

Original Article

Insecticidal Activity of *Salvia officinalis*, *Pistacia vera* and *Eucalyptus* sp. Extracts Against *Phlebotomus papatasi*: A Potential Plant-Based Approach for Vector Control

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Abstract

Background: Insecticides are essential for controlling *Leishmania* vectors, but their extensive use leads to adverse environmental effects, particularly on non-target species. Given the health challenges associated with synthetic insecticides and the dire need for safe and sustainable alternatives, plant-based insecticides offer a promising solution. This study explores the insecticidal potential of *Salvia officinalis*, *Pistacia vera* and *Eucalyptus* sp. as environmentally friendly alternatives for controlling *Phlebotomus papatasi*.

Methods: Methanolic extracts of *S. officinalis*, *P. vera* and *Eucalyptus* sp. were prepared using the maceration method. The extracts were analyzed using titration methods for bioactive compounds, including flavonoids, alkaloids, tannins, saponins and antioxidant properties. Susceptibility tests on *Ph. papatasi* were performed using WHO standard kits, with median lethal time (LT₅₀) and median lethal dose (LD₅₀) values calculated by probit analysis.

Results: Phytochemical analysis revealed the presence of flavonoids in *S. officinalis* and *Eucalyptus* sp. None of the extracts contained alkaloids, but all contained tannins. *Pistacia vera* was the only extract containing saponins. The LD₅₀ values after 24 hrs for *S. officinalis*, *Eucalyptus* sp. and *P. vera* were 0.156, 0.576 and 0.41 µg/ml, respectively. The LT₅₀ values for *S. officinalis*, *Eucalyptus* sp. and *P. vera* at 1.6 µg/mL were 11.9, 12.5 and 14.4 hrs, respectively.

Conclusion: Plant-derived insecticides are gaining attention due to their potential to mitigate the environmental and health risks posed by synthetic insecticides. The findings suggest that *S. officinalis*, *P. vera* and *Eucalyptus* sp. extracts may serve as effective biopesticides, contributing to integrated vector management strategies for leishmaniasis control.

Keywords: Sage; Pistachio bark; *Eucalyptus* leaves; *Phlebotomus papatasi*; Bioinsecticides

Introduction

Insecticides play a substantial role in controlling sand flies, the primary vectors of leishmaniasis, particularly in domestic and peri-domestic environments (1). However, concerns about their adverse effects on non-target organisms, environmental impact, insect resistance and compliance with ecological protection standards have led to restrictions or bans on the use of chemical insecticides for vector control in certain regions (2). In this context, botanical pesticides and plant-derived compounds have emerged as promising alternatives. These natural substances are not only environmentally

friendly but also exhibit insect-repellent, anti-feeding and insecticidal properties (3, 4). Typically, such compounds are extracted using organic solvents and then applied in diluted forms. Because of its diverse climate and topography, Iran is home to a wide array of medicinal plants, many of which possess natural insect-repellent properties. Several plant extracts have demonstrated efficacy in repelling sand flies and may contribute to reducing the transmission of leishmaniasis (5). Among them, *Salvia officinalis*, *Pistacia vera* and *Eucalyptus* sp. are three native plant species known for their pharmaco-

logical and therapeutic potential (6–8). *Salvia officinalis* (Family: Lamiaceae), commonly known as sage, thrives in warm and temperate climates. The genus *Salvia* includes over 900 species globally, with 17 species native to Iran (6). Widely cultivated in Iran, *P. vera* (Family: Anacardiaceae) possesses medicinal properties attributed to various parts of the plant, including the bark, which is used in traditional medicine (7). *Eucalyptus* sp. (Family: Myrtaceae) comprises more than 700 identified species and is well-known for its antimicrobial, insecticidal and therapeutic applications (8). This study aims to investigate the bioactive compounds and insecticidal properties of *S. officinalis*, *P. vera* and *Eucalyptus* sp., with a focus on their potential effectiveness against *Ph. papatasi*, the primary vector of the zoonotic cutaneous leishmaniasis in Iran.

Materials and Methods

Study area

Kerman Province is one of the thirty-one provinces of Iran, located in the southeast of the country. The city of Kerman is located at 32°38'N, 51°40'E, at an elevation of 1,764 meters above sea level (9).

