Michael P. B., and Coral B.. "Complex interplay between sphingolipid and sterol metabolism revealed by perturbations to the leishmania metabolome caused by miltefosine." *Antimicrobial agents and chemotherapy*, vol. 62, no. 5, 2018.
- [2] J. Zeng, Q.-W. Chen, Z.-Y. Yu, J.-R. Zhang, D.-L. Chen, C. Song, J. Luo, C. Zhang, S.-L. Wang, and J.-P. Chen, "Regulation of intrinsic apoptosis in cycloheximide-treated macrophages by the Sichuan human strain of Chinese Leishmania isolates," *Acta Tropica*, vol. 153, pp. 101–110, 2016.
- [3] E. Torres-Guerrero, M. R. Quintanilla-Cedillo, J. Ruiz-Esmenjaud, and R. Arenas, "Leishmaniasis: a review," *F1000Res.*, vol. 6, p. 750, 2017.
- [4] A. Balahbib, N. E., A. S., Y. B., and A. B, "Antileishmanial Properties of Moroccan Medicinal Plants and Mechanism Insights of their Main Compounds." .
- [5] M. Lipoldová and P. Demant, "Genetic susceptibility to infectious disease: lessons from mouse models of leishmaniasis," *Nat. Rev. Genet.*, vol. 7, no. 4, pp. 294–305, 2006.
- [6] W. C. Marquardt, R. S. Demaree, and R. B. Grieve, *Parasitology and Vector Biology*, 2nd ed. San Diego, CA: Academic Press, 1999.
- [7] M. J. Al-Obaidi and H. B. Ali, "Evaluation of the surveillance and epidemiological aspects of cutaneous leishmaniasis in Babylon province, Iraq," *Iraqi J. Sci.*, pp. 793–800, 2021.
- [8] B. Majeed, J. Sobel, A. Nawar, S. Badri, and H. Muslim, "The persisting burden of visceral leishmaniasis in Iraq: data of the National Surveillance System, 1990-2009," *Epidemiol. Infect.*, vol. 141, no. 2, pp. 443–446, 2013.
- [9] N. Salam, W. M. Al-Shaqha, and A. Azzi, "Leishmaniasis in the middle East: incidence and epidemiology," *PLoS Negl. Trop. Dis.*, vol. 8, no. 10, p. e3208, 2014.
- [10] M. M. Salih and K. H. Zghair, "The Cytotoxic Effect of ZnO Nps Against the Intracellular Amastigotes of *Leishmania donovani* in vitro," *Iraqi J. Sci.*, vol. 58, no. 4C, 2017.
- [11] P. A. Farias-Junior *et al.*, "Leishmanicidal activity of carvacrol-rich essential oil from *Lippia sidoides* Cham," *Biol. Res.*, vol. 45, no. 4, pp. 399–402, 2012.
- [12] E. E. Zijlstra, F. Alves, S. Rijal, B. Arana, and J. Alvar, "Post-kala-azar dermal leishmaniasis in the Indian subcontinent: A threat to the South-East Asia Region Kala-azar Elimination Programme," *PLoS Negl. Trop. Dis.*, vol. 11, no. 11, p. e0005877, 2017.
- [13] A. Oryan, "Plant-derived compounds in treatment of leishmaniasis," *Iran. J. Vet. Res.*, vol. 16, no. 1, pp. 1–19, Winter 2015.
- [14] M. R. Santin, A. O. dos Santos, C. V. Nakamura, B. P. Dias Filho, I. C. P. Ferreira, and T. Ueda-Nakamura, "In vitro activity of the essential oil of *Cymbopogon citratus* and its major component (citral) on *Leishmania amazonensis*," *Parasitol. Res.*, vol. 105, no. 6, pp. 1489–1496, 2009.
- [15] M. Mounni *et al.*, "Antifungal activity and chemical composition of seven essential oils to control the main seedborne fungi of cucurbits," *Antibiotics (Basel)*, vol. 10, no. 2, p. 104, 2021.
- [16] K. Moore-Neibel, C. Gerber, J. Patel, M. Friedman, and S. Ravishankar, "Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens: Lemongrass oil against *Salm. enterica*," *J. Appl. Microbiol.*, vol. 112, no. 3, pp. 485–492, 2012.

- [17] G. Shah, R. Shri, V. Panchal, N. Sharma, B. Singh, and A. S. Mann, "Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass)," *J. Adv. Pharm. Technol. Res.*, vol. 2, no. 1, pp. 3–8, 2011.
- [18] G. F. Santoro, M. G. Cardoso, L. G. L. Guimarães, J. M. Freire, and M. J. Soares, "Anti-proliferative effect of the essential oil of *Cymbopogon citratus* (DC) Stapf (lemongrass) on intracellular amastigotes, bloodstream trypomastigotes and culture epimastigotes of *Trypanosoma cruzi* (Protozoa: Kinetoplastida)," *Parasitology*, vol. 134, no. Pt 11, pp. 1649–1656, 2007.
- [19] M. Machado, P. Pires, A. M. Dinis, M. Santos-Rosa, V. Alves, L. Salgueiro, C. Cavaleiro, and M. C. Sousa, "Monoterpenic aldehydes as potential anti-*Leishmania* agents: Activity OF *Cymbopogon citratus* and Citral on *L. infantum*, *L. tropica* and *L. major*" *Experimental Parasitology*, vol. 130, no. 3, pp. 223–231, 2012.
