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Original paper

A study on the *in vitro* and *in vivo* effects of aqueous and alcoholic extracts of *Salvia mirzayanii* on *Leishmania major*

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ABSTRACT. *Salvia mirzayanii* contains anti-hyperglycemic, antimicrobial, antioxidant, anti-inflammatory, and neuroprotective effects. The purpose of this study was to evaluate the anti-leishmanial efficacy of aqueous and alcoholic extracts of *S. mirzayanii* (both *in vitro* and *in vivo*) against *Leishmania major*. Aqueous and alcoholic extracts of *S. mirzayanii* were prepared and tested on *L. major* promastigotes and amastigotes. MTT test was used to evaluate the cytotoxicity of the plant against *L. major*. Flow cytometry was performed to assay apoptosis induced by 50 and 100 µg/ml of extracts on the promastigotes and macrophages. For the *in vivo* assay, the therapeutic effects of aqueous and alcoholic extracts of *S. mirzayanii* were tested in BALB/c mice. After 72 h, the IC50 value of aqueous and alcoholic extracts of *S. mirzayanii* against *L. major* promastigotes was 6.04 and 4.47, respectively. The inhibitory concentration (IC50) of aqueous and alcoholic extracts of *S. mirzayanii* to amastigotes were determined to be 47.78 µg/ml and 33.58 µg/ml, respectively. Flow cytometry revealed that the apoptosis of promastigotes using 100 µg/ml of aqueous and alcoholic extracts of *S. mirzayanii* was 5.81% and 5.39%, respectively, while apoptosis induced at 200 µg/ml were 5.09% and 70.71%, respectively. Lesion size was significantly decreased in *in vivo* experiments, and the survival rate of the treated mice improved in contrast to the control group. Given the efficacy of aqueous and alcoholic extracts of *S. mirzayanii* on promastigotes both *in vitro* and *in vivo* condition, the plant could be considered as a candidate source for the treatment of leishmaniosis.

Keywords: *Salvia mirzayanii*, aqueous and alcoholic extract, *Leishmania major*, BALB/c mice, *in vitro*, *in vivo*

Introduction

Leishmaniosis is a widespread tropical disease caused by protozoan *Leishmania* parasites, primarily affecting developing countries [1–3]. Leishmaniosis is spread through the bite of infected sandflies, including *Lutzomyia* and *Phlebotomus* species [4]. The illness is categorized clinically into cutaneous leishmaniosis (CL, most common), mucocutaneous (MCL), and visceral leishmaniosis (VL) types [5]. Studies showed the prevalence to be 12 million patients globally, with 350 million people being vulnerable to leishmaniosis. Approximately 0.5 million of VL and 1–1.5 million new cases of CL are reported annually [6–8].

Due to the lack of adequate vaccination and care, climate warming, conflict, and displacement, leishmaniosis affects many people worldwide [9–11]. Chemotherapy is currently a successful method for treating leishmaniosis. The first medication option for *Leishmania* infections is pentavalent antimonial compounds, including sodium stibogluconate and meglumin antimonials (Glucantime) [12,13]. While the next prescribed drugs are amphotericin B, paromomycin, and pentamidine, amphotericin B is considered to be teratogenic and not safe for pregnant women [14]. The emergence of resistance to pentavalent antimonials, which are widely used to treat leishmaniosis, is confirmed by recent data [15].

Some variables that limit their acceptability by patients are high toxicity, cost, drug resistance, multiple systemic adverse effects, painful injection, and long-term cure [16].

The World Health Organisation (WHO) has reported that nearly 80 percent of the World's population relies on traditional treatments for fulfilling their primary health care needs [17]. Due to the importance of new anti-leishmaniosis medicines derived from the WHO's natural sources, recently, several studies have been carried out [6]. Natural products are being used this way, and many products have shown to have anti-leishmanial activity [18,19].

There are approximately 200 genera and 3000 species in the family Lamiaceae [20]. With over 900 different species worldwide and around 60 species in Iran, *Salvia* is the largest genus in the family Lamiaceae [21]. Traditionally, *Salvia* species have been used to cure bronchitis, hemorrhage, colds, measles, and menstrual conditions as well as more than sixty different illnesses ranging from epilepsy to aches [20,22].