Preparation of hydroalcoholic extracts

Fresh aerial parts of *S. officinalis*, *P. vera* and *Eucalyptus* sp. were collected from plantations around Kerman City during Spring 2023, shade-dried and finely ground into powder. For each plant, 100 g of the powdered material was extracted with 400 mL of absolute ethanol. The mixture was maintained at 50 °C for 10 minutes to facilitate initial extraction and then allowed to stand at room temperature for 48 hours to complete the maceration process. The extracts were subsequently filtered, concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use. Working solutions were prepared in concentrations ranging from 0.01% to 1.6% (w/v) for biological and chemical analyses.

Extraction and identification of bioactive compounds

Flavonoids were identified by placing 0.5 g of each extract into separate test tubes, mixing with distilled water and 10 mL of methanol using a vortex. The samples were divided into four groups: Group 1 received ammonia (results were observed after 5 minutes); Group 2 was treated with lead acetate; Group 3 was mixed with 0.5 g of magnesium powder, 2 drops of 2N hydrochloric acid and 10 drops of concentrated hydrochloric acid after 30 minutes; Group 4 served as the control (10). The presence of flavonoids was determined by semi-quantitative titration.

Alkaloids were identified by heating 0.5 g of pulverized plant material with hydrochloric acid and water. After cooling, specific reagents (iodine, Mayer's, or Dragendorff's) were added to the extract and the formation of a precipitate and its coloration was observed (11).

Tannins were identified by mixing 0.5 mL of plant extract with 10 mL of Ethanol, filtering and potentially diluting the solution. The solution was then tested with chloroform blue or green coloration indicated the presence of tannins. Lead acetate was used for confirmation in a separate test (12). The presence of Tannins was confirmed by color changes and precipitation upon the addition of lead acetate.

Saponins were identified by mixing 0.5 g of plant powder with boiling water, shaking briefly and incubating vertically for 30 minutes. After cooling and shaking again, two droplets of hydrochloric acid 2N were added to the mixture (13).

Antioxidants were identified by diluting 100 ml of each plant extract in methanol to a total volume of 1000 ml. Then, 50 µL of each diluted extract and 150 µL of DPPH (2, 2-di-phenyl-1-picrylhydrazyl) were added to culture plate wells. The control group, containing methanol without plant extracts, received only DPPH. After 10 minutes, absorbance was measured using a spectrophotometer (517 nm) (14). Steroids were identified by mixing 2 mL of

each extract with 1 mL of chloroform, dehydrating with 1 mL of anhydrous sodium sulfate, and dividing the solution into two parts. The first part received an equal volume of acetic anhydride and 1 ml. concentrated sulfuric acid, while the second part was treated with sulfuric acid alone, added slowly from the side of the test tube (15). The formation of green-blue circles in the first part served as an indication of steroids, whereas the change in solution color to brownish-red in the second part indicated the terpenoids.

Collection of sand flies

Sand flies were collected in Borkhar, Isfahan Province, during the summer season in 2023, from their resting places around rodent burrows using an aspirator and immediately transferred to cages. The cages were then transported to the insectarium of Tarbiat Modares University of Medical Sciences. The sand flies were kept under controlled conditions at 27 ± 5 °C, 70% relative humidity, and a photoperiod of 10:14 hours light: dark (16).

Susceptibility test

Bioassay papers were prepared using extract concentrations of 0.4%, 0.8% and 1.6%, following the protocol established by the World Health Organization (WHO) (17). For each concentration, four replicate papers were prepared, while methanol was used as the negative control. Susceptibility assessments, including median lethal time (LT₅₀) and median lethal dose (LD₅₀) determinations, were conducted using WHO-standard kits and protocols. The assays were performed on adult, non-blood-fed female sand flies, collected from the field and maintained on a 20% sugar solution.

Using standard WHO test tubes, sand flies were exposed to filter papers impregnated with hydroalcoholic extracts of *S. officinalis*, *P. vera* and *Eucalyptus* sp. at specified concentrations. The control group was exposed to methanol-treated papers. Experiments were conducted separately for each plant extract and concentration,

with four replicates for each treatment and a single replicate for the control group, in accordance with WHO guidelines (18). Following their exposure to treatments, the sand flies were kept under controlled insectarium conditions (27 ± 5 °C, $70\pm5\%$ relative humidity) for 24 hours (16). Mortality data were recorded at the end of the holding period. Specimens from both test and control groups were subsequently identified morphologically (19). Statistical analyses and graphical representations were limited to data on *Ph. papatasi*.

