Phytochemical Screening, in Vitro Antilishmanial Activity Of Conyza Canadensis Extract By Neopterin

Abstract:

Background: Leishmaniasis is a parasitic disease caused by the Leishmania parasite, transmitted to humans through the bite of an infected sand-fly. The disease manifests in four primary clinical manifestations: visceral, cutaneous, diffuse cutaneous, and post-kalaazar dermal. The disease's clinical manifestations depend on the Leishmania species and their immune response. Current antileishmanial drugs include amphotericin B, antimonials, sitamaquine, pentamidine, paromomycin, and miltefosine. However, these drugs have drawbacks, such as resistance to pentavalent antimonials and nephrotoxicity. The World Health Organization suggests using plants as a healing agent with good efficacy and affordability. Conyza Canadensis, a biennial plant, has been used for wound healing and has shown efficacy against gram-positive and gram-negative bacteria.

Objective: The study aimed to investigate the anti-leishmanial activity of extracts from Conyza Canadensis, on Iraqi strain of Leishmania tropica.

Material and method : The phytochemical compounds were found in the extraction process, with total alkaloid content, phenolic compounds, flavonoid content, amino acid analysis, and total glycosides. The extract was prepared into stock solutions and sub-cultured in RPMI-1640. The cells were then incubated in tissue culture flasks and treated with different concentrations of SSG and aqueous/alcoholic extracts. The anti-leishmanial activity of the extracts was then evaluated using the sandwich enzyme-linked immunosorbent assay (ELISA) technique. The results showed that the extracts had anti-leishmanial activity.

Result: the study shows that Iraqi C. canadensis plant extract contains alkaloids, gallic acid, apigenin, chlorogenic acid, caffeineic acid, quercetin, p-cumaric acid, and GSH, which reduce neopterin levels and activate immune response against L. tropica.

Conclusion: Conyza Canadensis extracts show anti-leishmanial activity, correlated with neopterin levels, promising as an alternative therapy for leishmaniasis. Further research is needed for effectiveness.

Keywords: Apigenin, Caffeic acid, *Chlorogenic acid, Conyza Canadensis*, Gallic acid,

Introduction:

Leishmaniasis is a parasitic disease caused by the Leishmania parasite, which is transmitted to humans through the bite of an infected sand-fly [1]. In humans, leishmaniasis can manifest in a variety of ways, from superficial, inflammatory lesions on the skin to more serious and sometimes lethal infections of the internal organs. There are four primary clinical manifestations of the disease: visceral (LV or calazar), cutaneous (LCM), diffuse cutaneous (DCL), and post-kalaazar dermal (PKDL). The clinical manifestations depend on the Leishmania species[2]. *Leishmania spp.* has two stages: promastigote (PRO) and amastigote (AMS). The Phlebotomus sand fly acts as both an intermediate host and a vector during the life cycle. Different *Leishmania types* elicit different immune responses.[3]. During the life cycle of Leishmania, the AMS undergoes binary fission in the midgut of the sand-fly and eventually transforms into a PRO. The PRO then migrates to the pharynx of the sand-fly and prepares for transmission to a new host. When the sand-fly feeds on the blood of a vertebrate host, the PRO is injected into the host's skin.[4], [5]. It is believed that a sand-fly entire life cycle lasts for approximately ten days [6].

The sand-fly injects metacyclic PRO and saliva into the host's bloodstream during blood-feeding. The saliva contains biologically active components that modify the immune response and influence parasite infection. Neutrophils and monocytes/macrophages then infiltrate the bite site [7] .

When PRO comes into contact with host cells, macrophages engulf them. Once inside, PRO undergoes a process of differentiation into AMS and proliferates within phagolysosomes. Leishmania must adapt metabolically and resist host immune system assaults [8]. Eventual macrophage lysis and the subsequent release of infectious stages that can invade other cells. The parasite's life cycle within the human host persists until another sand-fly acquires a blood meal from the afflicted individual, completing the cycle [9].

Macrophage and monocyte activation and release The biosynthesis of NEO, a pteridine, from guanosine triphosphate reflects the cellular immune system going into overdrive. The pro-inflammatory IFN-γ produced after T-lymphocyte activation is the primary factor that triggers NEO formation [10]. When the parasite infection is active, NEO release increases in VL as a result of activated macrophages and an increase in macrophage load. As the infection wanes, however, NEO release decreases [11].

The quantity of artificially produced NEO is directly correlated with the quantity of IFN-γ and indirectly suggests an elevation in TNF-α. There is a strong connection between the excessive synthesis of NEO neopterin and the stimulating impact of these cytokines on the metabolic activity of immune cells. Furthermore, NEO plays a part in the mechanism by which activated macrophages exert their cytotoxic action. NEO levels are indicative of the interaction between several cytokines in the monocyte/macrophage population. [12] .

Current antileishmanial drugs include amphotericin B, antimonials, sitamaquine, pentamidine, paromomycin, and miltefosine. Immunomodulatory antileishmanial drugs boost the innate immune system [13]. The treatment regimen has significant drawbacks. Pentavalent antimonials are the primary treatment for visceral leishmaniasis. Pentavalent antimonials (SbV) are available commercially as sodium stibogluconate (SSG) and meglumine antimoniate (MA). Resistance reduced the efficacy of this treatment [14]. Despite its effectiveness in treating visceral leishmaniasis resistant to pentavalent antimony, the injection and harmful side effects of pharmaceuticals make them toxic and sometimes fatal. [15]. Leishmanicidal efficacy of amphotericin B (AmB) reduces treatment failures and relapses. This medicine is not recommended as a first treatment since it causes nephrotoxicity and requires parenteral delivery [18,][9].

The World Health Organization (WHO) claims that using plants is the only viable path towards evolving a healing agent with a good efficacy in terms of safety and affordability to address various health issues. Clinical experiments have yielded empirical data indicating the efficacy of specific indigenous flora in combating cutaneous leishmaniasis and their potential utility in facilitating wound healing by applying herbal ointments. When native plants are abundant, they may be more cost-effective than synthetic medications. This circumstance can benefit developing nations where the prevalence of the disease is high [17].

The wealth herb grows naturally in various regions, including North of Baghdad, Baquba, Kut, Rustam, Mosul, Abu Ghriab, Rowanduz, Za'franiya, Pushtashan, Qerna Qaw valley, North-east of Zakho, and 50 km from Basra to Nassiriya. [18]. Conyza canadensis is a biennial plant. [19]. HPLC analysis of its extract confirms the presence of quercitrin, quercetin, apigenin, p-coumaric acid, and caffeic acid [20].

C. canadensis has been employed for the management of wounds, swellings, arthritis-related pain, inflammation, diarrhoea, and microbial infections such as urinary and respiratory tract infections (20, [3]). Various studies have demonstrated the efficacy of the plant's ethanolic, methanolic, chloroform, and ethyl acetate fractions against both gram-positive and gram-negative bacteria. The text is enclosed in tags. Furthermore, the plant exhibits noteworthy pharmacological activity, including anti-inflammatory, anticoagulant, anti-gastric ulcer, anti-diabetic, antioxidant, anti-cancer, and mutagenesis activities. [20].

Aim of study: The study aimed to determine the anti-leishmanial properties of Leishmania tropica of extracts from the Iraqi Conyza Canadensis.

