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RESEARCH ARTICLE

# Phytochemistry and *in vitro* growth inhibitory action of *Corchorus olitorius* leaves against *Leishmania tropica*

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## Abstract

*Corchorus olitorius* L. (Malvaceae) is a nutritional, edible vegetable, as its leaves have a high mineral content, including calcium and iron, as well as vitamins such as thiamine (B1), riboflavin (B2), folic acid, ascorbic acid (C) and tocopherol (E). Also, it is rich with essential phytochemicals such as cardiac glycosides, flavonoids, phenolics, fatty acids and terpenes, which are known for their pharmacological importance, like antioxidant, anti-inflammatory, antibacterial activities, antiviral, analgesic, antidiabetic and antitumor. This study aimed to evaluate the antileishmanial activity of *C. olitorius* hexane extract towards cutaneous leishmaniasis prepared by the extraction of 20 g of dried powdered leaves, followed by preliminary phytochemical analysis by GC-MS and TLC. Six different concentrations were assessed (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) and compared with Pentostam®, a commonly prescribed antileishmanial medication. The GC-MS chromatogram demonstrated that the hexane extract is rich in terpenes (Phytol) and fatty acids (linolenic and palmitic acid) and TLC confirms the presence of β-sitosterol as having an $R_F$ value similar to the standard. IBM software's one-way analysis of variance (ANOVA) test was used to calculate the rate of inhibition for the 6 concentrations. The inhibition rate was more significant in the first 3 concentrations, 1000, 500 and 250 µg/mL, giving 78.96 %, 76.99 % and 68.88 % inhibition rate respectively, proving the anti-antileishmanial effect, as compared to positive control's inhibition value, which was 67.23 % and the hexane fraction used to treat *L. tropica* was effective at low concentration of 0.127 mg/mL. The hexane extract contains a good percentage of linolenic, palmitic acid and phytol. TLC and GC-MS confirm the presence of β-sitosterol and the antileishmanial qualities are similar to Pentostam® treatment.

**Keywords:** antileishmanial activity; *Corchorus olitorius*; hexane extract; *Leishmania tropica*

## Introduction

Leishmaniasis is a vector-borne infection widely spread in different regions around the world, in Europe, Africa, Asia and America, with newly reported cases in Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Colombia, Ethiopia, India, Iran, Peru, South Sudan, Sudan and Syria (1). It is caused by flagellated protozoans of the genus *Leishmania*, which can be transmitted in 2 ways: known as anthroponotic transmission (human to human) or zoonotic transmission (animal to human) (2). Leishmaniasis is classified as an emerging and uncontrolled disease, since it causes 2 million new cases each year, making it a public health problem (3).

Generally, no vaccine is available for use in humans, with the development of resistance toward some drugs (first-line drugs, such as Glucantime, Pantostam® and Pentacarinat) and some have serious adverse effects (second-line drugs, such as Pentamidine and Amphotericin B) (4, 5). In addition, there has been a continuous increase in the incidence rate of cutaneous leishmaniasis in Iraq, leading to the search for new antileishmanial agents to combat this disease (6).

Nowadays, herbal medicines are a powerful approach for discovering more effective treatments and around 80 % of the world's population depends upon natural products as a primary source of medication (7). *Corchorus* is a genus of shrub, subshrub, or annual and perennial herb that contains about 50-60 species, mostly found in Africa. One of the known edible species is the *Corchorus olitorius* L. plant of the family Malvaceae, which is a good source of active constituents of numerous pharmacological activities and is found in tropics, subtropics and warm-temperate regions (8-10). The plant is an annual herb, tall with a height of 2-4 m. It could be without branches or with only a few side divisions. The leaves are alternately distributed, simple, lanceolate, finely serrated, or with lobed margins. *C. olitorius* contains small, yellow flowers (2-3 cm in diameter) with 5 petals (11). The known common names are bush okra, nalta jute, jute mallow, Jew's mallow, ewedu, melokhyia and moroheiya (9).