Results

Biologically active substances

In this study, hydroalcoholic extracts of *S. officinalis*, *Eucalyptus* sp. and *P. vera* were prepared using the maceration method and various concentrations (0.4, 0.8 and 1.6 mg/L) were formulated. Extraction from 200 g of dried plant material yielded 30.7 g, 29 g and 29.5 g of methanolic extract for *S. officinalis*, *Eucalyptus* sp. and *P. vera*, respectively.

Flavonoid screening using ammonia yielded positive only for *Eucalyptus* sp., whereas *P. vera* and *S. officinalis* tested negative. However, when tested with lead acetate, all three extracts tested positive. One interpretation of these results suggests that *S. officinalis* may have a higher flavonoid content than the other two extracts, while an alternative interpretation indicates that flavonoid levels are compatible across all extracts.

Alkaloid detection was performed using Mayer's, Dragendorff's and iodine reagents. No precipitate formation was observed in any of the extracts, indicating the absence of alkaloid compounds. The semi-quantitative analysis confirmed the presence of tannins in all three extracts, with *Eucalyptus* sp. exhibiting the highest concentration, as indicated by a pronounced blue-green coloration in the chlorophyll test. However, results from the lead acetate test suggested similar tannin levels among the three plant extracts. The standard

test for saponins revealed that only *P. vera* extract contained saponin compounds, while both *Eucalyptus* sp. and *S. officinalis* tested negative. Antioxidant activity was assessed using the DPPH radical scavenging assay. The reduction of DPPH free radicals was visually confirmed by a color change from purple to yellow, indicating antioxidant capacity (Table 1).

Median lethal time (LT₅₀) values for *Phlebotomus papatasi* mortality

The LT₅₀ results at concentrations of 0.4, 0.8 and 1.6 µg/mL are presented in Figures 1–3. At the concentration of 0.4 µg/mL, the *S. officinalis* extract induced 50% mortality in a longer time compared to *P. vera* and *Eucalyptus* sp., indicating lower efficacy at lower

doses. However, at 0.8 µg/mL, all three extracts exhibited comparable LT₅₀ values, suggesting similar insecticidal activity at higher concentrations. The LT₅₀ values for *S. officinalis*, *Eucalyptus* sp. and *P. vera* at 1.6 µg/mL were 11.9, 12.5 and 14.4 hrs, respectively.

Median lethal dose (LD₅₀) values for *Phlebotomus papatasi* mortality

To determine the percentage lethality of *S. officinalis*, *P. vera* and *Eucalyptus* sp. extracts, three concentrations (0.4, 0.8 and 1.6 µg/ml) were tested on adult *Phlebotomus papatasi* in four replicates. The LD₅₀ values after 24 h were 0.156, 0.576 and 0.410 µg/ml for *S. officinalis*, *Eucalyptus* sp. and *P. vera*, respectively (Figs. 4–6).