Material and Method:

The U937monocyte was supplied by "National Cell Bank of Iran." The cells were grown in "Roswell-Park Memorial Institute (RPMI-1640)" liquid medium (Gibco, UK) containing 10% fetal bovine serum (Gibco, UK) Phorbol Myrestate Acetate (PMA) (InvevoGenes, USA). Stibogluconate (Pentostam®) (GSK, UK), *Conyza Canadensis* was harvested from the mountains of Sulymaniyah at the end of the winter, and neopterin level was measured by ELISA Kit (Elabscience/USA). The Iraqi strain of Leishmania tropica was obtained from the parasitology laboratory, graduate studies, department of biology, college of science, and the University of Baghdad. The strain was then cultured in a biphasic medium (Nove-MacNeal-Nicolle) (NNN).[22]

1: Finding of the phytochemical compound in the extraction of Conyza Canadensis :

1:Total Alkaloid Content:

Extraction: A total of 20 grammes of plant material was subjected to extraction using methanol for a duration of 24 hours, utilising a Soxhlet equipment. The extract underwent filtration, and the methanol was removed through evaporation using a rotary evaporator set at a temperature of 45°C.

1. Qualitative Estimation (Test for Alkaloids):

1. Alkaloids were confirmed using Dragendroff's method. The extract was melted in dilute HCL, and Dragendorff's reagent was added. A crystalline precipitate indicated alkaloid presence. Positive samples were quantitatively evaluated[23].

2. Quantitative estimation of Alkaloid:

2. The residue was dissolved in 2N HCL, filtered, and combined with a Bromocresol Green solution and phosphate buffer. The resulting mixture was washed with chloroform and its pH level was adjusted using 0.1N NaOH. To create the standard curve, an Atropine standard solution was mixed with phosphate buffer and BCG solution, and then shaken with the extract and chloroform. The resulting solution was collected, and diluted with chloroform, and its absorbance was calculated at 470 nm in a UV-Spectrophotometer against the blank[24].

2: Determination of total phenolic compounds

Phenolic compounds were detected in an ethanolic extract using the Folin-Ciocalteu reagent. The mixture contained extract, reagent, and sodium carbonate. After 2 hours, the phenolic content was estimated using the absorbance at 765 nm and a calibration curve made with gallic acid [25].

3: analysis of Total flavonoid content

The flavonoid content was measured using the aluminum chloride colorimetric method. A sample of crude extract was mixed with $NaNO_2$ and $AlCl_3$ solutions, and NaOH was added to bring the final volume to 10 mL. After 15 min, absorbance was measured at 510 nm, and the flavonoid content was calculated as mg rutin equivalent per g dry weight[26].

4: Analysis of amino acid:

Solid samples weighing approximately 5 mg with a precision of 0.01 mg and liquid samples weighing approximately 100 mg with an accuracy of 0.01 mg were hydrolyzed with 1 ml of 6M hydrochloric acid solution at 100°C ± 20°C for 24 hours. After hydrolysis, the amino acid residues were dissolved in 100 µl of acetonitrile and derivative with 100 µl of OPA. The sample was then injected ten times (100 µl per injection) into a gas chromatograph with a C18-ODS column and a fluorescence detector (Ex = 445 nm, Em = 465 nm) using an isocratic flow of 50/50 (v/v) water (pH 7.0) and acetonitrile at a flow rate of 1.0 mL/min [27].

5: Total glycosides:

In order to ascertain the presence of glycosides, the extracted substance was blended with Baljet's reagent and allowed to sit for an hour. Following this, it was mixed with water and measured at a wavelength of 495 nm [28].

2: The preparation of the stock solution

1. The preparation of the Conyza canadensis aqueous extract stock solution (AqCC) involved dissolving 1 milligramme of extract in 10 millilitres of pyrogen-free Distel water. Subsequently, six serial dilutions were created, with concentrations of 62.5, 125, 250, 500, 1000, and 2000 micrograms per millilitre.
2. In order to create alcoholic extract of Conyza canadensis stock solution (AqCC), one milligramme of extract from Conyza canadensis was dissolved in three millilitres of methanol. The next step involved the creation of six different serial dilutions, each with a concentration of 62.5, 125, 250, 500, 1000, and 2000 µg/ml.
3. We employed a stock solution of stibogluconate (Pentostam®) SSG at a concentration of 100 mg/ml, and then proceeded to do six serial dilutions at concentrations of 62.5, 125, 250, 500, 1000, and 2000 µg/ml.

3: Cell lines preparing and sub-culturing

Monocyte cell lines have been sub-cultured for use as an in vitro model to evaluate the anti-leishmanial activity of AlCC and AqCC. The media used was RPMI-1640, supplemented with antimicrobial drugs such as gentamicin or penicillin (50µg/ml) and 5% fetal bovine serum. After that, the cells were kept in an incubator at 37 degrees Celsius for twenty-four hours. [29].

4: The development of a "macrophage-like" state in U937 monocyte:

To activate NF-κB, dissolve 5mg PMA powder in 1.5ml endotoxin-free water. Store at -20 °C, protected from light, avoiding repeated freeze-thaw cycles. Add 1µl PMA solution to U937 Monocyte cell lines and incubate at 37°C for 24 hours [30]. PRO was used to infect macrophages from U937 in a stationary growth phase at a ratio of 20:1. The mixture was incubated in tissue culture flasks at 34°C, 5% CO2, and 95% relative humidity. Following a period of twelve hours, non-internalized PRO was eliminated by washing the cells five times with RPMI in its simple form. The cells were further incubated for 96 hours in RPMI10% FCS. After treating the cell line with different concentrations of SSG and aqueous/alcoholic extracts, it was incubated at 37°C for 24 hours [31].

5: Detection of Neopterin by Enzyme-Linked Immunosorbent Assay (ELISA):

The study investigated the anti-leishmanial activity of extracts by introducing U973 macrophage cells into individual wells of a 96-well microplate. Following an overnight treatment, PRMO of L. tropica was given to macrophages and cultured at a temperature of 37 °C for 96 hr. Afterwards, the cell line was exposed to varying doses of SSG, as well as aqueous and alcoholic extracts, and then incubated at a temperature of 37 °C for 24 hours. Afterward, the liquid portion of the macrophage cells that had been treated was gathered from each well and transferred into distinct eppendorf tubes assigned to each group. The quantity of nitric oxide and neopterin was subsequently evaluated utilising the sandwich enzyme-linked immunosorbent assay (ELISA) technique. (34, [33]**.**

Statistical Analysis:

The data had been computed employing the SPSS VERSION.26 Student T-Test Software program. The threshold for statistical significance had been established at a P value of ($p \leq 0.05$).

Result:

The result of HPLC analysis of the extract of C. Canadensis:

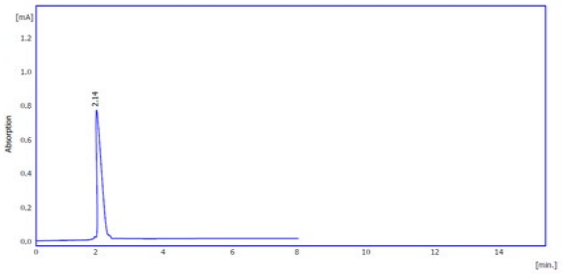
The outcomes of the revision indicate the presence of six unique peaks in the chloroform fraction of the Iraqi C. canadensis plant. These peaks were identified through the analysis of aqueous and alcoholic extractions, as depicted in "Figure. 2" and "Figure. 3", respectively. Each peak represents a different type of active compound present in the plant. To further validate the results, the retention time of each peak in the chloroform fraction was compared to the standard used, as shown in "Figure. 1". This comparison confirmed the presence of these active compounds in the plant, which are the same as those found in the standard. These findings are summarized in "Table 1". Samples show three peaks as shown in "Figure. 4" and "Figure. 5" at (3.5 -8) mint and in comparing it with the slandered curve of GSH, as shown in "Figure. 1 G", the present of GSH.

