PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.9238

eISSN 2348-1900

RESEARCH ARTICLE

Phytochemical analysis and *in vitro* antileishmanial activity of *Basella alba* extract cultivated in Iraq

Sara H Hachim[1*] & Nabaa M Ibrahim[2]

[1]Department of Salah Al-Din Health, Ministry of Health, Tikrit 34001, Iraq
[2]Department of Pharmacognosy and Medicinal Plant, University of Baghdad, Baghdad 00964, Iraq

*Correspondence email - sara.hamed2200@copharm.uobaghdad.edu.iq

Received: 01 May 2025; Accepted: 13 July 2025; Available online: Version 1.0: 09 September 2025

Cite this article: Sara HH, Nabaa MI. Phytochemical analysis and *in vitro* antileishmanial activity of *Basella alba* extract cultivated in Iraq. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9238

Abstract

Basella alba, widely known as Malabar spinach, is a fast-growing perennial native to tropical regions Worldwide. *B. alba* has been widely utilized in traditional medicine because of anti-inflammatory properties, laxative, diuretic and antioxidant properties. The current research focuses on determining the chemical composition of *B. alba* and evaluating its antileishmanial efficacy against the promastigote of *Leishmania tropica in vitro*. This study is the first to examine the potential of *B. alba* as a natural remedy for leishmaniasis, as its antileishmanial properties have not been previously investigated. A Soxhlet apparatus was used to obtain whole-plant extracts of *B. alba*. The MTT proliferation assay was employed to evaluate the *in vitro* antileishmanial efficacy of *L. tropica* promastigotes. Reverse phase-high -performance liquid chromatography (RP-HPLC) was utilized to identify and isolate lupeol and β-sitosterol in the petroleum ether fraction. Gas chromatography-mass spectrometry (GC-MS) was employed to perform a comprehensive chemical profile of the fraction. To confirm the isolated compounds' molecular structure and fragmentation patterns, structural characterization was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). To determine whether characteristic groups were found, Fourier-transform infrared spectroscopy (FTIR) was employed. The petroleum ether extract demonstrated dose-dependent anti-promastigote activity with a notable inhibition rate of 72 %, indicating its potential antileishmanial activity. The presence of bioactive components with established antileishmanial properties highlights the potential of *B. alba* as a natural antileishmanial agent. Further *in vivo* studies are needed to support these *in vitro* findings and confirm their therapeutic potential.

Keywords: HPLC; LC-MS/MS; *Basella alba*; *Leishmania tropica*

Introduction

Leishmaniasis is a parasitic disease that places a significant global population at risk, with more than 12 million people currently affected in 88 countries (66 in the old World and 22 in the new World). It presents with three clinical forms: cutaneous, mucocutaneous and visceral. The World Health Organization (WHO) estimates that 90 % of cutaneous leishmaniasis (CL) cases are found in just seven countries (Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria), which also estimates that approximately 1 to 1.5 million cases of CL and 500000 cases of the visceral type occur annually. In Bangladesh, India, Nepal, Sudan and Brazil, more than 90 % of reports of visceral leishmaniasis (VL) are found in agricultural regions and suburban settings.

Leishmania species such as *L. major, L. tropica, L. aethiopica, L. mexicana, L. amazonensis* and *L. braziliensis* are frequently responsible for CL, a dermal manifestation (1, 2). Amphotericin B, a polyene antibiotic and pentavalent antimonial sodium stibogluconate (pentostam) are first-line recommended treatments. These medications have substantial adverse effects, including nephrotoxicity, hepatitis, pancreatitis, nausea and vomiting. The proliferation of drug-resistant species is the biggest drawback of the aforementioned drugs. This is particularly evident with sodium stibogluconate, which has failure percentages of up to 65 % in endemic regions (3). However, plant extracts or chemicals produced from plants are probably going to be a great source of novel therapeutic treatments (4).

The Basellaceae family includes the fast-growing perennial plant known as Malabar spinach (*B. alba)*. It is also commonly known as Indian spinach, Ceylon spinach and vine spinach (5). Anticancer, antiviral, antioxidant, anti-inflammatory, anti-cholesterol, anti-ulcer, antibacterial, anti-hypoglycemic, wound healing and androgenic are just a few of the ailments that Malabar spinach has been used to treat since ancient times (6). Plants are utilized as laxatives in Ayurveda to cure skin conditions, hemorrhages, diarrhea, dysentery and other ailments. β-cyanin, carotenoids, bioflavonoids, β-sitosterol and lupeol exist in these plant species and are considered to have anti-inflammatory, anti-proliferative, antimicrobial and antioxidant properties (7). Because of their unique pharmacological properties, these naturally occurring substances-which are mostly derived from plant materials-have proved crucial in the drug development process. Their

broad range of pharmacological actions makes it possible to target many biological pathways, providing a broad spectrum approach to disease therapy (8).

The antileishmanial activity of *B. alba* against *L. tropica* was examined in this study. The petroleum ether extract was analyzed using LC-MS/MS, FT-IR and RP-HPLC. The effects of the plant extracts on *L. promastigotes* were assessed using these methods. The use of GC-MS, HPLC, FT-IR and LC-MS/MS enables a comprehensive and precise analysis of the chemical constituents in the extract. These methods also assist in determining the active ingredients that provide the extract of its anti-parasite biological effects.

Materials and Methods

Collection and authentication of plant materials

B. alba whole plant was acquired in September 2024 from a farm in the city of Baghdad (Fig. 1). The authenticity of *B. alba* was confirmed by Dr. Israa Abdulrazaq Majeed, Assistant Professor in the Department of Biology, University of Baghdad. A mechanical grinder was used to grind the plant into a coarse powder after it had been washed and allowed to dry undercover.

Extraction and fractionation

A Soxhlet apparatus with 1 L of 85 % aqueous ethanol was used to extract 100 g of the dried, powdered whole plant material for 12 hr. Next, 250 mL of distilled water was used to suspend the dried extract and washed three times in a separatory funnel using 250 mL each of petroleum ether, chloroform and ethyl acetate. Each solvent fraction was collected individually, dried with anhydrous sodium sulfate, purified and then dried completely using a rotary evaporator.

Chromatography using gas analysis of mass spectrometry (GC-MS)

The petroleum ether component of the plant was analyzed with a gas chromatograph (Agilent Technologies, USA (7820A) 5977E GC mass spectrometer) to identify its bioactive components.

Column of analysis: Agilent HP-5ms ultra unit (length 30 m × inner diameter 250 µm × film thickness 0.25 µm) under GS/MS conditions (9). GC line of inlet, 1 µL of injection volume and 11.933 psi of pressure 250 °C is the temperature. Extra heaters transporter, 310 °C gas 99.99 % is the injector. 250 °C is the temperature. Range of scan: m/z 25-1000.

Fig. 1. Iraqi cultivation of *B. alba.*

The splitless injection oven program started at 70 °C as the first ramp. Ramp 2, from 70 °C to 180 °C, 7 °C per min. Ramp 3, from 180 °C to 280 °C, 8 °C per min. Ramp 4, from 180 °C to 300 °C, 7 °C per min. Wait for seven min. The GC-MS analysis was performed at the Ibn Al-Bitar Research Center, Research and Development Authority, Ministry of Industry and Minerals.