The leaves of edible species of *C. olitorius* are rich in proteins, vitamins such as thiamine (B1), riboflavin (B2), folic acid, ascorbic acid (C) and tocopherol (E), specific hormone precursors and minerals (calcium and iron); therefore, it is widely used in the folklore medicine in the treatment of gonorrhoea, chronic cystitis, as an analgesic, febrifuge, anti-inflammatory, diuretic and cardiotonic (12-14). In addition to various bioactive compounds that have been documented from various parts of the plant, mainly flavonoids (quercetin, quercetrin, apigenin and astragalin), triterpenoids (oleanolic acid, urosolic acid, corosolic acid and

betulinic acid) and fatty acids (β-sitosterol, palmitic acid, steric acid and arachidic acid). These substances are responsible for several pharmacological effects, including antioxidant, antimicrobial and anticancer activity (15).

Moreover, the leaves of *C. olitorius* mainly exhibit protective effects, as antitumor, antifungal, gastroprotective, antinociceptive, antibacterial activities and antiviral activity against measles virus (11, 16-18). Because of the continuous high incidence rate and over more than a decade from 2009 until 2024, it has been proven through research that cutaneous leishmaniasis is endemic in several districts in rural areas in Iraq, including Alhaweja, Wasit, Alramadi, Diayla and Almuthanna (19-23). The uses and health advantages of *C. olitorius* leaves have been widely researched. However, data regarding the biological as well as pharmacological characteristics of special chemical compounds (terpenoid and steroid compounds) in the leaves are still limited. Furthermore, in Iraq, no studies have examined the terpenoid and steroid leaf fraction on cutaneous leishmaniasis. To address this gap, this study presents a framework for the first report investigating the plant's potentiality and efficacy towards cutaneous leishmaniasis through phytochemical investigations of its active constituents (terpenoids and steroids) by gas chromatography (GC) and thin layer chromatography (TLC), followed by assessing the antileishmanial activity.

## Materials and Methods

### Apparatus and instruments

The following were used: a rotatory evaporator type Buchi attached to a vacuum pump Buchi-Germany, an electrically sensitive balance (Sartorius, Germany), an Eppendorf centrifuge 5417R (Hamburg / Germany), a Gas Chromatography-Mass Spectrometer (GC-MS) type Agilent 7820A, an ELISA plate reader (AYAS/Hitech, Austria), a $CO_2$ incubator (SAYNO/ Japan), an inverted microscope (MEIJI TECHNO/Japan) and a laminar air flow (NAPCO/ France).

### Chemicals and reagents

Hexane solvent was purchased from BDH Limited/England. petroleum ether, chloroform, ethyl acetate and sulfuric acid were purchased from Alpha Chemika/India. Dimethyl sulfoxide (DMSO) was purchased from Santacruz Biotechnology/USA. β-sitosterol standard from Ghengdu Biopurify/China. MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5 2,5-diphenyltetrazolium bromide was purchased from Promega (USA).

### Collection of the plant material

Fresh leaves of *C. olitorius* were gathered from a local market in Baghdad city and Prof. Dr. Sukaena Abass was responsible for the plant authentication and registered at BUH No. 16815. After being thoroughly cleaned to remove unnecessary materials, the leaves were dried by air in a shaded place. Next, an electric blender was used to powder the dried leaves. After that, the powders were ready for extraction and kept in a firmly sealed container.

### Preparation of *C. olitorius* extract

Twenty g of pulverized plant samples were extracted by the Soxhlet apparatus, using 400 mL of hexane as a single solvent extraction. The temperature of the solvent was adjusted to the required boiling point (68 °C). The experiment process took a total of 4 to 5 hr. Once the experiment had finished, as the leaves became colorless and the siphon tube contained colorless extract, the mixture was filtered, collected, evaporated and the hexane extract was saved for analysis (24).

### Phytochemical analysis of *C. olitorius* hexane extract

#### Phytochemical analysis of hexane extract by GC-MS

GC-MS investigation was used to determine the chemical components of the *C. olitorius* extract. Ten mg from n-hexane extract obtained by Soxhlet extraction method was diluted separately with 10 mL of ethanol and then centrifuged for 2 min at 14000 rpm and 25 °C the supernatant was taken to be filtrated through 0.45 µm pore size disposable filters before utilization for analysis by GC/MS. Helium gas with a purity of 99.99 % was used as a carrier gas (1.3 mL/min). The oven was programmed firstly on ramp 1 with 65 °C and ramp 2 started from 60 °C reaching 180 °C at a rate of 8 °C/min, then ramp 3 from 180 °C to 300 °C at a rate of 7 °C/min, finally ramp 4 at 300 °C, then hold for 5 min. The analytical coiled column used was 30 m length and a film thickness equal to 0.25 µm. The volume injected was 1 µl under a pressure of 11.933 PSI, the GC inlet line temperature was 250 °C, while the auxiliary heaters temperature was at 320 °C and the injector temperature at 250 °C, scan range between 25-1000 and splitless injection type was used. Depending upon the date embedded in the MS machine library, the chemical investigation of the compounds in each sample was accomplished.