Table 1 Retention time of the active compound in comparison with the standard

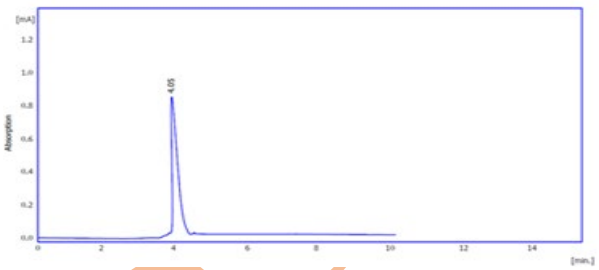
Name	**Retention time in a chloroform fraction in mint**		
	Standard	AlCC	AqCC

Gallic acid	2.14	2.14	2.19
Apigenin	11.95	11.98	11.95
Chlorogenic acid	4.05	4.09	4.08
Caffeic acid	6.1	6.01	6.00
Quercetin	8.00	8.07	8.01
p-Coumaric acid	9.92	9.92	9.91
GSH	3.89	3.88	3.80

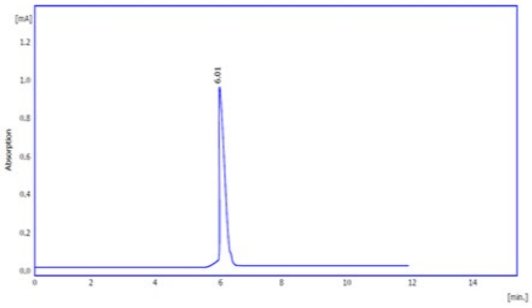
A



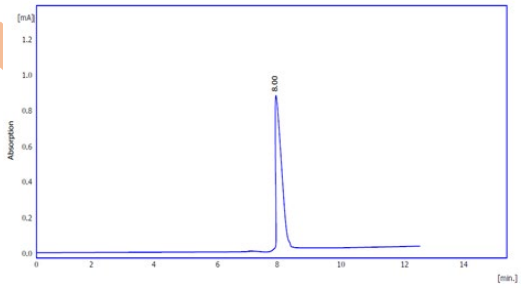
B



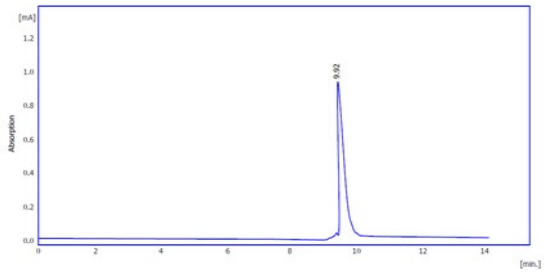
C



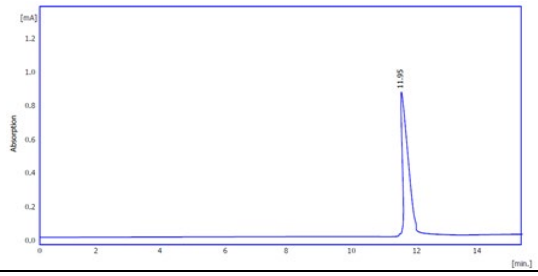
D



E



F



G

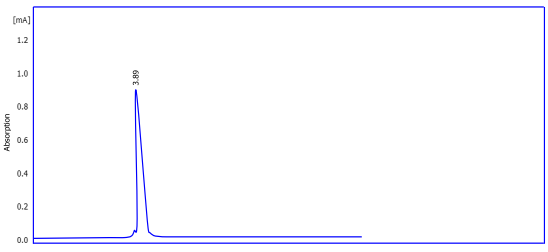


Figure 1 shows the HPLC chromatogram of quercitrin, and apigenin standards(A \gallic acid B\ Chiorganic acid, C\ caffic acid, D\ qurecetin, E\ p-cumaric acid, and F\ apigenin G\ GSH)