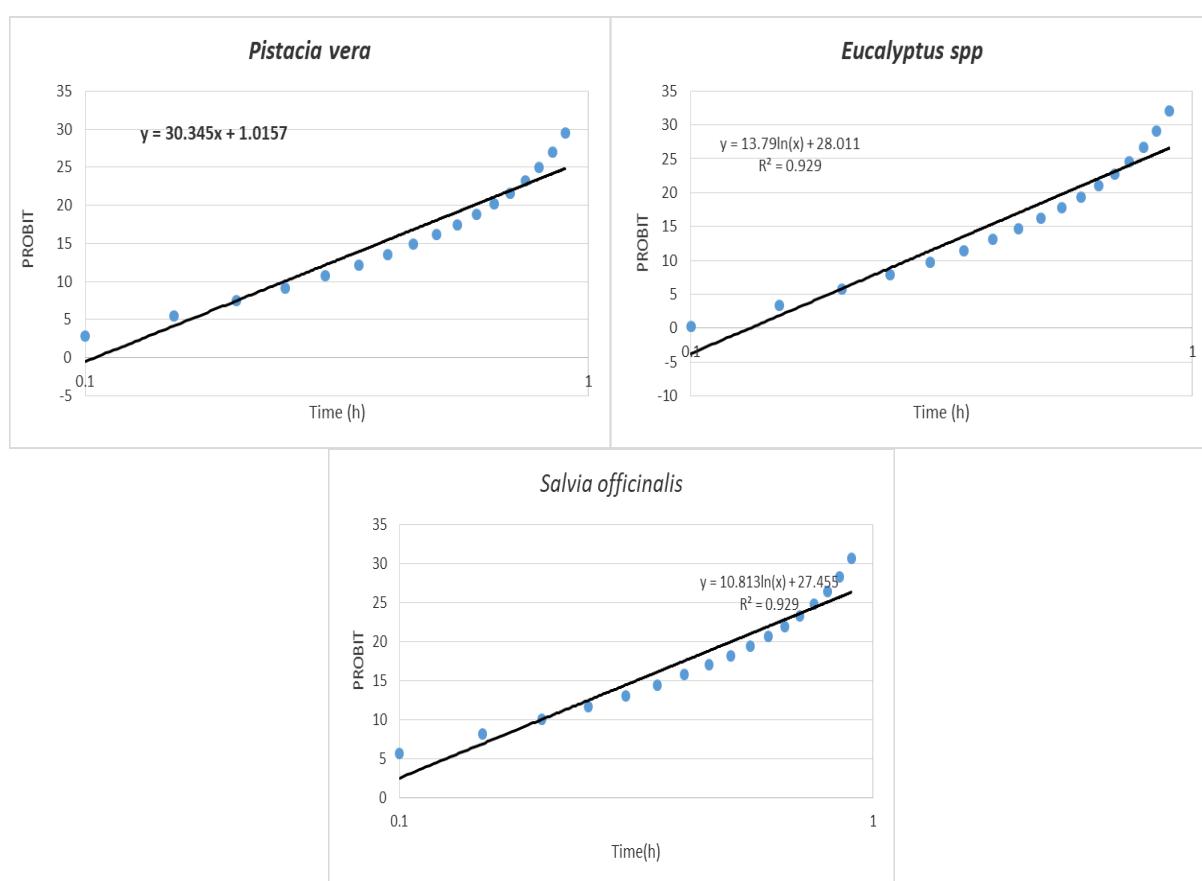


Fig. 1. Probit regression lines for the determination of the median lethal time (LT₅₀, in hours) of hydroalcoholic extracts from *Salvia officinalis*, *Pistacia vera* and *Eucalyptus* sp. methanolic extracts against *Phlebotomus papatasi* (Borkhar strain) from Isfahan Province, 2023, at a concentration of 0.4 µg/mL