Reverse phase-high performance liquid chromatography (RP-HPLC)

For HPLC separation, the Nucleosil C18 column (Supelco, 5 µm, 250 mm× 4.6 mm id) was used. Applying an injection volume of 100 µL and a flow rate of 1 mL/min, acetonitrile-water (20:80 v/v) was used as the mobile phase in isocratic mode for the detection. The wavelength was detected 260 nm (10). The most prevalent kind of HPLC is reversed-phase HPLC. The stationary phase in reversed-phase chromatography is generally non-polar, whereas the mobile phase is moderately polar (11).

Liquid chromatography-tandem mass spectrometry (LC MS/MS)

The criteria employed for the LCMS/MS analysis at the Jordan University of Science and Technology utilized the Shimadzu (Japan) instrument model, comprising: GL Science C18 column (100 mm × 4.6, 5 µm particle size), ESI positive ionization mode, 50800 m/z scan range, 5500 V ion source voltage, 5 µL injection volume, 1 mL/min flow rate and a 25 min run duration (12). The isocratic mobile phase utilized consisted of acetonitrile, methanol (50:50 v/v), water and 0.1 % formic acid (13).

Fourier transform infrared (FTIR) spectra

The Shimadzu FTIR spectrometer was used to record the infrared (IR) spectra of isolated chemicals in the transmittance mode, with wave numbers ranging from 500 to 4000 cm^{-1}. The thin disk was then prepared under anhydrous circumstances using a Pulver's mold and press that contained around 1 mg of material (14). The measuring tool is located at the Ministry of Sciences and Technology's Environmental and Water Research Department.

Parasite culture preparation

Several confirmed patients collected samples of *Leishmania* from a medical facility in Baghdad and delivered them to the Biological Technology Research Center, College of Science, University of Al-Nahrain. The organism was cultivated on "Roswell-Park-Institute-Park-Memorial" (RPMI) medium together with serum 12 %, fetal calf fetal for five days at 25 °C until the parasite concentration averaged 105 parasites per mL in a hemocytometer to assess the impact of the plant extract (15, 16).

Plant extract concentration preparation

When evaluated against *L. tropica*, the petroleum ether fraction's antileishmanial efficacy was comparable to that of pentostam, a commonly used drug. Dimethyl sulfoxide (DMSO) was utilized as an extract dissolver. A maximum of 20 µL of DMSO was added. To achieve the required strength of 2000 µg/

mL. Distilled water (DW) was subsequently included. The dilutions that followed were made using this solution (DW). They were 2000, 1000, 500, 250, 125 and 62.5 µg/mL. The selected concentration was based on previous literature (17).

Making a positive control

The pentavalent antimonial (sodium stibogluconate injection 100000 µg/mL) from Glaxo Smith Kline UK was utilized as a positive control. A concentration of 100 µg/mL was achieved by diluting the sodium stibogluconate injection (100000 µg/mL) many times. After that, six µL were added to each well that had previously held one mL of RPMI and one mL of *L. tropica* inoculum. To assess variance, this was carried out in two wells consecutively (15).

Activity assessment of samples against *L. tropica*

The experiments were conducted in triplicate. To evaluate the antileishmanial activity, *Leishmania promastigotes* were added to each well of a 96-well flat-bottom plate. A total of 30 wells were used: 18 wells received 10 µL (in triplicate) of the previously prepared serial dilutions of the tested fractions; 6 wells were treated with sodium stibogluconate as a positive control; 3 wells served as negative controls (with no treatment) and 1 well was treated with 50 % DMSO.

After incubation for 24 hr at 25 ± 1 °C, 10 µL of MTT dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well. The plates were then incubated for an additional 4 hr at the same temperature to allow for the formation of formazan crystals by metabolically active cells.

Subsequently, DMSO was added to each well to solubilize the formazan crystals. The level of formazan formation, which is directly proportional to the number of viable cells, was quantified by measuring the absorbance at 490 nm using an ELISA microplate reader (17, 18). A higher absorbance indicates a greater number of viable cells due to increased metabolic activity.

Statistical analysis

The data were displayed as mean ± SD (standard deviation) and each experiment was performed in three replicates. Microsoft Excel 2019 was used for statistical analysis and graphical representation.

Calculation of IC_{50}

The efficacy related to a substance in suppressing a particular physiological activity is indicated by its half-maximum inhibitory concentration (IC_{50}) (19). In this investigation, dose-response curves created in Microsoft Excel were used to calculate the petroleum ether fraction's IC_{50} values against *L. tropica*. To determine the rate of inhibition at different concentrations of the petroleum ether component, the following formula was used (3):

% Inhibition Rate= (OD Control-OD Test/OD control) × 100

Results

Amount and percentage yield of the fractions

Using petroleum ether for extraction and fractionation, various percentages of *B. alba* extracts were produced. Aqueous ethanol and petroleum ether extract amounts and yield percentages are shown in Table 1.

Table 1. The amounts and percentage of aqueous ethanol and petroleum ether extracts that were produced

Plant extract fraction	The amount in grams	The yield percentage
Aqueous ethanol	24	24 %
Petroleum ether	3	3 %

Lupeol and β-sitosterol isolation using RP-HPLC analysis

By using RP-HPLC to analyze the petroleum ether extract of *B. alba*, two major components were identified based on their chromatographic profiles: lupeol and β-sitosterol (Fig. 2). To confirm their presence in the extract, the retention times of these compounds were compared with those of pure standards (Table 2). The chromatographic profiles of the standards and isolated compounds are shown in (Fig. S1-S4). The retention times closely matched, supporting the identification. These findings are consistent with previous studies reporting the presence of terpenoids such as lupeol and β-sitosterol in *B. alba* (7).

Gas chromatography/mass spectroscopy investigation of *B. alba*

Bioactive phytochemical components were detected by GC-MS analysis in the petroleum ether extract of the entire Iraqi *B. alba* plant. Molecular weight, chemical formulas and retention time were used to identify the compound. Five major compounds were identified with similarity values above 90 %, providing high confidence in identification (Fig. S5-S10). Molecular ion peaks (M^+), base peaks, characteristic fragment ions (m/z), retention times, peak areas, exact masses, chemical formulas and compound classification based on elution behavior on the HP-5 ms capillary column (11). Ultra inert column are all summarized in Table 3. Standard mass spectrometry was used to evaluate the fragmentation patterns to support the interpretation of the fragmentation patterns for each compound (20-24).

Tandem mass spectrometry combined with liquid chromatography (LC-MS/MS)

Different molecular ion peaks for lupeol (m/z 427 [M+H]+) and β -sitosterol (m/z 415 [M+H]+) were revealed by LC-MS/MS analysis. Fig. 3 and 4 illustrate the fragmentation patterns that support their structural identities and these are consistent with previously published studies (20-24). The original LC-MS/MS spectra are provided in the supplementary material (Fig. S11, S12).