S. mirzayanii (Marv-e-Talkh in Persian) is a known native species of the genus *Salvia* and grows in the southern regions of Iran. *S. mirzayanii* has been used to treat spasms, diabetes, gastrointestinal disorders, inflammation, and infections in Iranian folk medicine, especially in the provinces of Hormozgan, Shiraz, and Kerman [23]. Local therapists still use *S. mirzayanii* as a traditional treatment for leishmaniosis patients in Larestan, (Fars-Iran). *S. mirzayanii* essential oils contains alpha-guaiene (2.23%), linalyl acetate (2.55%), 7-epi-alpha-selinene (3.23%), linalool (3.35%), epi-alpha-cadinol (4.20%), alpha-cadinol (4.58%), globulol (5.12%), δ-cadinene (5.46%), bicyclo-germacrene (6.47%), γ-cadinene (6.64%), alpha-terpinyl acetate (9.64%), 1,8-cineol (11.23%), and 5-neo-cedranol (15.48%) [24]. Anti-hyperglycemic, antimicrobial, antioxidant, anti-inflammatory and neuroprotective effects have been seen in pharmacological trials for various *S. mirzayanii* formulations [25,26]. However, there is no research reporting the anti-leishmaniosis impact of *S. mirzayanii*.

In the present study, the anti-leishmaniosis effects of aqueous and alcoholic extracts of *S. mirzayanii* were investigated both under *in vitro* and *in vivo* conditions.

Materials and Methods

Plant collection

Aerial sections of *S. mirzayanii* were collected in February and March 2019 from Larestan county, Shiraz, Iran. The species was certified by the Department of Traditional Medicines, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Iran to be *S. mirzayanii*. The plant's voucher (#1120) is kept in the herbarium of Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Iran. The specified sections of the plant were dried after collection at room temperature in the shade without heat.

Preparation of extracts and medicines

For preparing aqueous extraction, the powdered *S. mirzayanii* (50 grams) was macerated in distilled water (200 ml) at room temperature (25°C) for 72 hours. Then, Whatman paper was used to filter the mixtures. For alcoholic extract, 50 grams of powdered *S. mirzayanii* was percolated with 500 ml of ethanol 80–85% at room temperature for 72 hours. The extracts were centrifuged (5000 rpm) for a half-hour, and Whatman paper was used to filter the supernatants.

A concentration of 1 µg/ml of amphotericin B and 100 µg/ml of Glucantime (Glucantime® Rhone-Merieux, France) was chosen as the medication option for leishmaniosis in this report.

Promastigotes assay

L. major promastigotes of the Iranian standard strain (MRHO/IR/75/ER) were cultured in RPMI 1640 (Gibco, Germany) with antibiotics (100 µg/ml streptomycin and 100 IU/ml penicillin) and 10 percent fetal bovine serum (FBS; Gibco, Germany) at room temperature (24±2°C). To investigate the anti-leishmanial effects, *L. major* promastigotes in the logarithmic growth phase (2×10^6 parasites/ml) added 100 µl to 96-well plates, then 100 µl of 7 specific dilutions (6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) of the *S. mirzayanii* extracts were also used to treat the promastigotes for 24, 48, 72 h at 24±2°C. After incubation, the number, and morphology of the promastigotes were analyzed under an optical microscope, using Hemocytometer slide and compared with control groups, including those not treated and treated with glucantime (100 µg/ml) and amphotericin B (1µg/ml) to determine the anti-leishmanial effects. Experiments were done in triplicates.

Amastigote assay

In this study, a 12-well culture plate was seeded with macrophages and incubated for 24 h at 37°C under 5% CO_2. Non-adherent cells were washed with phosphate buffer salt (PBS). To acquire amastigotes, macrophages were contaminated with promastigotes (1:10) for 24 h. The extra non-penetrating promastigotes was washed and removed with PBS. Afterwards, *S. mirzayanii* extracts (6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) were added to contaminated macrophages. After incubation for 72 h, coverslips were fixed and the samples stained using methanol and Giemsa. Mean amastigote-infected macrophages have been determined using an optical microscope and compared with control groups.