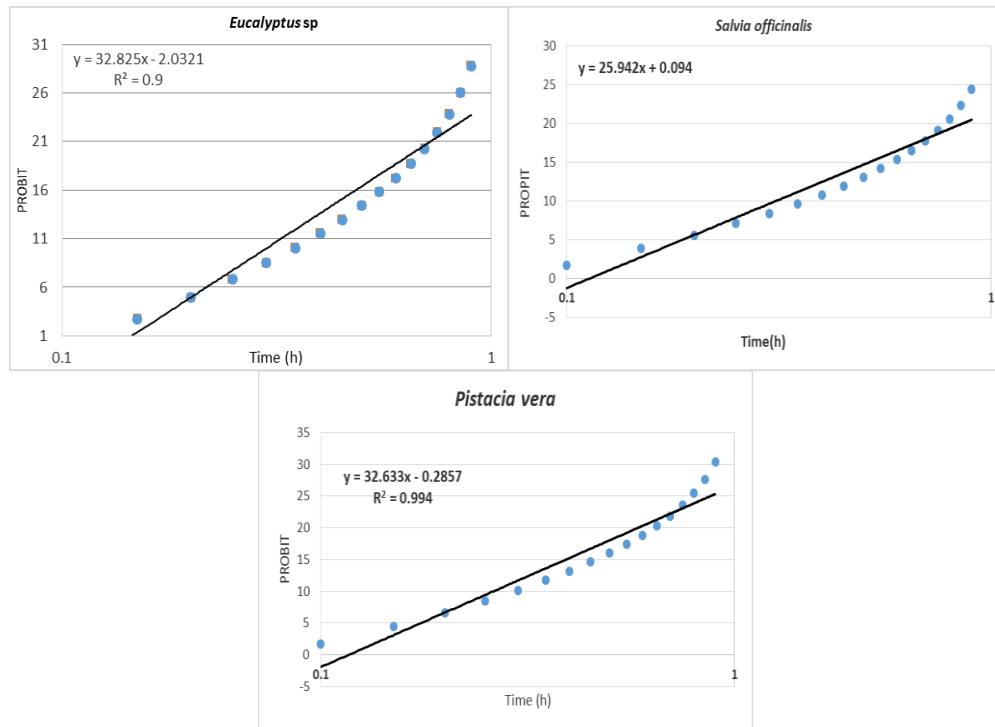


Fig. 2. Probit regression lines for the determination of the median lethal time (LT_{50} , in hours) of hydroalcoholic extracts from *Salvia officinalis*, *Pistacia vera* and *Eucalyptus* sp. methanolic extracts against *Phlebotomus papatasii* (Borkhar strain) from Isfahan Province, 2023, at a concentration of $0.8 \mu\text{g/mL}$

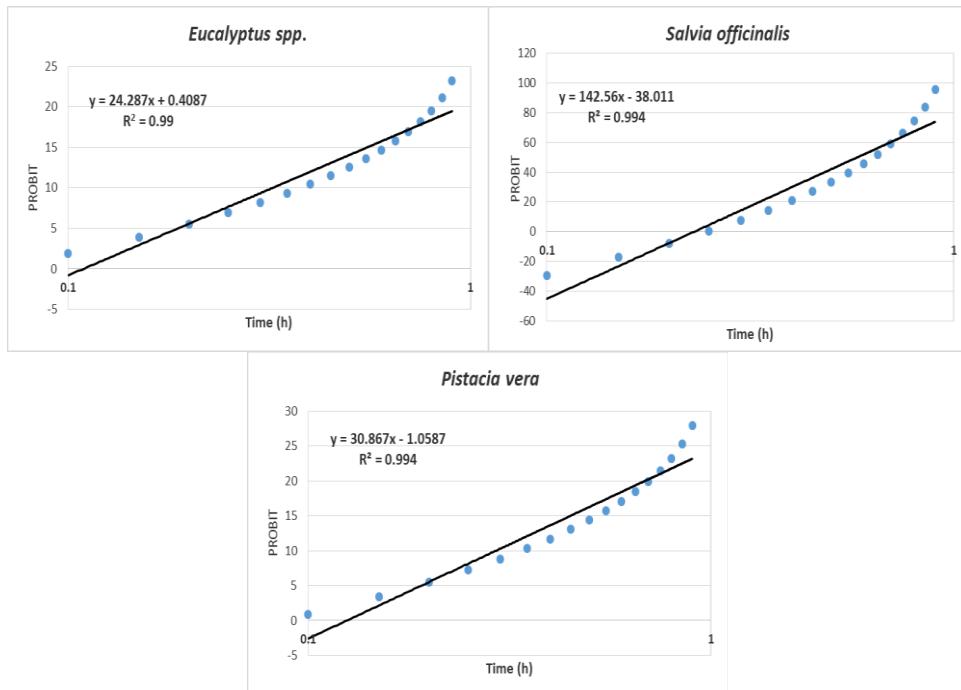


Fig. 3. Probit regression lines for the determination of the median lethal time (LT_{50} , in hours) of hydroalcoholic extracts from *Salvia officinalis*, *Pistacia vera* and *Eucalyptus* sp. methanolic extracts against *Phlebotomus papatasii* (Borkhar strain) from Isfahan Province, 2023, at a concentration of $1.6 \mu\text{g/mL}$

Table 1. Determination of dose-dependent antioxidant activity of *Eucalyptus* sp., *Pistacia vera* and *Salvia officinalis* methanolic extracts using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay against *Phlebotomus papatasi*, Borkhar strain, Isfahan Province, 2023

Plant name	Antioxidant capacity (% inhibition of scavenging activity)		
	0.4 μ g/mL	0.8 μ g/mL	1.6 μ g/mL
<i>Salvia officinalis</i>	0.082	0.065	0.066
<i>Eucalyptus</i> sp	0.089	0.085	0.080
<i>Pistacia vera</i>	0.117	0.095	0.102
Control group	0.495	0.508	0.421