Table 2. Retention time and peak area of the isolated compounds

Standard used	Retention time of standard peak (min)	Area of standard	Retention time of isolated compound (min)	Area of isolated compound
Lupeol	5.60	622.45	5.70	1014.55
β-sitosterol	6.00	419.80	6.09	1389.77

Table 3. GC/MS analytical results were obtained from the petroleum ether fraction of the sample

Compound name	Retention time in Min.	Chemical formula	Molecular weight g/mol	Molecular ion peak	Base peak	Other Fragment m/z
Squalene	29.267	$C_{30}H_{50}$	410.730	$[C_{30}H_{50}]^+$ m/z:410	$[C5H9]^+$ m/z :69	231 $[C_{17}H_{27}]^+$ 149 $[C_{11}H_{17}]^+$ 95 $[C_7H_{11}]^+$ 81 $[C_6H_9]^+$
α-Tocopheryl acetate	32.955	C29H50O2	430.71	$[C_{29}H_{50}O_3]^+$ m/z :430	$[C_{10}H_{13}O_2]^+$ m/z:165	207$[C_{13}H_{19}O_2]^+$ 136$[C_8H_8O_2]^+$ 91 $[C_7H_7]^+$ 43 $[C_2H_3O]^+$
Stigmasterol	35.748	$C_{29}H_{48}O$	412.702	$[C_{29}H_{48}O]^+$ m/z:412	$[C_4H_7]^+$ m/z:55	119$[C_9H_{11}]^+$ 133$[C_{10}H_{13}]^+$ 91$[C_7H_7]^+$ 43 $[C_3H_7]^+$
β-Sitosterol	36.048	C29H50O	414.718	$[C29H50O]^+$	$[C_3H_7]^+$ m/z = 43	55 $[C_4H_7]^+$ 81 $[C_6H_9]^+$ 95 $[C_7H_{11}]^+$ 315$[C_{22}H_{35}O^+]$
Lupeol	36.670	$C_{30}H_{50}O$	426.729	$[C_{30}H_{50}O]^+$ m/z:426	$[C_3H_7]^+$ m/z = 43	55 $[C_4H_7]^+$ 68 $[C_5H_8]^+$ 109$[C_8H_{13}]^+$ 207 $[C_{15}H_{27}]^+$

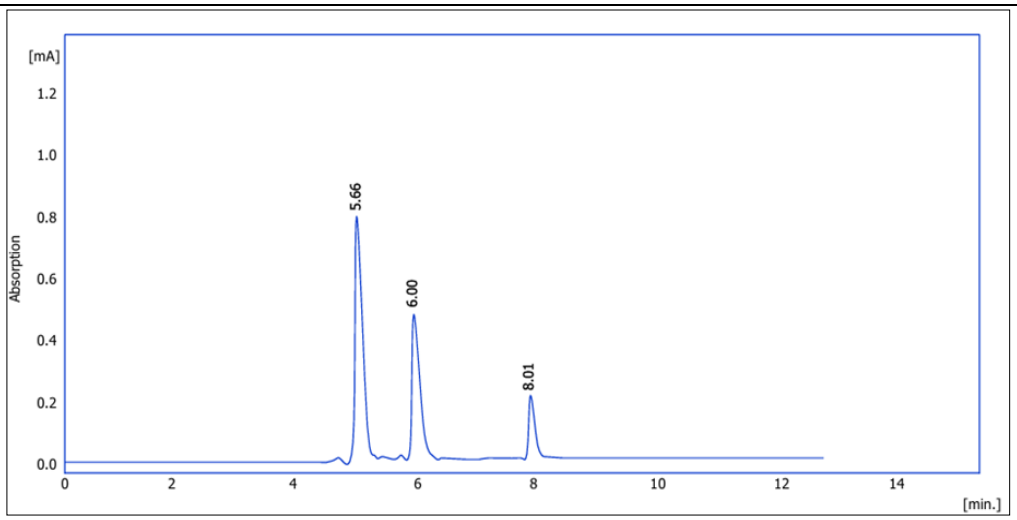

Fig. 2. Iraqi *B. alba* petroleum ether fraction HPLC chromatogram.

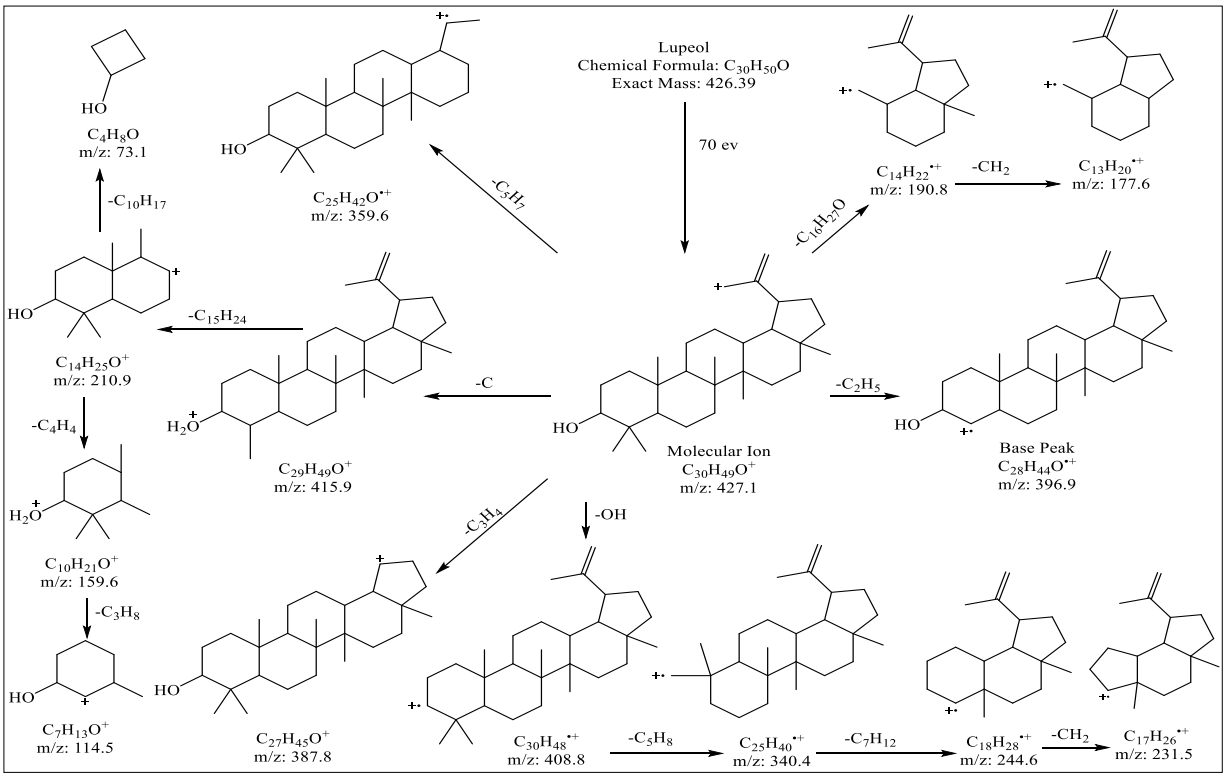

Fig. 3. Lupeol LC-MS/MS spectrum interpretation identification of molecular ions and fragmentation pattern.