MTT assay for macrophages

The MTT solution was prepared by mixing 5 mg of MTT powder (Sigma Chemical Co., Germany) with sterile PBS (1 ml) in a dark room. For macrophage inhibition, murine macrophage cell line (J774) was used for MTT assay (Sigma Aldrich, USA). The cells were cultured in RPMI 1640 (Gibco, Germany) with antibiotics (100 µg/ml streptomycin and 100 IU/ml penicillin) and 10% FBS under 5% CO_2 at 37°C. After seeding cells in 96-well plates, (2×10^6/ml), *S. mirzayanii* extracts added at various concentrations (6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) and glucantime (50µg/ml) and amphotericin B (1µg/ml) was used as the positive control at 37°C for 72 h. To allow the cells to convert tetrazolium salt into insoluble formazan, 20 µl (5 mg/ml) MTT was added to each well, and the plate was incubated at 37°C for five h. DMSO (100 µl) was added to each well in a dark place at 25°C, and the crystals of formazan are solubilized in DMSO. ELISA reader (Stat Fax, USA) was used to measure the optical density at 540 nm. The percentage of macrophage inhibition rates was calculated using the following formulas in both treated and untreated groups [27,28].

Macrophage inhibition (%) = 100 - Macrophage viability (%)

Macrophage viability (%) =(AT-AB)/(AC-AB)×100

AT: Absorbance of treated cells

AB: Absorbance of the blank

AC: Absorbance of untreated (control) cells

MTT assay for L. major promastigotes

The inhibition of *L. major* promastigotes was evaluated using the MTT assay, and promastigotes (2×10^6/ml) were cultured as briefly stated earlier. *L. major* promastigotes were cultured in plates containing serial dilutions of *S. mirzayanii* (6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) 24°C for 72 h. On each well, 5 mg/ml (20 µl) MTT was added. The incubation was carried out at 25°C for five hours. DMSO (100 µl/well) was added to each well after draining the supernatant. ELISA reader (Stat Fax, USA) was used to read the optical density at 540 nm. The percentage of inhibition of *L. major* promastigotes was calculated using the aforementioned formulas [27,28].

Flow cytometry analysis

Annexin-V can be used in this method to differentiate between apoptotic (only annexin-V positive as early apoptosis/lower right – both annexin-V and PI-positive as late apoptosis/upper right), necrotic (PI-positive/upper left) and living cells (both annexin-V and PI negative/lower left) [27]. As mentioned before, promastigotes (2×10^6/ml) treated with *S. mirzayanii* (100, 200 µg/ml) and untreated *L. major* promastigotes were centrifuged for 10 min at 1400g with cold PBS solution. 5 µl PI and 5 µl annexin V plus 500 µl buffer (IQ Products BV, Groningen, Netherlands) were added to samples. After incubation at 25°C for 20 minutes, the samples were analyzed using a flow cytometer (BD FACSCanto II, USA), and FlowJo software was used to analyze the data.

In vivo assay

In this study, six- to eight-week-old BALB/c female mice (from Razi Institute, Karaj, Iran) were used. Mice were kept in standard cages at ambient humidity, room temperature, and 12-hour normal light-dark cycle and fed ad libitum. Animal experimentation was carried out upon the approval of the Animal Care Committee of Tarbiat Modares University, Tehran, Iran. Using stationary phase promastigotes (2×10^6) at a volume of 100 µl, mice were subcutaneously injected at the tail base. Nodules were observed in the injection site after one month. The ointment was prepared by combining aqueous and alcoholic extracts of *S. mirzayanii* (400 µg/ml) with vitamin A ointment. Mice were divided into five groups (N=5 mice):

1. Untreated mice (negative control)
2. Mice treated with 60 mg/kg of glucantime (positive control)
3. Mice treated with vitamin A ointment
4. Mice treated with aqueous extract of *S.*