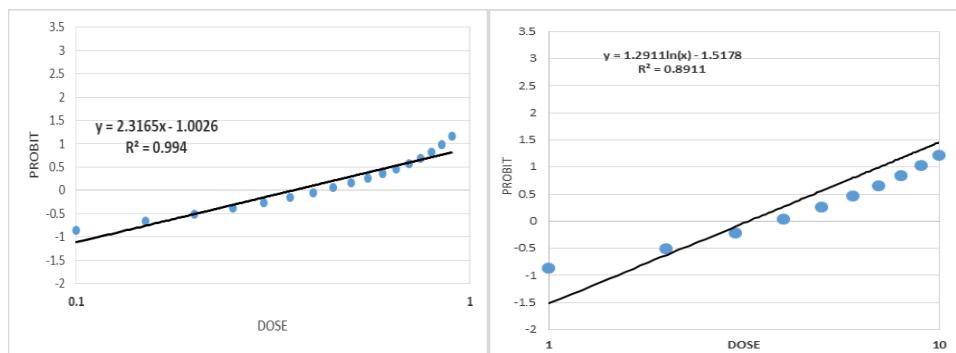


Fig. 4. LD₅₀ values of *Salvia officinalis* at 8 h and 24 h for concentrations of 0.4, 0.8, and 1.6 μ g/mL

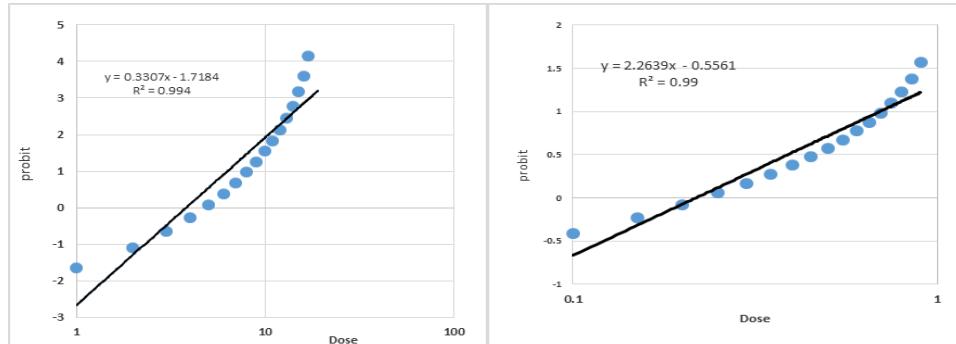


Fig. 5. LD₅₀ values of *Eucalyptus* sp. at 8 h and 24 h for concentrations of 0.4, 0.8, and 1.6 μ g/mL

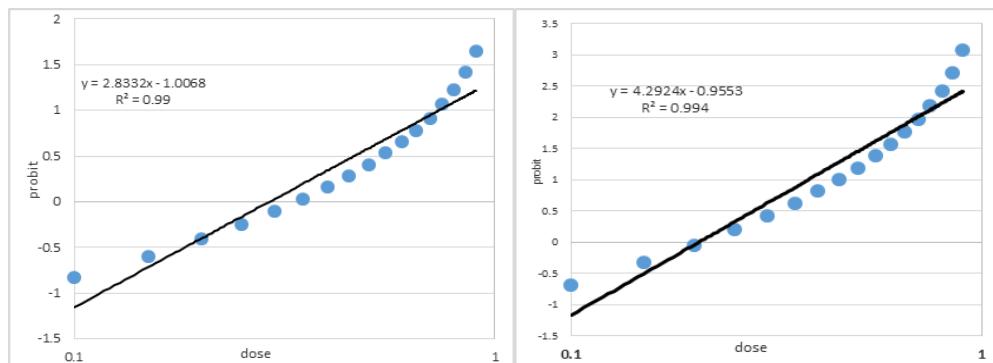


Fig. 6. LD₅₀ values of *Pistacia vera* at 8 h and 24 h for concentrations of 0.4, 0.8 and 1.6 μ g/mL