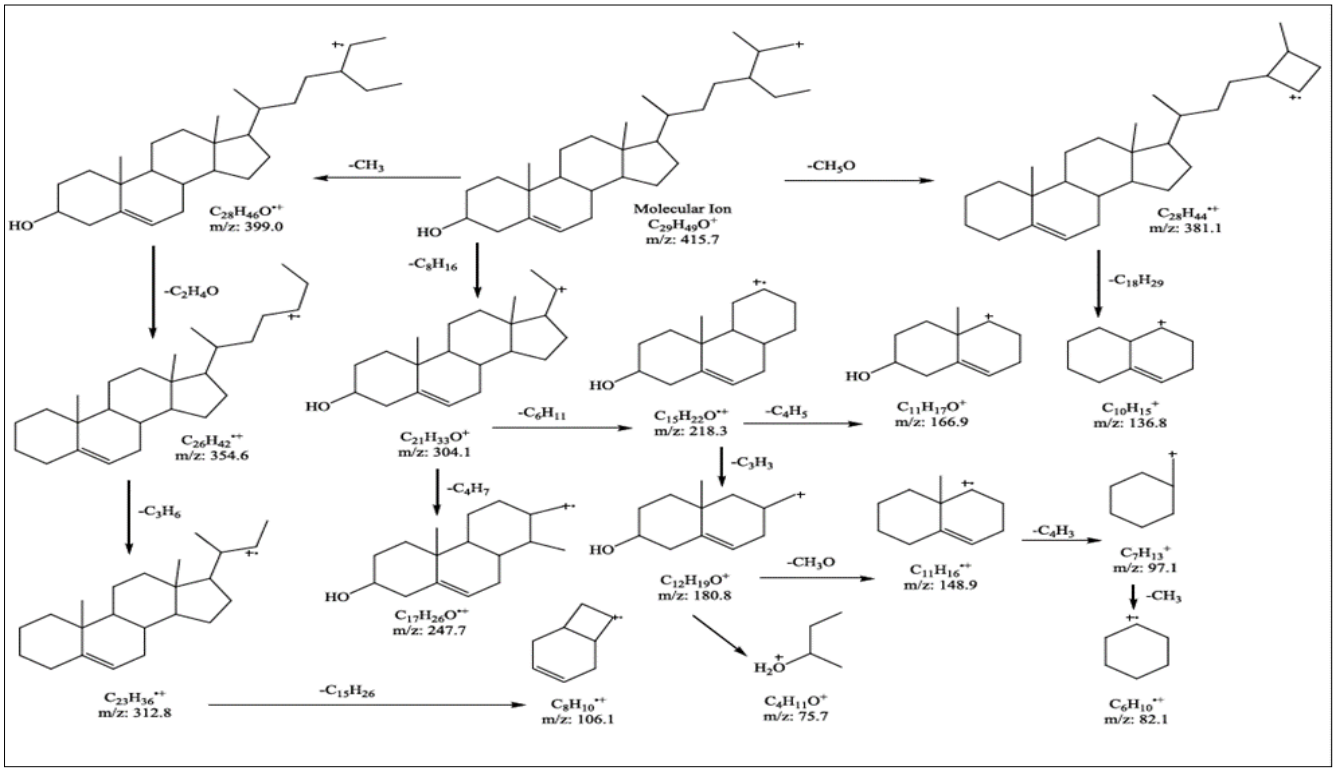

Fig. 4. β-Sitosterol LC-MS/MS spectrum interpretation identification of molecular ions and fragmentation pattern.

FT-IR spectrum

The separated compounds' FT-IR spectra displayed distinctive absorption bands that matched the lupeol and β-sitosterol standards. The infrared spectra were consistent with those from earlier research (25, 26). The compounds' effective functional groups are further described in the accompanying table of characteristic FT-IR absorption bands. Fig. 5 displays the FT-IR spectrum for lupeol, while Fig. 6 depicts its chemical structure. Table 4 provides specifics on how to interpret the absorption bands. Regarding β-sitosterol, FT-IR spectrum and the chemical structure illustrated in Fig. 7, 8. Table 5 lists the appropriate absorption band assignments.

Petroleum ether fraction's antileishmanial activity

Fig. 9 shows the *L. tropica* inhibition rate for each of the six concentrations. This study used dose-response curves to compute petroleum ether fraction's IC_{50} values against *L. tropica* (1157 µg/mL). A dose-dependent response was suggested by the high positive correlation (R^2 = 0.9101) found between concentration and inhibition rate. After 1000 µg/mL, the inhibitory effect peaked, increasing only slightly at 2000 µg/mL (72 % to 61 % inhibition). This implies a concentration barrier, over which additional increases provide declining rewards. The IC_{50} reflects the potency of a substance in achieving a 50 % reduction in parasite viability at lower concentrations, while the 70 % inhibition in 2000 µg/mL, shows the extract's effectiveness at a high concentration. The findings showed that the differences in inhibition percentages, suggesting that the fraction may have a dose-dependent effect in inhibiting *Leishmania* growth (Fig. 10).

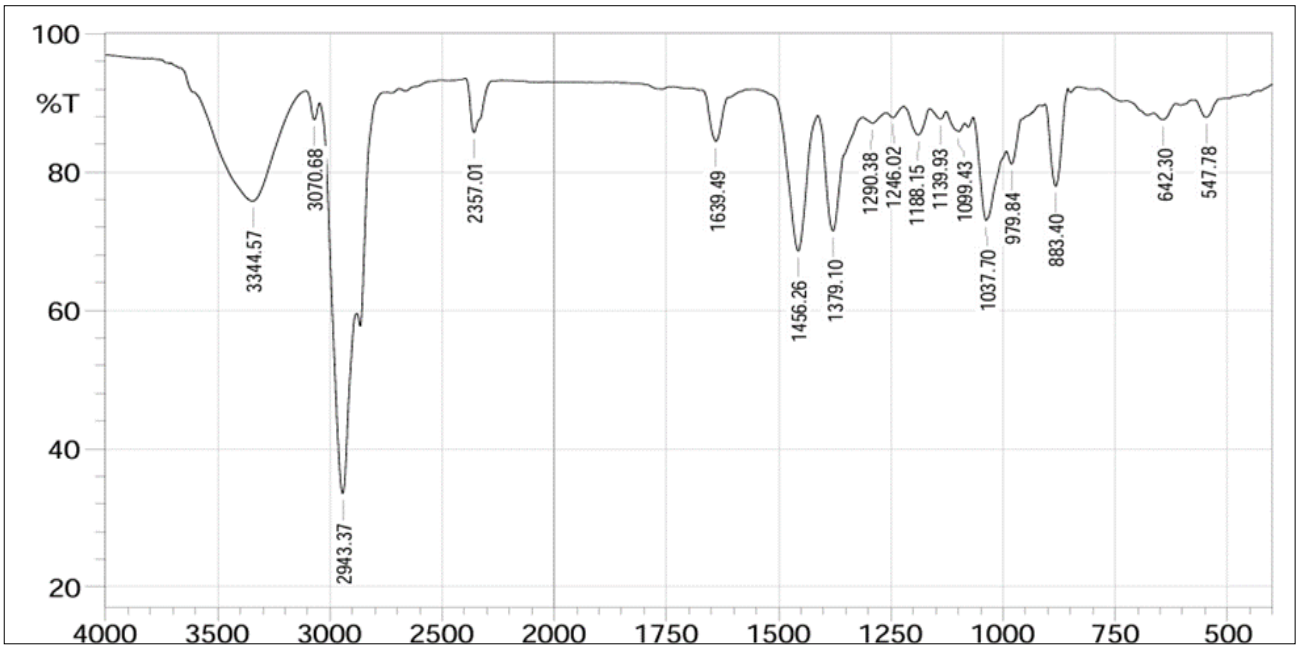

Fig. 5. FTIR spectra of isolated lupeol from petroleum ether fraction.