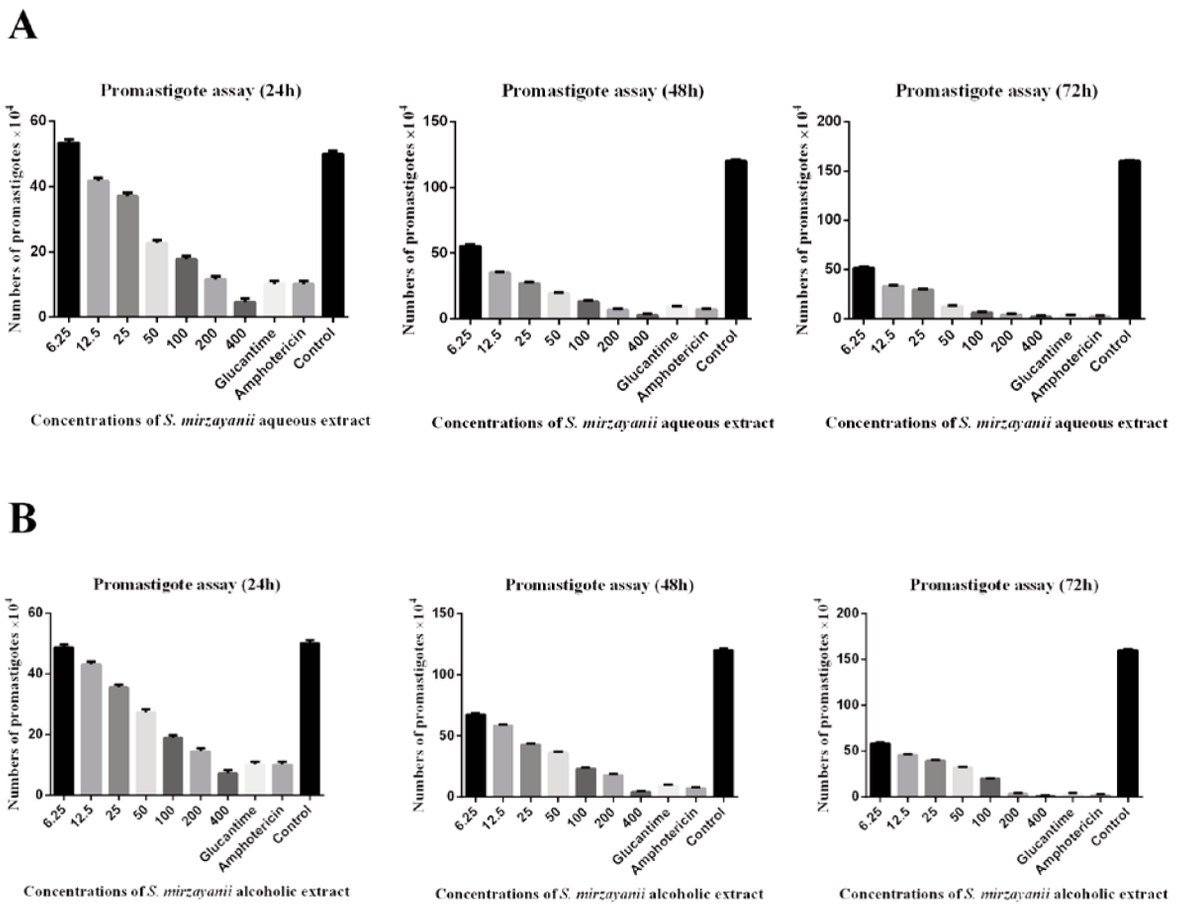

Figure 1. A,B. Mean and standard deviation of the number of promastigotes of *L. major* exposed to concentrations of aqueous and alcoholic extracts of *S. mirzayanii* for 24, 48, 72 h. There are statistical differences in all groups with the control group ($P < 0.05$).

mirzayanii (400 µg/ml) + vitamin A

5. Mice treated with alcoholic extract of *S. mirzayanii* (400 µg/ml) + vitamin A

After 28 days, each group's mice were checked to measure parasite burden and lesion size [27,28].

Statistical analysis

Data were analyzed using GraphPad Prism 5.0 (Graphpad Inc., USA). One-way ANOVA was used to compare the mean differences in different groups. To ensure data normalization, the Kolmogorov-Smirnov test was used. The values are displayed as the mean ± SD, and *p*-value < 0.05 was considered significant.