#### Phytochemical analysis of hexane extract by analytical TLC Plates

Thin-layer chromatography (TLC) is an affinity-based method used to estimate the steroid (β-sitosterol) of *C. olitorius* qualitatively. Aluminum TLC plates (20 × 20 cm) coated with silica gel GF 254 were activated in an oven heated to 110 °C for 30 min, then left to cool andused for sample application. Ethyl acetate:petroleum ether (7:4) and chloroform:petroleum ether (18:2) were used as developing solvent systems for β-sitosterol detection (assigned as S1 and S2 respectively), which filled the TLC chamber to a depth of less than 1 cm for 15 min. to ensure the saturation of the jar (25). A fine glass capillary tube was used to apply 1-2 drops of the hexane extract and β-sitosterol standard onto the plate, followed by the development process, during which the mobile phase tries to elute the sample as much as possible to achieve the separation. Detection was done by spraying the plate with 10 % sulphuric acid ($H_2SO_4$) (spray reagent for steroids) and heating at 100 °C for 5 min. The positions of the separated spots were marked with a pencil and the retardation factor ($R_F$) values were calculated according to the following mathematical equation: $R_F$ value = distance moved by the active ingredient/ distance moved by the mobile phase.

### Assessing antileishmanial activity of *C. olitorius* hexane extract

#### Inoculum preparation

The leishmania samples were taken from patients who had been clinically diagnosed with leishmaniasis and then submitted to the University of Al-Nahrain's College of Science at the Research Biological Technology Centre. The organism was grown on a Roswell Park Memorial Institute (RPMI) medium with 12 % calf fetal serum. The parasite samples were then kept at 25 °C for 3 consecutive days to reach a mean concentration of $10^5$ parasites/ mL as measured under a microscope (26). This allowed researchers to assess the effectiveness of the plant extract against the cutaneous leishmania parasite.

### Preparation of C. *olitorius* hexane extract concentration

The anti-parasitic efficacy of the hexane extract was evaluated against *L. tropica* (causes cutaneous leishmaniasis) and the outcomes were contrasted with those of the commonly prescribed antileishmanial medication, Pentostam®. 1 mg of hexane extract was kept for 6 repeated dilutions and DMSO was used as an extract solubilizer with a maximum amount that did not exceed 20 µL of 100 % v/v, followed by the addition of distilled water to get the required 1 mg/mL concentration to prepare the necessary dilutions. Six concentrations were obtained (1000, 500, 250, 125, 62.5 and 31.25) µg/mL (27).

### Positive control preparation

Standard antileishmanial drug known as Pentavalent antimonial was used as a positive control, using the sodium stibogluconate injection from GlaxoSmithKline in the UK at 100 mg/mL. One mL of sodium stibogluconate was diluted with up to 10 mL of distilled water to get a 10 mg/mL solution. Subsequently, 6 µL of this solution were introduced into each well, along with 1 mL of RPMI and *L. tropica* inoculum (2).

#### Assessment of C. *olitorius* hexane extract for leishmaniasis

A flat-bottom plate with 32 wells was utilized to assess the antileishmanial activity. *Leishmania* culture was added to each well. Then, 8 wells were filled with 10 mL of the previously prepared concentrations, totalling 24 wells filled with the plant extract. Furthermore, 3 wells were labelled as negative controls, meaning the *Leishmania* culture was not exposed to any extra substances. Three wells received positive control of sodium stibogluconate. DMSO 50 % was used to fill the remaining wells as a blank. For 24 hr, the plate was kept in an incubator set at 25 ± 1 °C. After this time, 10 µL of MTT dye was added to each well and in an incubator, the plate was kept for 4 hr at 25 °C to measure the metabolic rate. Finally, DMSO was used as a solubilizing agent and added to every well for targeting the purple dye MTT found in living things (26, 28).

#### Scanning

The ELISA spectrophotometer measures the optical density of each well at 490 nm. The quantity of biological material present is closely correlated with the intensity of the purple color. As a result, a higher ELISA reading absorbance indicates a better degree of precision in quantifying living matter.