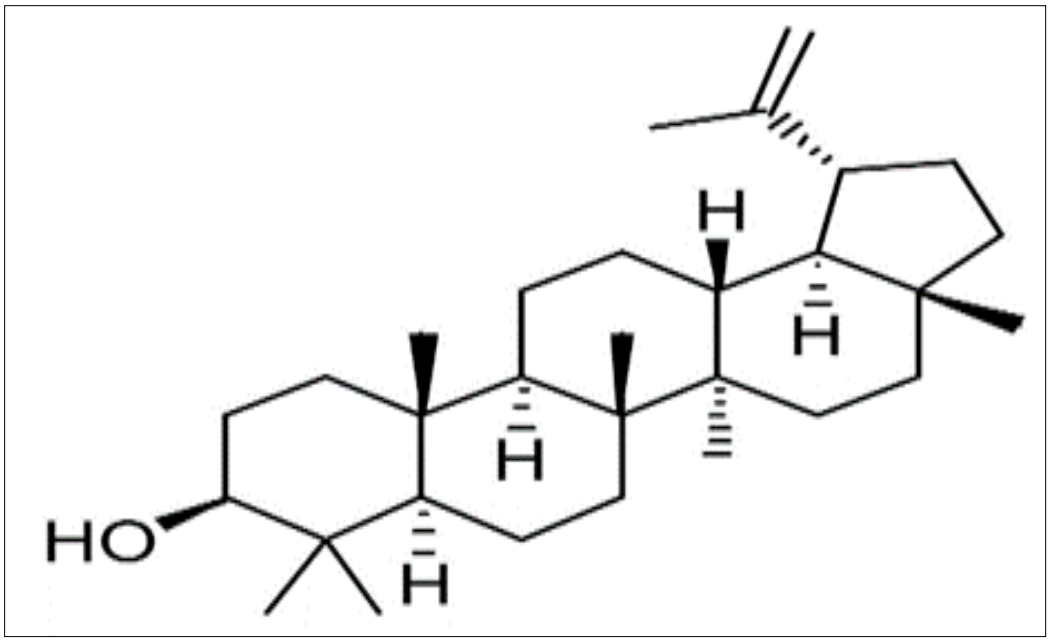

Fig. 6. Isolated lupeol structure from petroleum ether fraction.

Table 4. The isolated compound lupeol's characteristic FTIR absorption bands (cm^{-1})

Functional group	Frequency wave number(cm^{-1})	Interpretation
O-H	3342.57	Stretching of the O-H vibration in alcohol
C-H	3070.68	Stretching vibration of alkene C-H
C-H	2943.8	Aliphatic stretching vibration of the C-H
C-H	1456.26	The C-H alkane bending vibration
C=C	1639.49	C=C alkene vibration stretching
C-O	1099.43, 1246.0, 1290.3	Stretching of the C-O vibration in alcohol

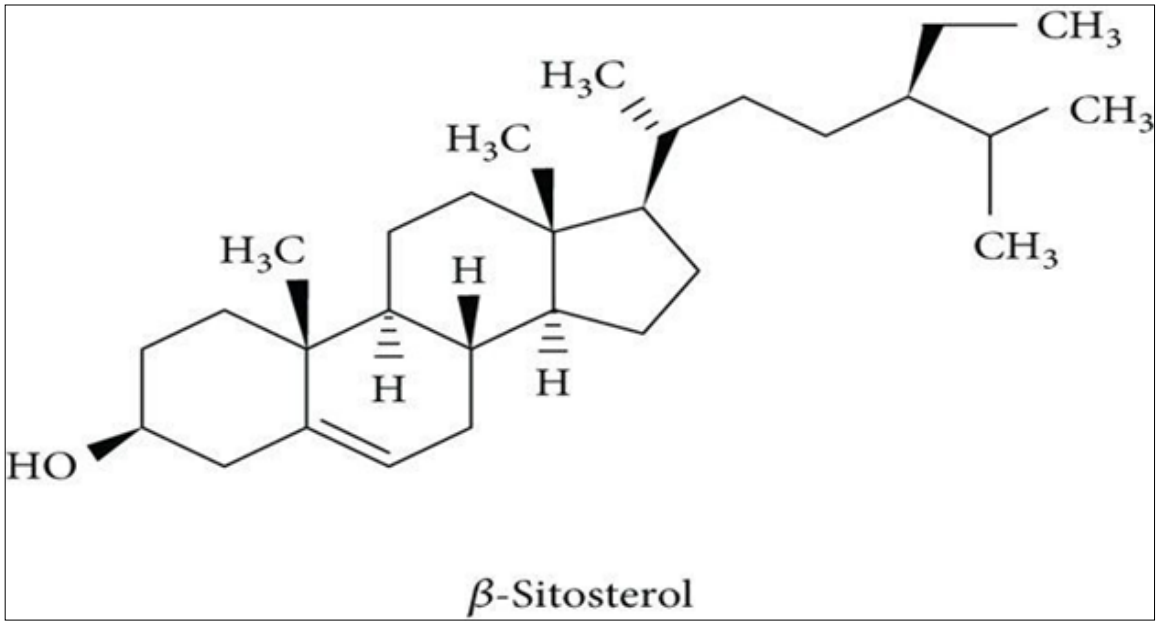

Fig. 7. Isolated β-sitosterol structure from petroleum ether fraction.

Table 5. The isolated substance β-sitosterol's characteristic FTIR absorption bands (cm^{-1})

Functional group	Frequency wave number(cm^{-1})	Interpretation
O-H	3352.3	Broadband stretching vibration of an alcoholic group
C-H	2944.52	Alkane C-H stretching
C=C	1660.71	Alkene groups stretching (C=C)
C-H	1458-1371	Bending vibration of isopropyl
C-O	1371.39	C-O alcohol stretching
C-C	1056.09	C-C stretching