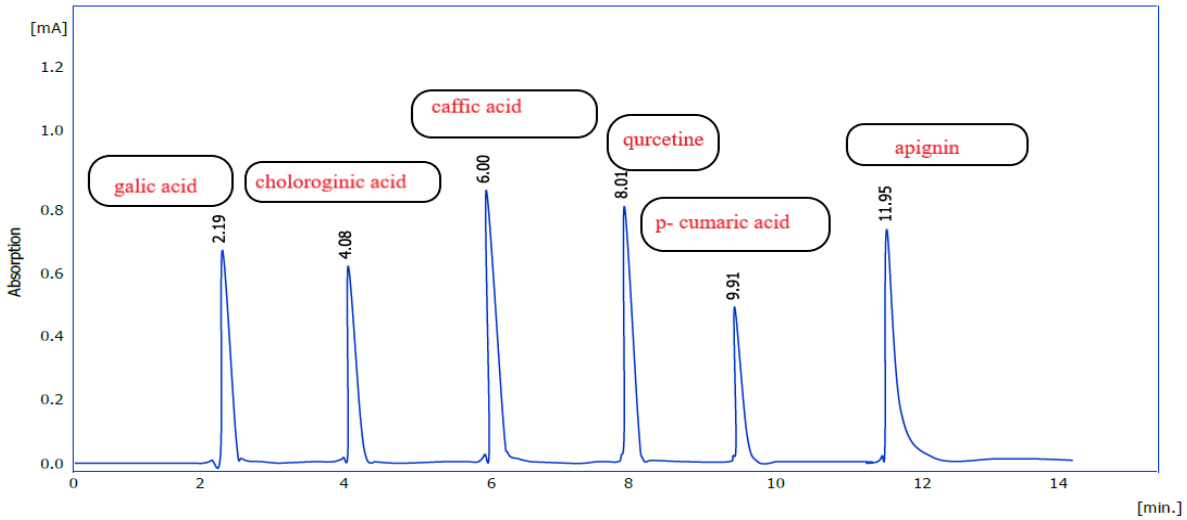


Figure 2 illustrates the HPLC chromatogram of AqCC

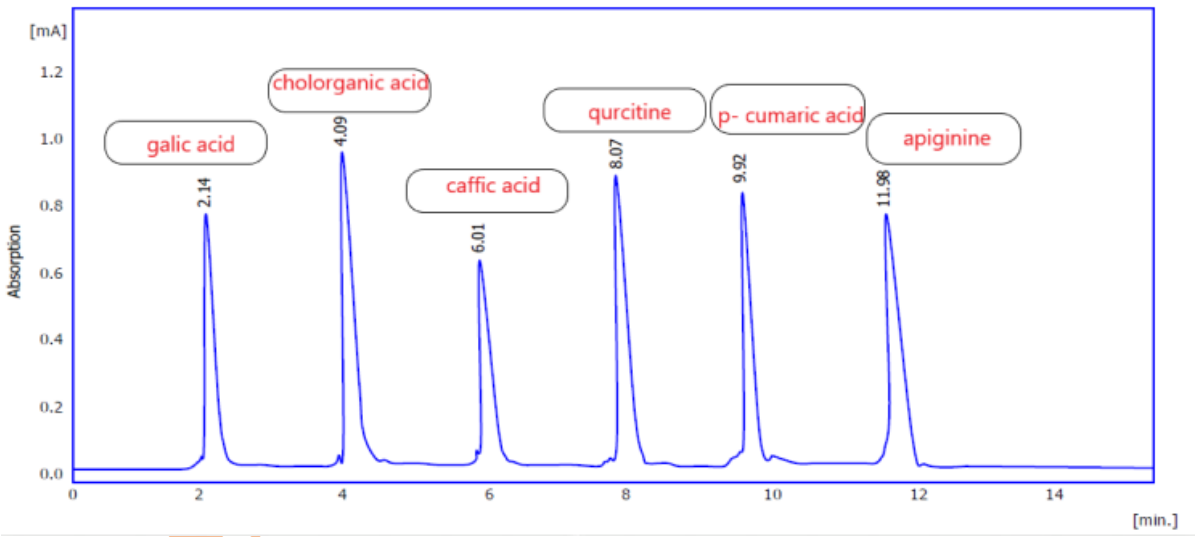


Figure 3 shows the HPLC chromatogram of AlCC

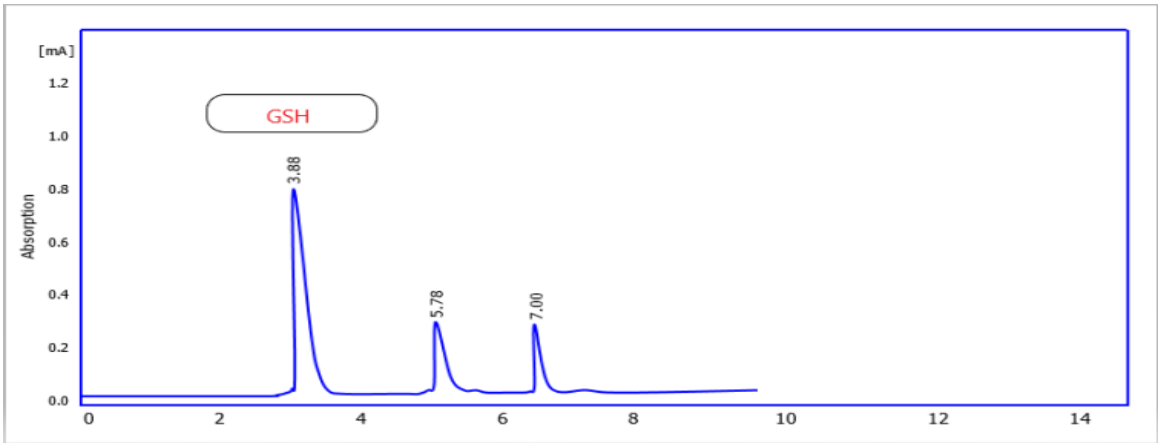


Figure 4 illustrates the HPLC chromatogram of AlCC for amino acid

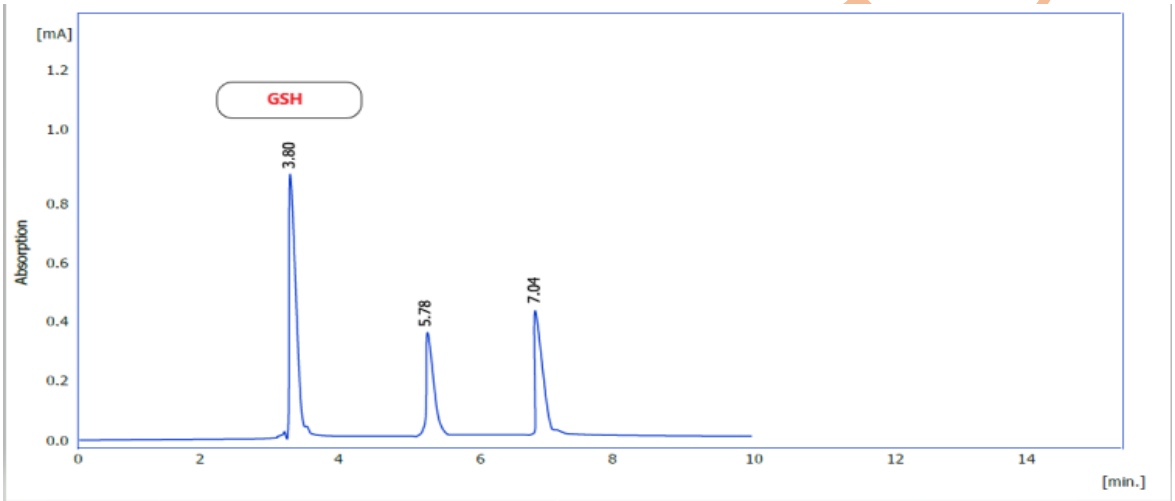


Figure 5 illustrates the HPLC chromatogram of AqCC for amino acid

Table 2 the active compound present within extracts

No	Name	AqCC	AlCC
1	Total-phenolic content (mg Gallic / gm)	140.15	98.58
2	Total-flavonoid content (mg Rutin / gm)	98.2	62.15
3	Total-alkaloid content %	25.6	15.99
4	Total-glycoside content %	11.2	6.58
5	Gallic acid (µg / gm)	111.5	74.5
6	Apigenin (µg / gm)	80.9	42.9
7	Chlorogenic acid (µg / gm)	74.6	52.1
8	Caffeic acid (µg / gm)	30.5	16.5
9	Quercetin (µg / gm)	65.9	43.9
10	p-Coumaric acid (µg / gm)	151.4	108.9
11	GSH (µg / gm)	63.5	41.9

Analysis of the effect of compounds on neopterin:

1: The effect of SSG on NEO in U937 macrophage cell-line :

The administration of SSG leads to a significant decrease in NEO levels in the U937 macrophage cell line when linked to the control-group ($p < 0.05$), as demonstrated in Figure 6.