### Statistical analysis

After calculating the rate of inhibition of the 6 concentrations by using IBM software's one-way analysis of variance (ANOVA) test, we confirmed the significance of the results by comparing the means. We can then use the least statistically significant differences (LSD) and *p*-value to determine if the inhibition rate significantly differs from that of sodium stibogluconate. A half-maximum inhibitory concentration ($IC_{50}$) indicates a substance's ability to block a particular biological or biochemical activity. This work utilized the dose-response curves generated in Microsoft Excel to calculate the $IC_{50}$ values of the hexane extract against *L. tropica* (28). First, the formula (Eqn. 1) was used to compute the percentage inhibition rates at different concentrations of the hexane portion.

% Inhibition Rate =

(Control value-sample value/ control value) × 100 (Eqn. 1)

Next, using Excel's scatter chart tool, the inhibition rates were displayed versus the concentrations. The scatter chart was enhanced with a logarithmic trendline to get the $IC_{50}$ values (29). The concentration at which 50 % inhibition occurred was determined using the logarithmic trendline equation (Eqn. 2)

$$y = a \ln(x) + b \quad \text{(Eqn. 2)}$$

## Results and Discussion

### Extraction

Extraction is essential in separating, identifying and obtaining valuable compounds from different plants. The nature of the target compound determines the selection of a suitable technique and an appropriate solvent to achieve maximum efficiency and purity. Soxhlet extraction of the *C. olitorius* leaf by hexane solvent yields 5 g of hexane extract and the following equation (Eqn. 3) calculates the hexane extract's proportion yield, which is equal to 25 %.

Percentage yield = weight of extract/weight of sample × 100 (Eqn. 3)

### Preliminary identification of the hexane fraction of *C. olitorius* by chromatographic techniques

#### Preliminary identification of the hexane fraction of C. olitorius by GC-MS

GC-MS analysis of the hexane extract was used to identify the different components of *C. olitorius* that demonstrated bioactivity. The peak area of various compounds and the retention time were determined by GC through compound fragmentation into small fragments, resulting in different peaks. While a MS provides mass information and structural identification, which is supplied from the data library (30). The results revealed the presence of hexadecenoic acid methyl ester, dibutyl phthalate, hexadecanoic acid, phytol, 9,12,15-octadecatrienoic acid, squalene and β-sitosterol, as shown in Fig. 1 and Table 1.

The result demonstrates that hexane extract contains essential secondary metabolites like fatty acids, terpenes and phytosterols. These compounds are known for their pharmacological activity; for example, palmitic acid possesses some biological activity as an antioxidant, hypocholesterolemic, nematicide and pesticide (31). Hexadecanoic acid methyl ester is a type of fatty acid ester that exhibits a good antibacterial effect against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* and *K. pneumoniae)* (32, 33). This activity is exerted in 4 mechanisms, starting by targeting the cell membrane of the bacteria, then interfering with cellular energy production, inhibiting the enzyme activity and, finally, causing bacterial cells' lysis. This activity is greatly affected by fatty acids' structure, length of the carbon chains, the number, position and orientation of the double bond (34). In addition, hexane extract is rich in alpha-linolenic acid (omega-3 polyunsaturated fatty acid) with a peak area of 22.20 %, which is known for its anti-inflammatory effect, antitumor, treating autoimmune disorders and stroke (35). Furthermore, phytol is an acyclic alcohol of diterpene frequently available in certain aromatic plants and *C. olitorius* hexane extract contains phytol with a peak area of 20.87 %, representing the second-highest peak.

**Table 1.** Bioactive components identified in the hexane extract of *C. olitorius* by GC-MS

| Peak No. | Retention time in min | Peak area % | Chemical formula | Compound name | Similarity index | Class of compounds |
|---|---|---|---|---|---|---|
| 1 | 20.372 | 2.91 | $C_{17}H_{34}O_2$ | Hexadecenoic acid methyl ester | 97 % | Fatty acid methyl ester |
| 2 | 20.796 | 3.89 | $C_{16}H_{22}O_4$ | Dibutyl phthalate | 97 % | Phthalate ester |
| 3 | 20.977 | 14.15 | $C_{16}H_{32}O_2$ | Palmitic acid (Hexadecanoic acid) | 90 % | Fatty acid |
| 4 | 22.726 | 20.87 | $C_{20}H_{40}O$ | Phytol | 90 % | Acyclic diterpenoid |
| 5 | 23.133 | 22.20 | $C_{18}H_{30}O_2$ | linolenic acid | 99 % | Fatty acid |
| 6 | 29.927 | 4.77 | $C_{30}H_{50}$ | Squalene | 99 % | Triterpene |
| 7 | 30.048 | 4.75 | $C_{29}H_{50}O$ | Beta -sitosterol | 96 % | Phytosterol |