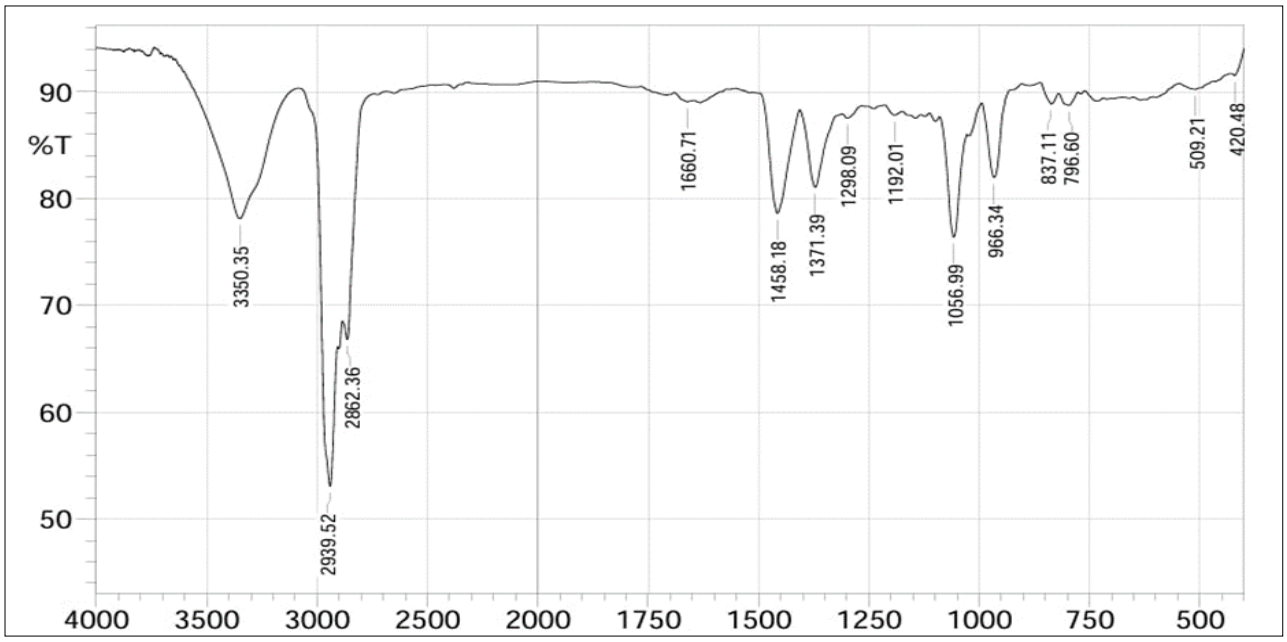

Fig. 8. FTIR spectra of isolated β-sitosterol from petroleum ether fraction.

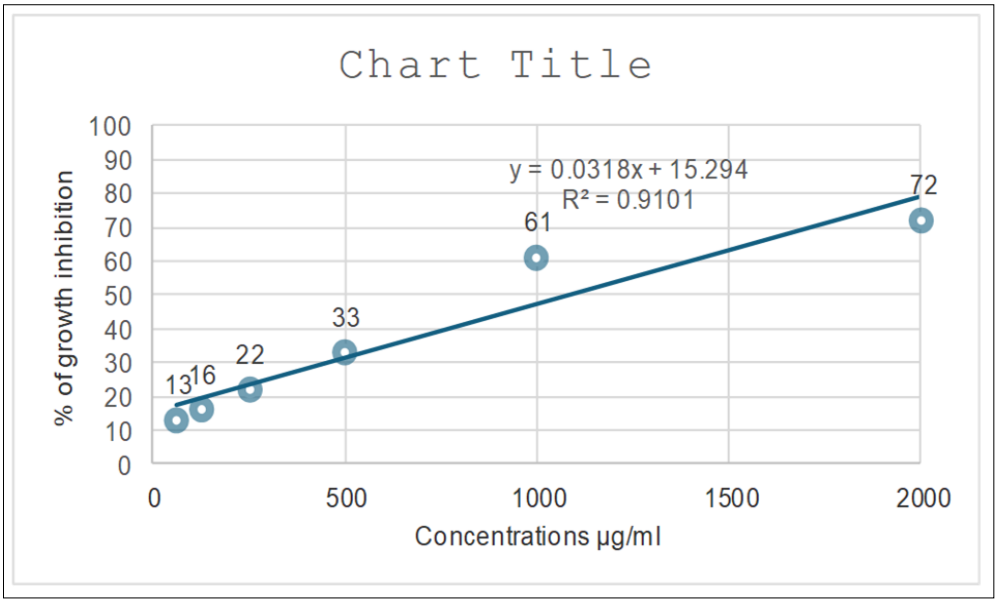

Fig. 9. Growth of inhibition percentages of *L. tropica* promastigotes obtained with various dilutions of petroleum ether fraction (µg/mL).

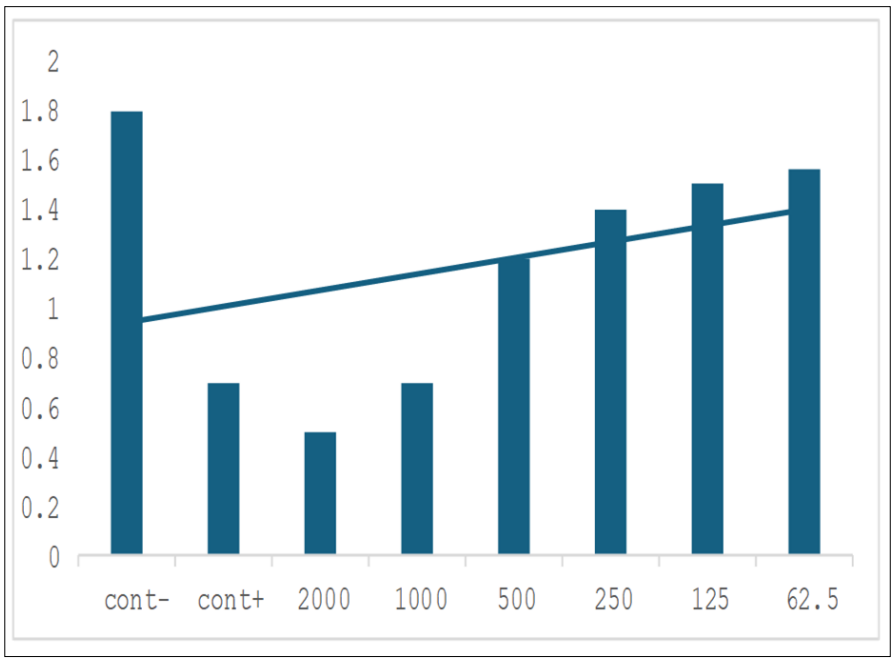

Fig. 10. To compare the effects of the positive control (pentostam) with each of the six concentrations of fraction.

Discussion

This study demonstrates the antileishmanial potential of *B. alba*, as its petroleum ether extract inhibited *Leishmania* replication in the MTT assay. The phytochemical research confirmed the existence of many bioactive substances with antimicrobial, anti-inflammatory and antiparasitic effects, such as β-sitosterol, lupeol, squalene, α-tocopheryl acetate and stigmasterol. Prior research has demonstrated that β-sitosterol generated from plants has been thoroughly investigated for several types of leishmaniasis. *In vitro* studies have demonstrated the antileishmanial activities of phytosterols, such as stigmasterol and β-sitosterol that were extracted from the fruit peel of *Musa paradisiaca*. These phytosterols specifically inhibit the development of promastigotes and amastigotes of *L. infantum* Chagasi (27).

Nevertheless, the precise mode of action of β-sitosterol is yet unclear. *Leishmania* species and strain variations, phenotypic variety, clinical presentation and geographic origin are likely to contribute to variations in its efficacy (28, 29). There are several secondary metabolites from plants that have target-specific anti-parasite properties. By directly targeting parasites and indirectly influencing the immune system, terpenes, for instance, have shown antileishmanial action. Through the release of cytokines, they stimulate a pro-inflammatory response that results in the generation of NO and ROS, ultimately lowering the parasite load. Remarkably, terpene-based therapies have demonstrated minimal toxicity and in some case, equal or greater effectiveness as prescribed drugs (30).