- [20] Council of Europe. Methods of pharmacognosy. In: European Pharmacopoeia. European Department for the Quality of Medicines, Strasbourg, 1997
- [21] G. Mohammadpour, E. T. Marzony, and M. Farahmand, "Evaluation of the anti-*Leishmania major* activity of *Satureja bakhtiarica* essential oil in vitro," *Nat. Prod. Commun.*, vol. 7, no. 1, pp. 133–136, 2012.
- [22] P. W. Sylvester, "Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability," *Methods Mol. Biol.*, vol. 716, pp. 157–168, 2011.
- [23] A. Bahuguna, I. Khan, V. K. Bajpai, and S. C. Kang, "MTT assay to evaluate the cytotoxic potential of a drug," *Bangladesh J. Pharmacol.*, vol. 12, no. 2, p. 8, 2017.
- [24] N. Cary. "Statistical Analysis System, User's Guide. Statistical. Version 9. SAS. Inst." *Inc. USA* (2012).
- [25] H. Iqbal. "Comparative efficacy of *Aloe vera* and *Tamarix aphylla* against cutaneous leishmaniasis." *International Journal of Basic Medical Sciences and Pharmacy (IJBMS)*, vol. 2, no. 2, 2012.
- [26] M. A. O. Dawood, M. F. El Basuini, A. I. Zaineldin, S. Yilmaz, M.T. Hasan, E. Ahmadifar, A. M. El Asely, H. M. R. Abdel-Latif, M. Alagawany, N.M. Abu-Elala, H. V. Doan and H. Sewilam. "Antiparasitic and antibacterial functionality of essential oils: An alternative approach for sustainable aquaculture," *Pathogens*, vol. 10, no. 2, p. 185, 2021.
- [27] D. S. Alviano, A. L. S. Barreto, F. d. A. Dias, I. d. A. Rodrigues, M. d. S. d. S. Rosa, C. S. Alviano, and R. M. d. A. Soares "Conventional therapy and promising plant-derived compounds against trypanosomatid parasites," *Front. Microbiol.*, vol. 3, p. 283, 2012.
- [28] P. S. Luize, T. S. Tiunan, L. G. Morello, P. K. Maza, T. Ueda-Nakamaru, B. P. D. Filho, D. A. G. Cortez, J. C. P. de Mello, and C. V. Nakamura. "Effects of medicinal plant extracts on growth of *Leishmania (L.) amazonensis* and *Trypanosoma cruzi*," *Rev. Bras. Ci. Farm.*, vol. 41, no. 1, pp. 85–94, 2005.
- [29] R. Irkin and M. Korukluoglu, "Effectiveness of *Cymbopogon citratus* L. essential oil to inhibit the growth of some filamentous fungi and yeasts," *J. Med. Food*, vol. 12, no. 1, pp. 193–197, 2009.
- [30] L. Mayaud, A. Carricajo, A. Zhiri, and G. Aubert, "Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics," *Lett. Appl. Microbiol.*, vol. 47, no. 3, pp. 167–173, 2008.
- [31] S. S. Ranade, and P. Thiagarajan. "Lemon Grass." *Int. J. Pharm. Sci. Rev. Res*, vol. 35, pp. 162-167, 2015.
- [32] N. Anjur., and S. F. Sabran. "Potential of Lemongrass (*Cymbopogon citratus*) Extract as Antiparasite in *Tilapia* (*Oreochromis niloticus*) Culture." *copyright© 2019 by Innovation Centre in Agritechology for Advanced Bioprocessing, Universiti Teknologi Malaysia*, vol. 53, 2019.
- [33] M. Machado, G. Santoro, M. C. Sousa, L. Salgueiro, and C. Cavaleiro, "Activity of essential oils on the growth of *Leishmania infantum* promastigotes," *Flavour Fragr. J.*, vol. 25, no. 3, pp. 156–160, 2010.

- [34] M. Li, B. Liu, C. Bernigaud, K. Fischer, J. Guillot, and F. Fang, "Lemongrass (*Cymbopogon citratus*) oil: A promising miticidal and ovicidal agent against *Sarcoptes scabiei*," *PLoS Negl. Trop. Dis.*, vol. 14, no. 4, p. e0008225, 2020.
- [35] J. Sanchez-Suarez, I. Riveros, and G. Delgado, "Evaluation of the leishmanicidal and cytotoxic potential of essential oils derived from ten colombian plants," *Iran. J. Parasitol.*, vol. 8, no. 1, pp. 129–136, 2013.
- [36] V. C. S. Oliveira, D. M. S. Moura, J. A. D. Lopes, P. P. de Andrade, N. H. da Silva, and R. C. B. Q. Figueiredo, "Effects of essential oils from *Cymbopogon citratus* (DC) Stapf., *Lippia sidoides* Cham., and *Ocimum gratissimum* L. on growth and ultrastructure of *Leishmania chagasi* promastigotes," *Parasitol. Res.*, vol. 104, no. 5, pp. 1053–1059, 2009.
- [37] M. F. Halabi and B. Y. Sheikh, "Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract," *Biomed Res. Int.*, vol. 2014, p. 906239, 2014.