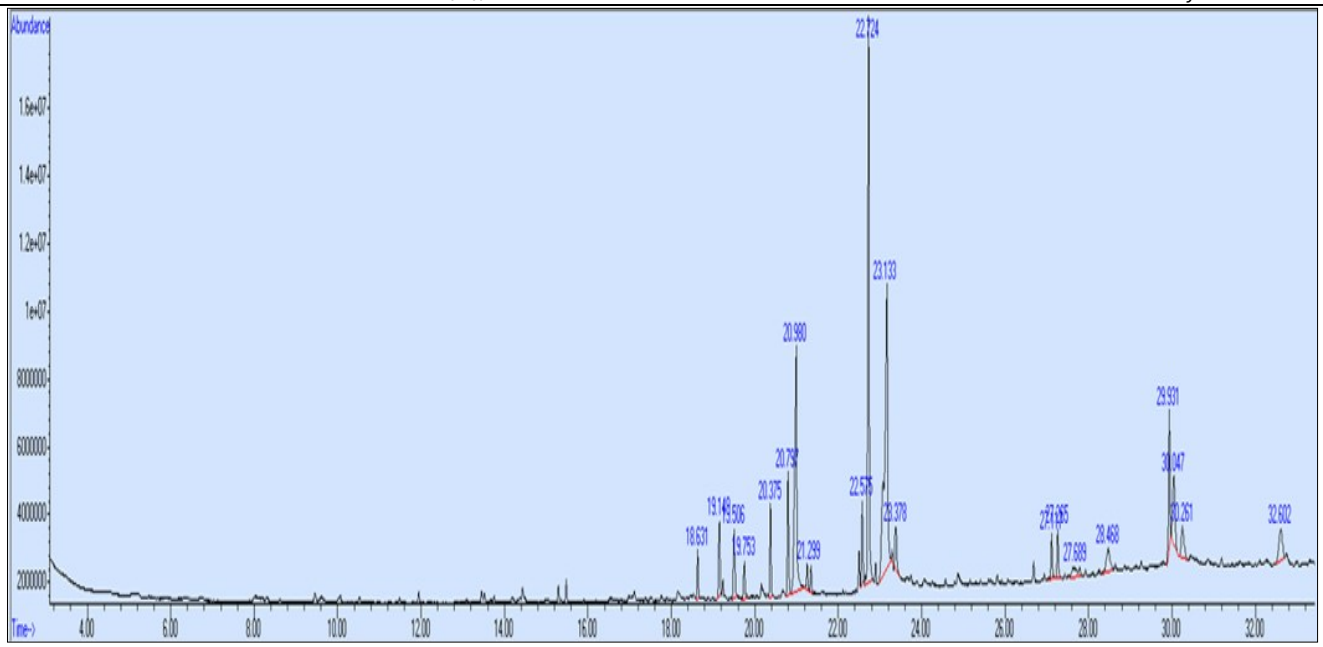


**Fig. 1.** GC-MS chromatogram of the hexane fraction of *C. olitorius*.

Linolenic and palmitic acids are fatty acids with the highest percentages of 22.2 and 14.15 respectively, eluted at 23.133 and 20.997 retention times, while β-sitosterol eluted at 30.048 retention time with a 4.75 peak area.