The antileishmanial properties of lupeol are believed to be influenced by its lipophilic nature, which may alter the structure and function of ion channels, receptors and enzymes, hence compromising parasite survival (31). For *L. tropica* infections, it has also been demonstrated that β-sitosterol, a phytosterol structurally like cholesterol, works better than mupirocin and ketoconazole, resulting in complete recovery with no visible scarring (32). This suggests that β-sitosterol might be a good alternative for treating cutaneous leishmaniasis. Furthermore, by increasing antioxidant enzyme activity and lowering lipid peroxidation, vitamin E (α-tocopheryl acetate) and squalene contribute significantly to the reduction of oxidative stress, strengthening the extract's protective properties (33-35). The extract contains sterols like stigmasterol and β-sitosterol, which not only prevent parasites from multiplying but also strengthen cellular membranes and make it more difficult for them to enter host cells (7).

To detect, isolate and validate the presence of lupeol and β-sitosterol in *B. alba,* a combination of RP-HPLC, GC-MS and LC-MS/MS analytical methods was used. For the separation of these non-polar phytosterols, RP-HPLC was crucial since it provided accurate retention data. By comparing their mass spectrum data with standard libraries, GC-MS offered additional evidence. Additionally, by using fragmentation patterns to enable high-sensitivity detection and structural confirmation, LC-MS/MS made it possible to accurately identify both lupeol and β-sitosterol. These substances highlight *B. alba*'s therapeutic significance and supporting its potential applications in pharmaceuticals. Based on research demonstrating that it offers the best extraction efficiency for a range of phytochemicals, 85 % aqueous ethanol was used. In comparison to pure ethanol or lesser concentrations, the inclusion of water at this concentration improves solvent polarity and cell penetration, resulting in better yields (36).

Since *B. alba* has not been previously investigated for its antileishmanial activity, we employed a broad concentration range in the cytotoxicity assay to maximize the possibility of detecting any biological activity. This strategy is supported by toxicological guidelines suggesting that *in vitro* tests are commonly performed at concentrations much higher than plasma C_{max} levels-often 20- to 200- fold higher-to trigger observable effects, as higher concentrations are typically needed in culture media to induce cytotoxicity compared to *in vivo* conditions (37, 38).

The petroleum ether fraction's comparatively high IC_{50} value might be the result of multiple compounds with various biological functions, including potential antagonistic interactions (39). The fraction still contains a variety of elements that may affect overall potency, although being more refined than a crude extract. To improve antileishmanial effectiveness, it is advised to further purify active ingredients such lupeol and β-sitosterol. Determining the therapeutic index and safety profile also requires assessing cytotoxicity on mammalian cell lines. To fully comprehend and maximize the fraction's potential as a natural antileishmanial agent, future research should concentrate on these areas.

Conclusion

This study examines the activity of *B. alba* against *L. tropica* for the first time. The RP-HPLC, GC-MS, FTIR and LC-MS/MS techniques were used to characterize the petroleum ether extract. Promising antileishmanial potential was shown by the extract's dose-dependent suppression of *L. tropica* promastigotes. The comparatively high IC_{50} value, however, indicates that greater doses are required to provide significant inhibition. Future studies should concentrate on separating and enhancing the extract's active ingredients in order to increase effectiveness. *B. alba* fractions might also be combined with other established antileishmanial drugs to explore potential synergistic effects. To evaluate the extract's pharmacokinetics, safety and therapeutic potential in animal models, *in vivo* research will be essential. These actions are necessary to confirm that *B. alba* is a safe and effective natural remedy for leishmaniasis.

Acknowledgements

We thank Department of Pharmacognosy and Medicinal Plants, teachers, pharmacy college and staff at Baghdad University for their assistance in providing the necessary facilities.