Discussion

One effective strategy for preventing insect-borne diseases involves the use of plant-derived compounds, which primarily function by reducing contact between disease vectors and humans. Due to the increasing resistance of insects to synthetic insecticides, coupled with growing concerns over their environmental impact and the risk of toxic or allergic reactions, there is a rising interest in the development of plant-based pesticides. Essential oils and plant extracts have emerged as promising alternatives to conventional insecticides, particularly in cases where resistance has rendered synthetic chemicals ineffective (20). Kerman Province, a major pistachio-producing region in Iran, generates substantial quantities of pistachio husks as agricultural waste. These green husks are rich in phenolic compounds and represent a valuable resource for the extraction of bioactive substances (21). *Salvia officinalis*, native to the Kerman region, has been widely documented for its antimicrobial, antioxidant and other medicinal properties (22). Furthermore, several studies have demonstrated the insecticidal potential of *S. officinalis*, highlighting its potential application in the development of biopesticides within integrated pest management (IPM) strategies (23). In the current study, *S. officinalis* extract exhibited stronger biocidal effects against *Ph. papatasi* than those of *Eucalyptus* and *P. vera*. This bioactivity against the primary vector of *Leishmania major* in Iran is yet another addition to sage's other successful records against a variety of pest species, including *Plodia interpunctella*, *Aphis fabae*, *Tribolium confusum*, *Calliphora vomitoria* and *Tetranychus urticae* (24–28). Similarly, the observed high efficacy of *Eucalyptus* extract against the tested sandflies aligns with the potent toxicity reported by Kumar et al. (30) against houseflies and the rapid action of *Eucalyptus* oils against head lice reported by Toloza et al. (31). The broad bioactivity of *Eucalyptus* extracts across a range of concentrations,

is likely due to the presence of bioactive terpenoids and other secondary metabolites that interfere with insect physiological functions (29). Kumar et al. (30) investigated the contact toxicity of *Eucalyptus globulus* extract against housefly larvae. Their findings demonstrated that LT_{50} values varied between 6.0 and 1.7 days. Our results indicated an LT_{50} value equal to 12.5 hours against the tested sandflies for *Eucalyptus* applied at 1.6 μ g/ml. In their study, Toloza et al. (31) assessed the fumigant and toxicological activities of essential oils from three *Eucalyptus* species, *Eucalyptus cinerea*, *Eucalyptus viminalis* and *Eucalyptus saligna* against permethrin-resistant human head lice. They reported the median knockdown time values (KT_{50}) of the species to be 12.0, 14.9 and 17.4 minutes, respectively. In a recent investigation, Mouna et al. (24) evaluated the efficacy of *Eucalyptus amaldolensis* extract at a 50% concentration. Their findings demonstrated notable biological activity, achieving a mortality rate of 93% and a repellence rate of 70% against *A. fabae*. Despite the demonstrated efficacy of certain medicinal plant extracts against various insects, their specific activity against sandflies, the primary vectors of leishmaniasis, remains underexplored. Our research directly addresses this gap by assessing the efficacy of three native medicinal plants in the most heavily stricken area in the country by leishmaniasis against one of the most potent vectors of the disease. Furthermore, our findings on the biocidal activity of the medicinal plants against a leishmanial vector complement the limited existing work, such as that by Coelho et al. (32), who showed that azadirachtin increased mortality in *Lutzomyia longipalpis*. Yet, research regarding the bioactivity of plant extracts as environmentally friendly herbal insecticide candidates against sandflies remains limited. Nowadays, natural compounds derived from plants, minerals and animals are increasingly examined by researchers and phar-

maceutical developers for various biomedical applications. This is due to their accessibility, lower side effect profiles, reduced cost, minimal drug interactions and the presence of diverse bioactive molecules with therapeutic potential. This study showed that extracts from *Eucalyptus* sp. and *S. officinalis* are effective as natural bioinsecticides and offer viable alternatives to synthetic chemicals. Further studies are necessary to determine the main active ingredients of the plant extracts and evaluate their potencies as marketable biopesticides.

Conclusion

Plant-based insecticides like *S. officinalis*, *P. vera* and *Eucalyptus* sp. exhibit promising insecticidal activity against *Ph. papatasi* offering environmentally friendly alternatives for leishmaniasis vector control. Their integration into pest management strategies could reduce reliance on harmful synthetic chemicals, supporting sustainable and health-conscious efforts.

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Ethical considerations

All experimental protocols in this study were conducted in accordance with the guidelines of the Institutional Ethics Committee of Tarbiat Modares University. The protocols were approved by the TMU Ethics Committee under the registry number TMU [IR.KMU.REC.1401.179].

Conflict of interest statement

The authors declare that there is no conflict of interest.

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