According to different research, Phytol, as well as its derivatives, are known for several crucial pharmacological activities such as cytotoxic, anti-inflammatory, anti-diabetic, anti-hyperalgesic, antibiotic chemotherapy, antimicrobial and anti-tumor effect (36, 37). Moreover, another exciting compound is squalene, a terpenoid hydrocarbon that accounts for 4.77 % of the peak area and is helpful in nutrition (present in high concentrations in virgin olive oils), cosmetic (skin hydrating, emollient activities and decrease skin's wrinkles, increase type I procollagen and minimize the DNA damage) and pharmaceutical or medicine applications (anticancer, antioxidant, anti-inflammatory and hypocholesterolemic) (38). Finally, numerous studies demonstrated the effects of phytosterols as anti-inflammatory, immunomodulatory and antioxidant properties. Also, it prevents apoptosis induction and inhibits the production of carcinogens and angiogenesis (39). GC-MS chromatogram showed a peak area of 4.75 % with a similarity index of 96 %, accounting for the identification of beta-sitosterol and further demonstration is recommended by using the TLC technique. So, *C. olitorius* leaves are dominated by fatty acids like palmitic and linolenic acid. Also rich in diterpenes like phytol and contains phytosterols (β-sitosterol) in minimal percentages, which are compatible with previous studies that have proven its significant fatty acid percentages (40).

### Identification of steroids of C. olitorius by TLC

TLC aims to identify a compound by comparing its $R_F$ value with a standard compound, obtaining a well-defined and well-separated spot. TLC analysis of the *C. olitorius* extract visualized by using 2 different solvent systems and the standard β-sitosterol, after spraying with 10 % $H_2SO_4$, showed characteristic spots and their $R_F$ values were comparable to those of the standard β-sitosterol, as depicted in Fig. 2 and Table 2.

The previous phytochemical analysis by 2 different techniques (GC-MS and TLC) demonstrate the presence of β-sitosterol (41). It is a natural metabolite, making up 65 % of the diet content. Various studies support the pharmacological importance of β-sitosterol, reported the anti-inflammatory response of β-sitosterol. The study revealed the *in vivo* effect of β-sitosterol in a delayed-type hypersensitivity model that can modulate cell-mediated oedema. At the same time, another study demonstrated the inhibitory effect on vascular adhesion and intracellular adhesion molecule 1 expression in TNF-alpha-stimulated human aortic endothelial cells. Cytotoxicity of β-sitosterol was examined against different cancer cell lines by MTT assay; the results showed antiproliferative and apoptosis activities in colon carcinoma, breast cancer and prostate cancer. Also, anti-diabetic, antioxidant, angiogenic, hypercholesterolemia, modulating the immune system and anthelminthic effects have been proven to β-sitosterol (42).

## Assessment of antileishmanial activity

Scientific research relies heavily on plant treatment and herbal medicine usage is expanding rapidly; therefore, *C. olitorius* is considered for antileishmanial assessment (43). The percentage of eliminated organisms from the fraction with its 6 concentrations

**Table 2.** *$R_F$ value* of β sterol standard and *$R_F$* value in the hexane fraction of *C. olitorius* plant

| No. of mobile Phase | Mobile phase composition | $R_F$ value of β sterol standard | $R_F$ value in hexane fraction |
|---|---|---|---|
| S1 | Ethyl acetate:petroleum ether (7:4) | 0.83 | 0.85 |
| S2 | Chloroform:petroleum ether (18:2) | 0.35 | 0.37 |