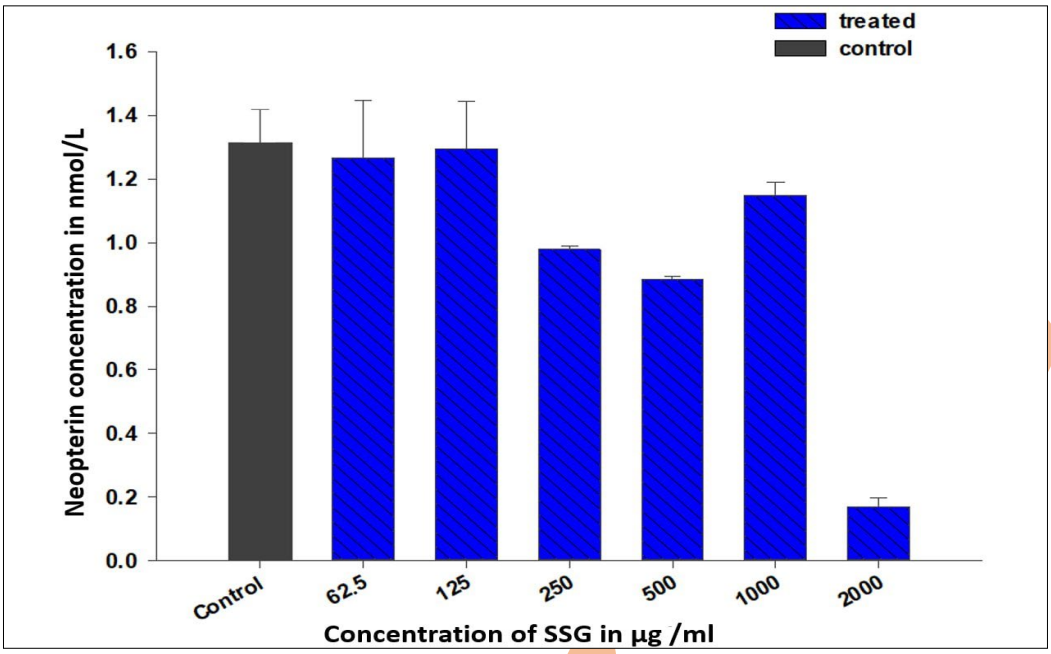


Figure 6 Effect of SSG in U937 macrophage cell line

2: The effect of SSG on NEO in U937 macrophage cell line infected with L. tropica.

The experiment revealed a substantial decrease ($p < 0.05$) in neopterin production at high concentrations (250, 500, 1000, and 2000 µg/ml) related to the control-group after a period of 24 hours of incubation, as illustrated in Figure 7.

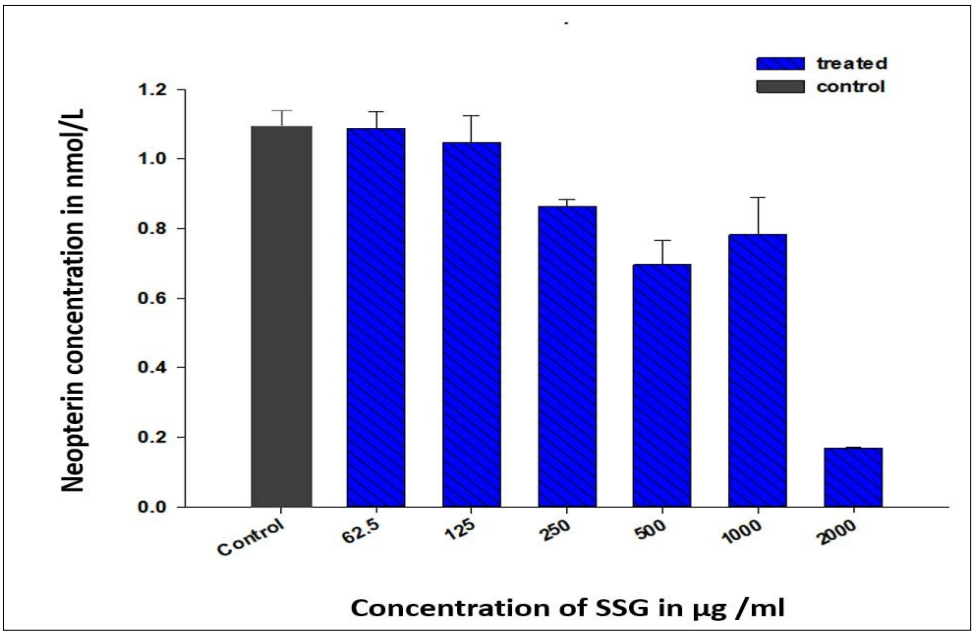


Figure 7 Effect of SSG in U937 macrophage cell line infected with Leishmania tropica

3: The influence of AqCC on NEO in U937 macrophage cell-line:
Evidence indicates that AqCC significantly reduces NEO levels in macrophages when compared to the control-group. NEO's decline was statistically significant at elevated concentrations (125, 250, 500, 1000, and 2000), with a p-value below 0.05. The decline is depicted in the diagram titled "Figure 8".

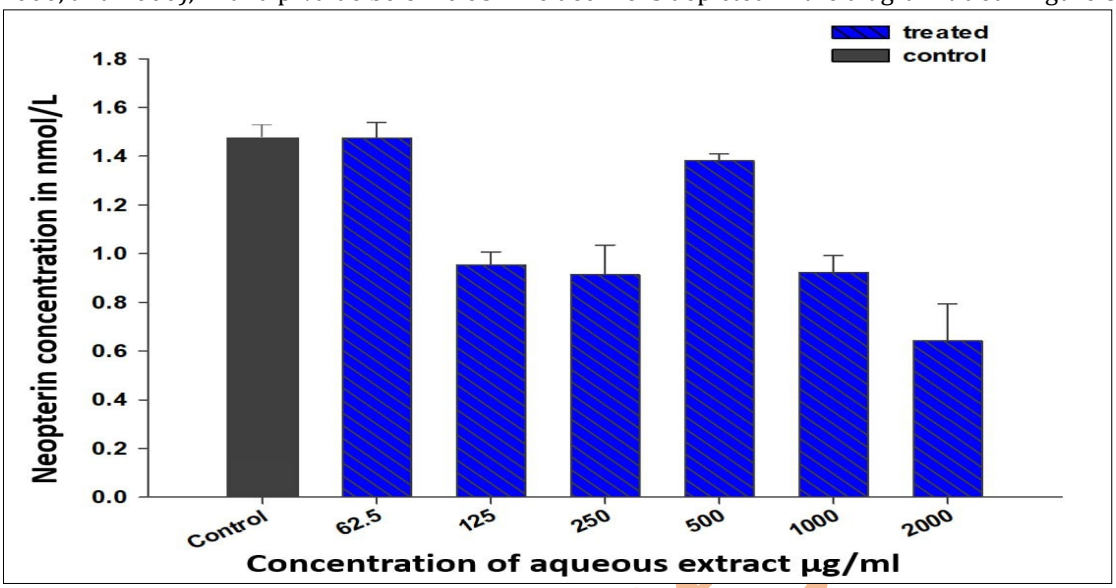


Figure 8 shows the effect of AqCC on U937Macrophage cell-line

4: The influence of AqCC on NEO in U937 macrophage cell-line infected with L. tropica :
The concentrations of AqCC at 1000 and 2000 µg/ml cause a substantial drop in NEO `levels compared to the normal group, with a p-value of ≤ 0.05. As seen in Figure 9.

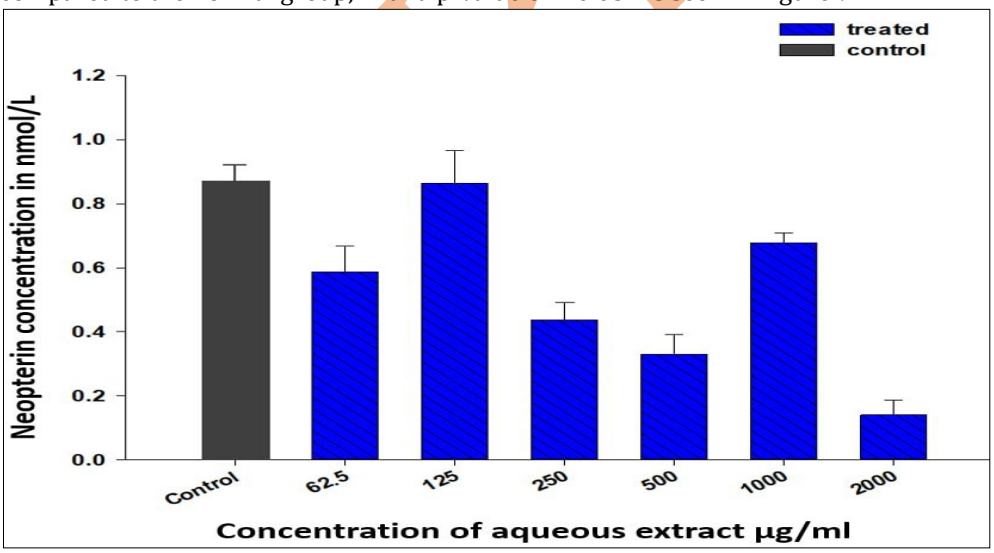


Figure 9 shows the effect of AqCC on U937Macrophage cell-line infected with L. tropica

5: The influence of AlCC on NEO in U937 macrophage cell-line:
AlCC markedly decreases the levels of NEO in the U937 macrophage cell-line in comparison to the control group. Figure 10 supports the findings.