Authors' contributions

The work was equally contributed by SHH and NMI. Both author reviewed the findings and endorsed the final draft of the paper. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Gramiccia M, Gradoni L. The current status of zoonotic leishmaniases and approaches to disease control. Int J Parasitol. 2005;35(11-12):1169–80. https://doi.org/10.1016/j.ijpara.2005.07.001
2. Aliaga L, Cobo F, Mediavilla JD, Bravo J, Osuna A, Amador JM, et al. Localized mucosal leishmaniasis due to *Leishmania (Leishmania) infantum*: Clinical and microbiologic findings in 31 patients. Medicine. 2003;82(3):147–58. https://doi.org/10.1097/01.md.0000076009.64510.b8
3. Jewely HM, Zuhair T. Evaluation of antileishmanial activity of *Osteospermum ecklonis* extract of aerial parts against *Leishmania donovani*: *In vitro*. Iraqi J Pharm Sci. 2022;31(Suppl):45–53. https://doi.org/10.31351/vol31issSuppl.pp45-53
4. Bravo JA, Sauvain M, Gimenez TA, Balanza E, Serani L, Laprevote O, et al. Trypanocidal withanolides and withanolide glycosides from *Dunalia brachyacantha*. J Nat Prod. 2001;64(6):720–25. https://doi.org/10.1021/np000527p
5. Roy SK, Gangopadhyay G, Mukherjee KK. Is stem twining form of *Basella alba* L. a naturally occurring variant? Curr Sci. 2010;99 (10):1370–75.
6. Moutusi S, Parivallal PB, Prasannakumar MK, Kiranmayee P. Morphological and molecular characterization of culturable leaf endophytic fungi from Malabar spinach: The first report. Stud Fungi. 2019;4(1):192–204. http://dx.doi.org/10.5943/sif/4/1/2
7. Abu-Irmaileh B, Al-Hroub HM, Rasras MH, Hudaib M, Semreen MH, Bustanji Y. Phytochemical composition and antiviral properties of *Achillea fragrantissima* methanolic extract on H1N1 virus. Pharmacia. 2025;72:1–9. https://doi.org/10.3897/pharmacia.72.e138108
8. Satar Al, Baaj A, Abdul-Jalil TZ. Phytochemical screening of petroleum ether fractions by GC/MS and isolation of lupeol from two different parts of Iraqi *Leucaena leucocephala*. Iraqi J Pharm Sci. 2022;31(Suppl):62–74. https://doi.org/10.31351/vol31issSuppl.pp62-74
9. Nandhini S, Ilango K. Simultaneous quantification of lupeol, stigmasterol and β-sitosterol in extracts of *Adhatoda vasica* Nees. leaves and its marketed formulations by a validated RP-HPLC method. Pharmacogn J. 2020;12(4):850-56.
10. Ali AH. High-performance liquid chromatography (HPLC): A review. Ann Adv Chem. 2022;6(1):10–20.
11. Ibrahem NM, Khadum EJ, Mutlag SH. Isolation of catechin and epigallocatechin from Iraqi *Rhus coriaria* by preparative high-performance liquid chromatography (PHPLC). Iraqi J Pharm Sci. 2022;31(2):271–82. https://doi.org/10.31351/vol31iss2pp271-282
12. Ezghayer MA, Ahmed OH, Tawfeeq MF. UPLC-ESI-MS/MS phytochemicals profiling of n-butanol, chloroform and hexane fraction of *Xanthium strumarium* fruit extract. Biomed Pharmacol J. 2024;17(2):1035–43.
13. Selvaraju R, Sakuntala P, Jaleeli KA. GC–MS and FTIR analysis of chemical compounds in *Ocimum gratissimum* plant. Biophysics. 2021;66(3):401–408. https://doi.org/10.1134/S0006350921030167
14. Al-Ogaili N. Synergistic effect of *Lawsonia inermis* and *Peganum harmala* aqueous extracts on *in vitro* growth of *Leishmania tropica promastigotes* compared to sodium stibogluconate. Al-Qadisiyah Med J. 2016;12(22):76–83.
15. Sereno D, Lemesre JL. Axenically cultured amastigote forms as an *in vitro* model for investigation of antileishmanial agents. Antimicrob Agents Chemother. 1997;41(5):972–76. https://journals.asm.org/doi/pdf/10.1128/aac.41.5.972
16. Rasool MS, Abdul-Jaleel TZ. Antileishmanial evaluation and phytochemical screening of Iraqi *Sanchezia speciosa* petroleum ether fraction *in vitro*. Iraqi J Pharm Sci. 2025;34(1):266–74. https://doi.org/10.31351/vol34iss1pp266-274
17. Wahyuni HS, Nugraha SE, Sumantri IB, Jap CC, Hijriyan TB. Unveiling the anticancer potential of the ethanolic extract from *Pometia pinnata*: Molecular dynamics targeting CHK1 and cytotoxicity study on MCF-7 cells. Pharmacia. 2024;71:1–15. https://doi.org/10.3897/pharmacia.71.e138556
18. Abdul-lalil TZ. Ultrasound-assisted extraction of fennel leaves: Process optimization, thin layer chromatography and cytotoxic activity of ethanolic extract. Iraqi J Pharm Sci. 2024;33(1):94-103. https://doi.org/10.31351/vol33iss1pp94-103
19. Mus'hib HK, Abdul-jalil TZ. Lupeol: Triterpene from Iraqi *Portulaca grandiflora* L. (Portulacaceae): Its extraction, identification (GC/MS), isolation (Combiflash) and structure elucidation. Iraqi J Pharm Sci. 2024;33(4SI):147-58. https://doi.org/10.31351/vol33iss(4SI)pp147-158
20. de Carvalho TC, Polizeli AM, Turatti IC, Severiano ME, de Carvalho CE, Ambrósio SR, et al. Screening of filamentous fungi to identify biocatalysts for lupeol biotransformation. Molecules. 2010;15 (9):6140-51. https://doi.org/10.3390/molecules15096140
21. Kosyakov DS, Ul'Yanovskii NV, Falev DI. Determination of triterpenoids from birch bark by liquid chromatography-tandem mass spectrometry. J Anal Chem. 2014;69:1264-69.
22. Abdullah Hussein Kshash, Omar Jamal Mahdi Al-Asafi, Hanaa Kaen Salih. Synthesis, characterization and investigation of mesomorphic properties of a new 2,5-bis-(4-alkanoyloxybenzylidene) cyclopentan-1 -one. Acta Chim Slov. 2022;69:519-25. https://doi.org/10.17344/acsi.2022.7360
23. Ezghayer MA, Kadhim EJ. UPLC-ESI-MS/MS and various chromatographic techniques for identification of phytochemicals in *Populus euphratica* Oliv. leaves extract. Iraqi J Pharm Sci. 2020;29 (1):94-114. https://doi.org/10.31351/vol29iss1pp94-114
24. McMurry J. Organic chemistry. Cengage Learning; 2016.
25. Azeez RA, Abaas IS, Kadhim EJ. Isolation and characterization of β-sitosterol from *Elaeagnus angustifolia* cultivated in Iraq. Asian J Pharm Clin Res. 2018;11(11):442-46. https://doi.org/10.22159/ajpcr.2018.v11i11.29030
26. Emaikwu V, Ndukwe IG, Mohammed R, Iyun ORA, Anyam JV. Isolation and characterization of lupeol from the stem of *Tapinanthus globiferus* (A Rich.) and its antimicrobial assay. J Appl Sci Environ Manage. 2020;24(6):1015-20. https://doi.org/10.4314/jasem.v24i6.1133
27. Silva AAS, Morais SM, Falcão MJC, Vieira IGP, Ribeiro LM, Viana SM, Andrade-Junior HF. Activity of cycloartane-type triterpenes and sterols isolated from *Musa paradisiaca* fruit peel against *Leishmania infantum chagasi*. Phytomedicine. 2014;21(11):1419-23. https://doi.org/10.1016/j.phymed.2014.05.005
28. Kariyawasam UL, Selvapandiyan A, Rai K, Wani TH, Ahuja K, Beg MA, Karunaweera ND. Genetic diversity of *Leishmania donovani* that causes cutaneous leishmaniasis in Sri Lanka: A cross-sectional study with regional comparisons. BMC Infect Dis. 2017;17:791. https://doi.org/10.1186/s12879-017-2883-x
29. Alcantara LM, Ferreira TC, Fontana V, Chatelain E, Moraes CB, Freitas-Junior LH. A multi-species phenotypic screening assay for leishmaniasis drug discovery shows that active compounds display a high degree of species-specificity. Molecules. 2020;25(11):2551. https://doi.org/10.3390/molecules25112551
30. Raimundo VD, Carvalho RPR, Machado-Neves M, de Almeida Marques-da-Silva E. Effects of terpenes in the treatment of visceral leishmaniasis: A systematic review of preclinical evidence.

Pharmacol Res. 2022;177:106117. https://doi.org/10.1016/j.phrs.2022.106117

31. Singh AP, Zhang Y, No JH, Docampo R, Nussenzweig V, Oldfield E. Lipophilic bisphosphonates are potent inhibitors of *Plasmodium* liver-stage growth. Antimicrob Agents Chemother. 2010;54(7):2987-93. https://doi.org/10.1128/aac.00198-10
32. Afedh AA. *In vivo* comparative study of the efficacy of β-sitosterol, ketoconazole 2 % and mupirocin for the treatment of cutaneous leishmaniosis. Ann Parasitol. 2022;68(2):233-39. https://doi.org/10.17420/ap6802.4313
33. Galli F, Bonomini M, Bartolini D, Zatini L, Reboldi G, Marcantonini G, et al. Vitamin E (alpha-tocopherol) metabolism and nutrition in chronic kidney disease. Antioxidants. 2022;11(5):989. https://doi.org/10.3390/antiox11050989
34. Cheng L, Ji T, Zhang M, Fang B. Recent advances in squalene: Biological activities, sources, extraction and delivery systems. Trends Food Sci Technol. 2024;104392.
35. Jie F, Yang X, Yang B, Liu Y, Wu L, Lu B. Stigmasterol attenuates inflammatory response of microglia via NF-κB and NLRP3 signaling by *AMPK* activation. Biomed Pharmacother. 2022;153:113317. https://doi.org/10.1016/j.biopha.2022.113317
36. Kumoro AC, Hartati I. Microwave assisted extraction of dioscorin from Gadung (*Dioscorea hispida* Dennst) tuber flour. Procedia Chem. 2015;14:47–55. https://doi.org/10.1016/j.proche.2015.03.009
37. He S, Wang X, Chen J, Li X, Gu W, Zhang F, et al. Optimization of ultrasonic-assisted extraction of steroidal saponins from *Polygonatum kingianum*. Molecules. 2022;27(5):1463. https://doi.org/10.3390/molecules27051463
38. Albrecht W. Which concentrations are optimal for *in vitro* testing? EXCLI Journal. 2020;19:1172.
39. Saab AM, Lampronti I, Finotti A, Borgatti M, Gambari R, Esseily F, et al. *In vitro* evaluation of the biological activity of lebanese medicinal plants extracts against herpes simplex virus type 1. Minerva Biotecnol. 2012;24(3):117–21.

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.