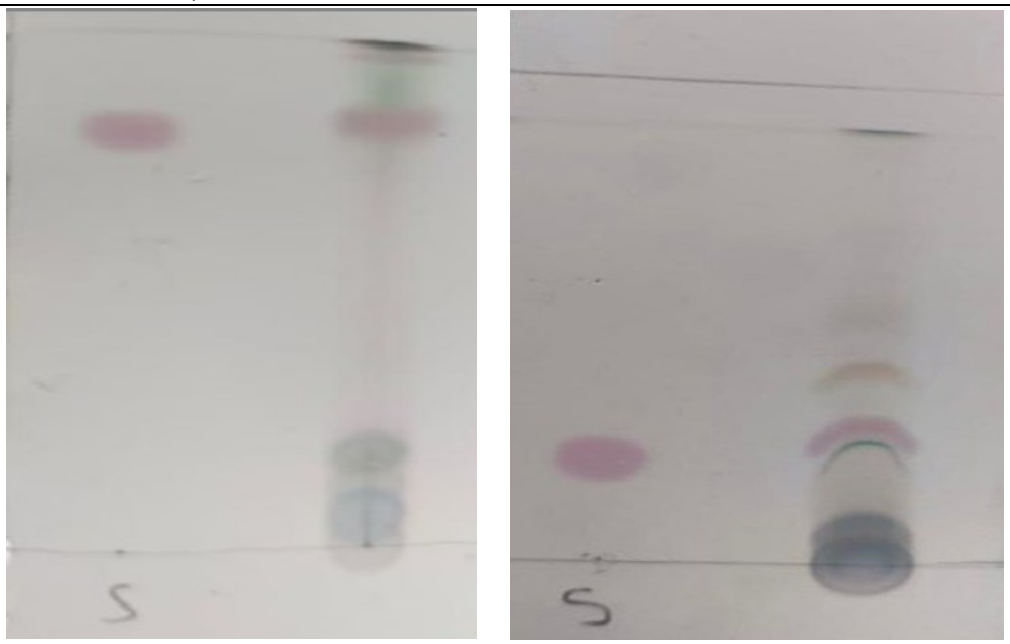


**Fig. 2.** Thin-layer chromatography for the hexane fraction of *C. olitorius* using silica gel GF254 as adsorbent with 2 solvent systems, A) S1 solvent and B) S2 solvent.

S (STD): β-sitosterol. β-sitosterol is demonstrated in 2 solvent systems with an $R_F$ value approximately equal to the β-sitosterol standard.

was calculated using the optical density (OD) data of the ELISA experiment by using Eqn. 1 (29).

For every concentration, the % inhibition rate was calculated and compared to the results of the positive and negative controls. The hexane percentage at 6 distinct concentrations was averaged and the inhibition rate of *L. tropica* was calculated. The results showed that the percentages of the IR were more significant in T1, T2 and T3, indicating a strong anti-leishmanial effect, as shown in (Table 3) andthis activity was compared to the positive control's inhibition value, which was 67.23 %. Fig. 3 shows the y-axis represented by the inhibition rate for *L. tropica,* with the x-axis represented by the

**Table 3.** Results for *L. tropica* overall concentration gradients, measured in % IR

| Sample name | Concentration mg/ml | % IR ± STDEV |
|---|---|---|
| T1 | 1 | 78.96 ± 0.007937 |
| T2 | 0.5 | 76.97 ± 0.015524 |
| T3 | 0.25 | 69.88 ± 0.022338 |
| T4 | 0.125 | 48.63 ± 0.01253 |
| T5 | 0.0625 | 38.59 ± 0.054994 |
| T6 | 0.03125 | 21.32 ± 0.043501 |

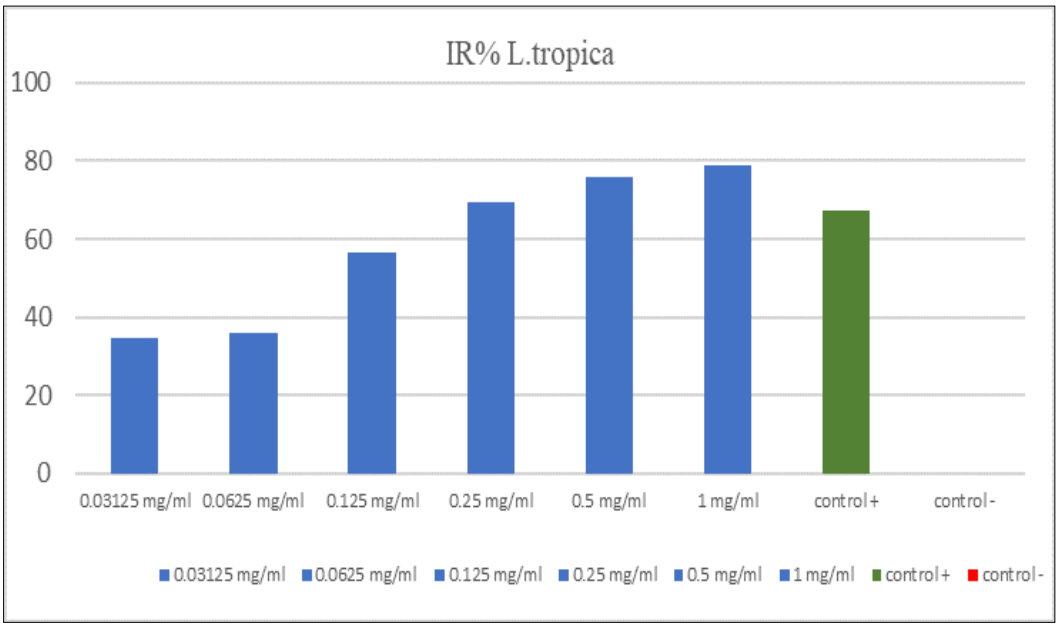


**Fig. 3.** % IR of *L. tropica* against the 6 concentrations of hexane extract.

The inhibition rate in specific concentrations (1, 0.5, 0.25) was higher than the inhibition rate of the positive control.