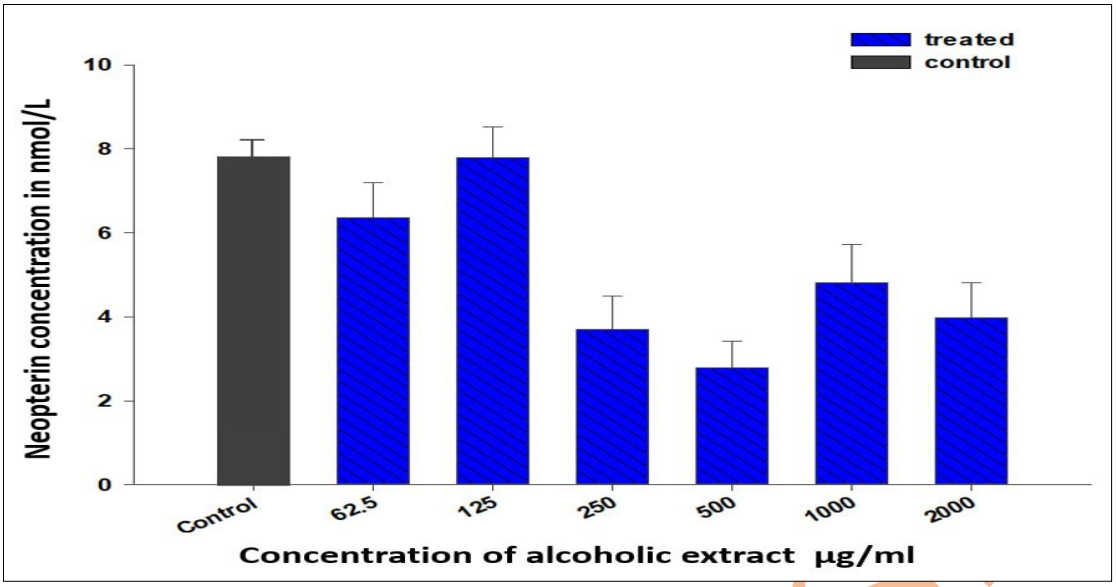


Figure 10- shows the influence of the AlCC on the U937Macrophage cell-line.

6: The influnce of AlCC on NEO in U937 macrophage cell-line infected with L. tropica:

In the macrophage cell line U937, the data shown in "Figure 11" demonstrates that AlCC is accountable for the immune response against L. tropica. Neopterin levels significantly decrease ($p < 0.05$) compared to the control-group .

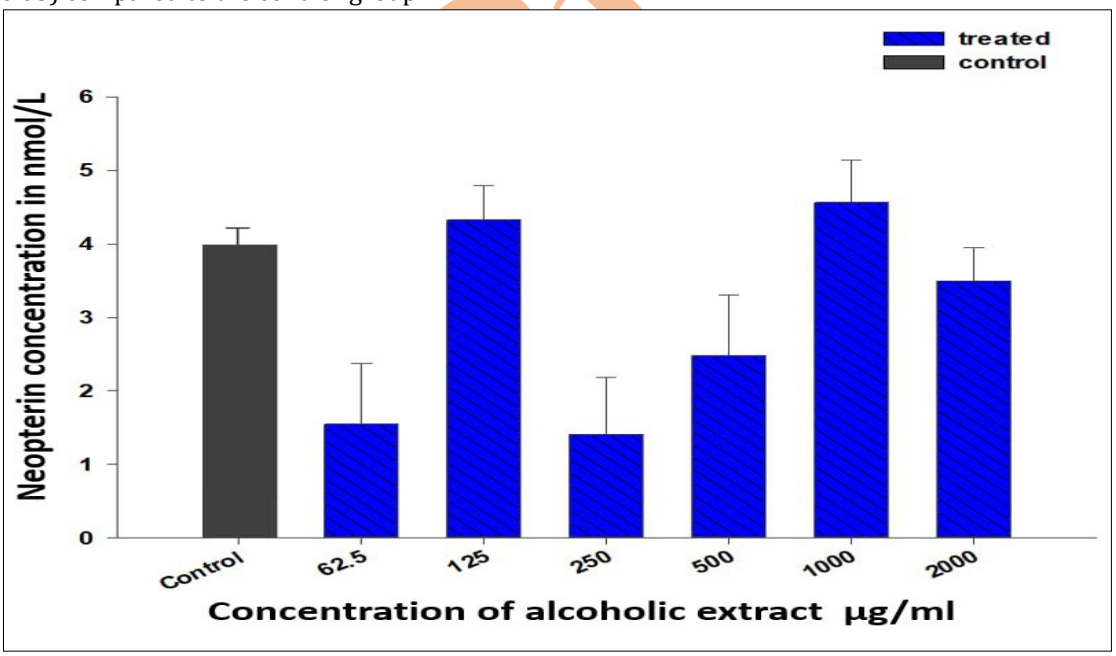


Figure 11- shows the influence of the AlCC on the U937Macrophage cell-line infected with L. tropica

Discussions:

NEO levels in L. tropica-infected individuals decreased significantly in response to treatment with SSG, AlCC, and AqCC at all concentrations, according to this study. On the contrary, a significant reduction in NEO concentrations was observed exclusively at elevated concentrations of 1000 and 2000 µg/ml AqCC. Serum NEO levels that exceed the upper limit of the established normal range are indicative of cell-mediated immunity activation. It is crucial to acknowledge that serum NEO does not exhibit disease specificity. Monitoring and evaluating the concentrations of it in the blood serum