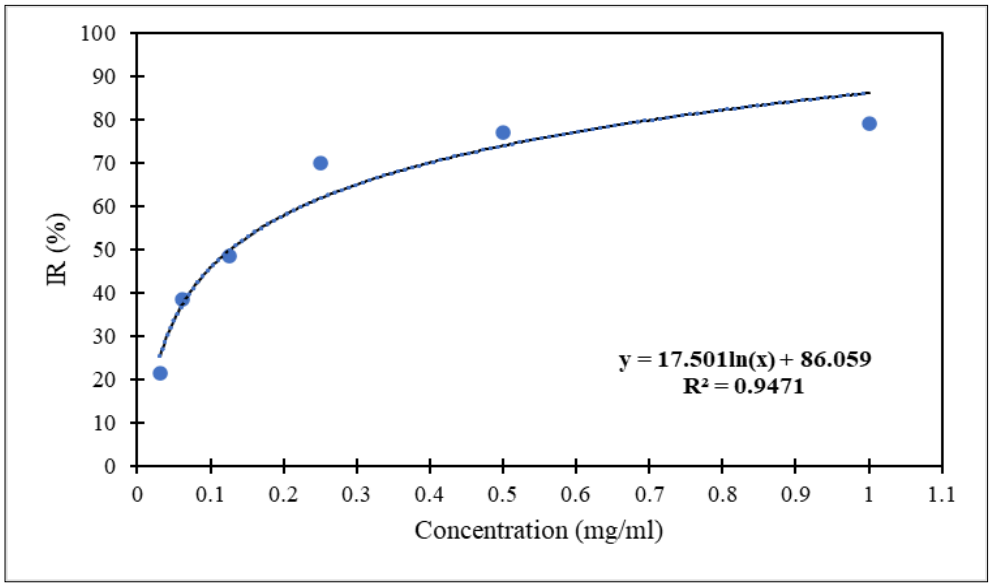


**Fig. 4.** $IC_{50}$ for hexane extract against *L. tropica*.

The hexane extract showed more activity at doses of 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL.

concentration and compares it to the positive control. Using a logarithmic equation, as shown in Fig. 4, the $IC_{50}$ used to treat *L. tropica* was 0.127 mg/mL.

According to previous research, 94 plant species distributed in 39 families have been known for their antileishmanial activities against different species, such as *L. tropica*, *L. major*, *L. aethiopica* and *L. donovani* and the leaves were the most commonly used parts at nearly 50 %. One of the reported families with antileishmanial activity is Malvaceae, with different plant species (*Ceiba pentandra*, *Cola acuminate*, *Cola cordifolia*, *Glyphaea brevis*) (44). Therefore, for the 1st time, the *C. olitorius* plant was assessed for antileishmanial activity using leaf part and hexane solvent to extract terpenes, including triterpenes, steroids, sesquiterpenes and diterpenes, which were frequently observed as leishmanicidal agents (45). The results exhibited that the hexane extract is rich in terpenes and the inhibition rate in specific concentrations (1, 0.5, 0.25) was higher than the inhibition rate of the positive control.

## Conclusion

According to this study, the phytochemical analysis by the GC-MS for the hexane extract of the *C. olitorius* plant showed that it is rich in non-polar phytoconstituents with more significant percentages of palmitic acid, linolenic acids and phytol compared to minor percentages of β-sitosterol, which was also confirmed by TLC analysis. In addition, it has been shown that hexane extract has antileishmanial qualities similar to Pentostam® treatment. In terms of its steroid and terpenoid composition, the hexane extract showed more activity than Pentostam® at doses of 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL. This not only validates the conventional claim about the plants but also provides a basis for further research into the active ingredients of these plants to develop safe and effective antileishmanial drugs.

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## Authors' contributions

TZAJ collected the sample, conducted the extraction procedure and the phytochemical analysis. NMI assessed the antileishmanial activity and the data analysis. MAA made the interpretation of GC-MS data and wrote the manuscript. All authors collaborated on the completion of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** There are no disclosed conflicts of interest for the authors.

**Ethical issues:** None

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