throughout an infectious disease can provide valuable insights into the disease's severity and the effectiveness of treatment [34]. VL is characterised by an increase in the number and activation of macrophages, which would lead to a rise in neopterin concentrations during the disease's active phase. Subsequently, as the parasite infection decreases, these levels would decrease. [11,12,13]. Antileishmanial activity has been observed in AlCC and AqCC, according to recent studies. This is confirmed by the presence of quercitrin, quercetin, apigenin, p-coumaric acid, and caffeic acid in the extract, as determined by HPLC analysis [20]. The results of this research are consistent with those of Monzote et al., who similarly documented the antileishmanial properties of p-coumaric acid [36]. This drug has been discovered to suppress the activity of three crucial enzymes involved in the advancement of Leishmania braziliensis: aldehyde dehydrogenase (ALDH), mitogen-activated kinase protein (MPK4), and DNA topoisomerase 2 (TOP2). This discovery is consistent with prior investigations that propose SSG functions by impeding DNA topoisomerase. Conversely, NEO levels in the blood serum can be measured throughout the course of treatment for VL, which can be used to determine the efficacy of therapy [10]. Increased levels of reactive oxygen species are associated with it. The determination of NEO concentrations enables an evaluation of the degree of oxidative stress and immunological activation [39]. Flavonoids, which are abundant in C. canadenesis, exert various biological effects, such as regulating enzymes responsible for the elimination of reactive oxygen species (ROS) [40]. Flavonoids are chemical compounds that possess the capacity to stimulate cellular apoptosis and autophagy. Moreover, they possess the capacity to hinder the growth and penetration of cancerous cells. In healthy cells, flavonoids serve as antioxidants, assisting in control of (ROS) concentrations. In cancer cells, nevertheless, they operate as potent pro-oxidants. By inhibiting the activity of pro-inflammatory signaling pathways and stimulating apoptotic pathways, flavonoids regulate the equilibrium of (ROS) [41]. Gallic acid (GA) demonstrated significant immunomodulatory characteristics. An increase in the capacity of macrophages to ingest and eliminate foreign particles, an enlargement of lysosomes, the release of nitrite, and elevated levels of calcium ions within the macrophages were indicators of these results [42]. Chlorogenic acid (CGA) demonstrates ant-leishmanial properties through its ability to eradicate the parasites and disrupt their cell cycle, resulting in detrimental and inhibitory effects on the PRO. In vitro, CGA eradicates intracellular AMO one hundred percent, demonstrating its efficacy in removing the parasites from the host cells. The enhanced functionality of macrophages facilitates the clearance process through the concurrent elevation of IL-12, TNF, and NO levels, and the reduction of IL-10 synthesis. Majumder et al. propose that CGA may function as an innovative and non-toxic chemical agent to address visceral leishmaniasis, thereby presenting a viable substitute for chemotherapy [43]. Anke et al., on the other hand, discovered that serum neopterin concentrations did not increase in patients with CL [44]; they conducted research on this population. However, the aforementioned statement contradicts this finding and suggests that the two sources are in conflict.

The Conclusion:

This study demonstrates the significant anti-leishmanial activity of Conyza Canadensis extracts and highlights the correlation between neopterin levels and Leishmania infection. These findings lend credence to the use of natural plant extracts as alternative therapies for leishmaniasis. Conyza Canadensis demonstrates promise because of its bioactive components and various pharmacological effects. There is a need for additional research to be conducted in order to comprehend the mechanisms of action and assess the effectiveness of the treatment in animal models and clinical trials. Developing cost-effective and accessible therapies is crucial, particularly in regions with limited resources. Continued exploration of Conyza Canadensis as a therapeutic candidate could lead to effective and sustainable solutions for leishmaniasis.

Conflicts of Interest

Considering the presentation of research, there is a complete absence of any potential conflicts of interest.

Funding

The research was not funded.

Ethics Statements

On the first of May in the year 2021, a local ethics commission at the College of Medicine at Kufa University gave their approval to both the study protocol and the informed consent statement.

Author Contribution

The authors affirm that the following constitutes their contribution to the paper: The study was conceived of and designed by Sarmad Nory Gany and Rana A. Ghaleb. Rana A. Ghaleb, Maysam Ali Ameen Awadh, and Anssam Ali Ameen were responsible for the collecting of data. Maysam Ali Ameen Awadh and Anssam Ali Ameen were responsible for the analysis and interpretation of the results. Maysam Ali Ameen Awadh was responsible for the writing of the draft article. The results were evaluated by all of the writers, and they gave their approval to the final version of the manuscript.

References

[1] H. Irshad, "Types and Treatments of Leishmaniasis," *Biomed. J. Sci. Tech. Res.*, vol. 42, no. 5, pp. 34037–34042, 2022, doi: 10.26717/bjstr.2022.42.006815.

[2] H. J. C. de Vries and H. D. Schallig, "Cutaneous leishmaniasis: a 2022 updated narrative review into diagnosis and management developments," *Am. J. Clin. Dermatol.*, vol. 23, no. 6, pp. 823–840, 2022.

[3] C. Ferreira, J. Estaquier, and R. Silvestre, "Immune-metabolic interactions between Leishmania and macrophage host," *Curr. Opin. Microbiol.*, vol. 63, pp. 231–237, 2021.

[4] M. Martínez-López, M. Soto, S. Iborra, and D. Sancho, "Leishmania Hijacks Myeloid Cells for Immune Escape.," *Front. Microbiol.*, vol. 9, p. 883, 2018, doi: 10.3389/fmicb.2018.00883.

[5] E. C. Saunders and M. J. McConville, "Immunometabolism of Leishmania granulomas," *Immunol. Cell Biol.*, vol. 98, no. 10, pp. 832–844, 2020.

[6] L. T. Y. Lazar and K. S. Abass, "Morphology, life cycle, pathogenesis and virulence factors of genus Leishmania: a review," *Plant Arch.*, vol. 20, no. 2, pp. 4057–4060, 2020.

[7] B. M. Scorza, E. M. Carvalho, and M. E. Wilson, "Cutaneous manifestations of human and murine leishmaniasis," *Int. J. Mol. Sci.*, vol. 18, no. 6, p. 1296, 2017.

[8] M. A. Sloan, K. Brooks, T. D. Otto, M. J. Sanders, J. A. Cotton, and P. Ligoxygakis, "Transcriptional and genomic parallels between the monoxenous parasite Herpetomonas muscarum and Leishmania," *PLoS Genet.*, vol. 15, no. 11, p. e1008452, 2019.

[9] R. K. Majeed, H. F. Muhammed, and H. M. Rahim, "Acute toxicity study of indomethacin and oxytetracycline in rabbits," *Med. J. Babylon*, vol. 15, no. 3, p. 219, 2018.

[10] F. F. V Hamerlinck, T. Van Gool, W. R. Faber, and P. A. Kager, "Serum neopterin concentrations during treatment of leishmaniasis: useful as a test of cure?" *FEMS Immunol. Med. Microbiol.*, vol. 27, no. 1, pp. 31–34, 2000.

[11] S. Palić, J. H. Beijnen, and T. P. C. Dorlo, "An update on the clinical pharmacology of miltefosine in the treatment of leishmaniasis," *Int. J. Antimicrob. Agents*, vol. 59, no. 1, p. 106459, 2022.

[12] N. B. Kuryltsiv and O. V. Zborovska, "Neopterin, a promising biomarker for the diagnosis of intraocular inflammation," *Blood*, vol. 78, p. 79, 2021.

[13] B. L. S. Campos *et al.*, "Analysis of iron superoxide dismutase-encoding DNA vaccine on the evolution of the Leishmania amazonensis experimental infection," *Parasite Immunol.*, vol. 37, no. 8, pp. 407–416, 2015.

[14] L. Kristanc, B. Božič, Š. Z. Jokhadar, M. S. Dolenc, and G. Gomišček, "The pore-forming action of polyenes: From model membranes to living organisms," *Biochim. Biophys. Acta (BBA)-Biomembranes*, vol. 1861, no. 2, pp. 418–430, 2019.

[15] J. Chakravarty and S. Sundar, "Current and emerging medications for the treatment of leishmaniasis," *Expert Opin. Pharmacother.*, vol. 20, no. 10, pp. 1251–1265, 2019.

[16] N. C. Brito, A. Rabello, and G. F. Cota, "Efficacy of pentavalent antimoniate intralesional infiltration therapy for cutaneous leishmaniasis: A systematic review," *PLoS One*, vol. 12, no. 9, p. e0184777, 2017.

[17] M. Bahmani *et al.*, "Leishmaniosis phytotherapy: Review of plants used in Iranian traditional medicine on leishmaniasis," *Asian Pac. J. Trop. Biomed.*, vol. 5, no. 9, pp. 695–701, 2015.

[18] N. A. Al-douri, "A survey of medicinal plants and their traditional uses in Iraq," *Pharm. Biol.*, vol. 38, no. 1, pp. 74–79, 2000.

[19] J. W. Kadereit and C. Jeffrey, *Flowering plants. Eudicots*. Springer, 2018.

[20] M. A. Abood and E. J. Kadhim, "Phytochemical investigation of some active components in Iraqi Conyza canadensis (Syn. Erigeron canadensis)," *IJDDT*, vol. 11, pp. 669–675, 2021.

[21] K. Biswas and S. N. Sinha, "Evaluation of the antibacterial activity of Conyza canadensis (L.) Cronquist collected from Nainital, Uttarakhand, India," *Int. J. Univ. Pharm. Biol. Sci*, vol. 3, pp. 46–53,

2014.
[22] N. Hai, R. P. Akhter, and I. Ali, “Isolation, maintenance and detection of Leishmania parasite by microscopy and culture technique,” in *2017 14th International Bhurban Conference on Applied Sciences and Technology (IBCAST)*, 2017, pp. 179–182.
[23] W. C. Evans, “Trease and Evans Pharmacognosy. 15th edtion,” *Edinburgh, Saunders*, vol. 249, 2002.
[24] W. C. Evans, “Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer,” *Anc. Sci. Life*, vol. 31, no. 4, p. 198, 2012.
[25] S. E. Laouini and M. R. Ouahrani, “Phytochemical screening, in vitro antioxidant and antibacterial activity of Rumex vesicarius L. extract,” *Sci. Study Res. Chem. Chem. Eng. Biotechnol. Food Ind.*, vol. 18, no. 4, pp. 367–376, 2017.
[26] S. Habibatni, A. F. Zohra, H. Khalida, S. Anwar, I. Mansi, and N. A. Awadh Ali, “In vitro antioxidant, Xanthine oxidase-inhibitory and in vivo Anti-inflammatory, analgesic, antipyretic activity of Onopordum acanthium,” *Int. J. Phytomed*, vol. 9, pp. 92–100, 2017.
[27] V. Sutariya, D. Wehrung, and W. J. Geldenhuys, “Development and validation of a novel RP-HPLC method for the analysis of reduced glutathione,” *J. Chromatogr. Sci.*, vol. 50, no. 3, pp. 271–276, 2012.
[28] Z. Tofighi, S. N. GHAZI, A. Hadjiakhoondi, and N. Yassa, “Determination of cardiac glycosides and total phenols in different generations of Securigera securidaca suspension culture,” 2016.
[29] C.-F. Kuo *et al.*, “Abrogation of streptococcal pyrogenic exotoxin B-mediated suppression of phagocytosis in U937 cells by Cordyceps sinensis mycelium via production of cytokines,” *Food Chem. Toxicol.*, vol. 45, no. 2, pp. 278–285, 2007.
[30] B. M. Sharp, “Conversion of the U937 Monocyte into" Macrophage-Like" Populations Exhibiting M1 or M2 Characteristics,” 2013.
[31] F. Puentes *et al.*, “Cultivation and characterization of stable Leishmania guyanensis complex axenic amastigotes derived from infected U937 cells.,” *Am. J. Trop. Med. Hyg.*, vol. 63, no. 1, pp. 102–110, 2000.
[32] E. S. Abamor, “Antileishmanial activities of caffeic acid phenethyl ester loaded PLGA nanoparticles against Leishmania infantum promastigotes and amastigotes in vitro,” *Asian Pac. J. Trop. Med.*, vol. 10, no. 1, pp. 25–34, 2017.
[33] A. E. Kip *et al.*, “Macrophage activation marker neopterin: a candidate biomarker for treatment response and relapse in visceral leishmaniasis,” *Front. Cell. Infect. Microbiol.*, vol. 8, p. 181, 2018.
[34] S. Bührer-Sekula, F. F. V Hamerlinck, T. A. Out, L. G. Bordewijk, and P. R. Klatser, “Simple dipstick assay for semi-quantitative detection of neopterin in sera,” *J. Immunol. Methods*, vol. 238, no. 1–2, pp. 55–58, 2000.
[35] S. Palić, P. Bhairosing, J. H. Beijnen, and T. P. C. Dorlo, “Systematic review of host-mediated activity of miltefosine in leishmaniasis through immunomodulation,” *Antimicrob. Agents Chemother.*, vol. 63, no. 7, pp. 10–1128, 2019.
[36] L. Monzote, W. H. P. Córdova, M. García, A. Piñón, and W. N. Setzer, “In-vitro and In-vivo Activities of Phenolic Compounds Against Cutaneous Leishmaniasis,” *Rec. Nat. Prod.*, vol. 10, no. 3, p. 269, 2016.
[37] S. P. Lopes, L. M. Yepes, Y. Pérez-Castillo, S. M. Robledo, and D. P. de Sousa, “Alkyl and aryl derivatives based on p-coumaric acid modification and inhibitory action against Leishmania braziliensis and Plasmodium falciparum,” *Molecules*, vol. 25, no. 14, p. 3178, 2020.
[38] A. Lucumi, S. Robledo, V. Gama, and N. G. Saravia, “Sensitivity of Leishmania viannia panamensis to pentavalent antimony is correlated with the formation of cleavable DNA-protein complexes,” *Antimicrob. Agents Chemother.*, vol. 42, no. 8, pp. 1990–1995, 1998.
[39] C. Murr, B. Widner, B. Wirleitner, and D. Fuchs, “Neopterin as a marker for immune system activation,” *Curr. Drug Metab.*, vol. 3, no. 2, pp. 175–187, 2002.
[40] R. H. Shareef, Z. F. Sharba, and E. N. Hameed, “The positive role of antioxidants on body immunity: An overview,” *Med. J. Babylon*, vol. 18, no. 3, p. 169, 2021.
[41] D. M. Kopustinskiene, V. Jakstas, A. Savickas, and J. Bernatoniene, “Flavonoids as anticancer agents,” *Nutrients*, vol. 12, no. 2, p. 457, 2020.
[42] M. M. de M. Alves *et al.*, “Gallic and ellagic acids: two natural immunomodulator compounds solve infection of macrophages by Leishmania major,” *Naunyn. Schmiedebergs. Arch. Pharmacol.*, vol. 390, pp. 893–903, 2017.

[43] N. Majumder, S. Ganguly, A. K. Ghosh, S. Kundu, A. Banerjee, and S. Saha, “Chlorogenic acid acts upon Leishmania donovani arresting cell cycle and modulating cytokines and nitric oxide in vitro,” *Parasite Immunol.*, vol. 42, no. 6, p. e12719, 2020.
[44] E. Anke, M. Balasegaram, J. H. Beijnen, J. H. M. Schellens, J. de Peter, and T. P. C. Vries, “Biomarkers to monitor therapeutic response in leishmaniasis: a systematic review 2,” 2014.