Results

Promastigote assay

Longer treatment times (72 h) and higher extract concentrations (200, 400 µg/ml) resulted in increased toxicity to promastigotes. The alcoholic extract of *S. mirzayanii* demonstrated a more significant lethal impact on promastigotes than aqueous extract (Fig. 1 A,B).

Amastigote assay

Generally, the results demonstrated that aqueous and alcoholic extracts of *S. mirzayanii* reduce the number of *L. major* amastigotes in macrophages. The percentage of macrophages infected with amastigotes are summarized in Fig. 2 A,B.

MTT assay for macrophages

MTT test showed that concentrations of 200 and 400 µg/ml of aqueous and alcoholic extracts of *S. mirzayanii* have toxic effects on cells after 72 h. However, they were less toxic than amphotericin and glucantime. Briefly, the results are shown in Fig. 3 A,B.

MTT assay for promastigotes

The percentage of parasite inhibition was evaluated using the MTT test. This test showed that concentrations of 200 and 400 µg/ml aqueous and

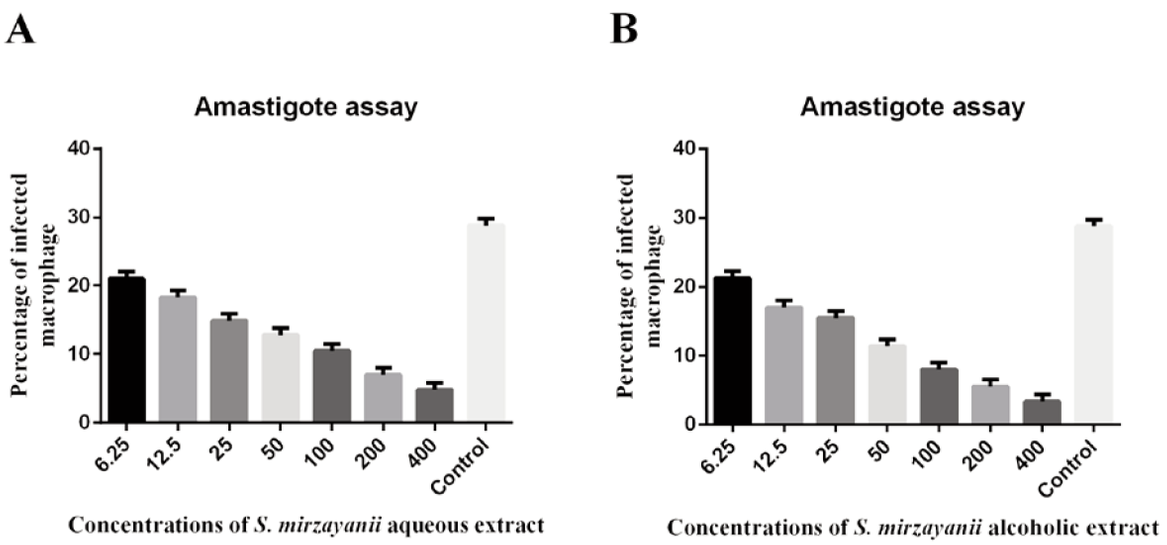

Figure 2. A,B. Mean and SD of percentage of infected macrophages with amastigote of *L. major* in treatment groups with aqueous and alcoholic extracts of *S. mirzayanii* and control group after 72 h

alcoholic extracts of *S. mirzayanii* induced more inhibitory effects on the parasite than amphotericin and glucantime (Fig. 4 A,B).

Flow cytometry analysis

In this study, concentrations of 100 and 200 µg/ml of aqueous and alcoholic extracts of *S. mirzayanii* were used for flow cytometry. In the samples treated with concentrations of 100 and 200 µg/ml of aqueous extract, the percentage of apoptosis was estimated at 8.92% and 35.39%, respectively. The rate of primary apoptosis was estimated at 16.37% and 80.19% in the samples treated with concentrations of 100 and 200 µg/ml of alcoholic extract respectively. However, in the control group that received no drugs, 99.70% of the cells were alive and 0.20% were apoptotic. The results of flow cytometry are shown in Fig. 5 A–C.

In vivo assay

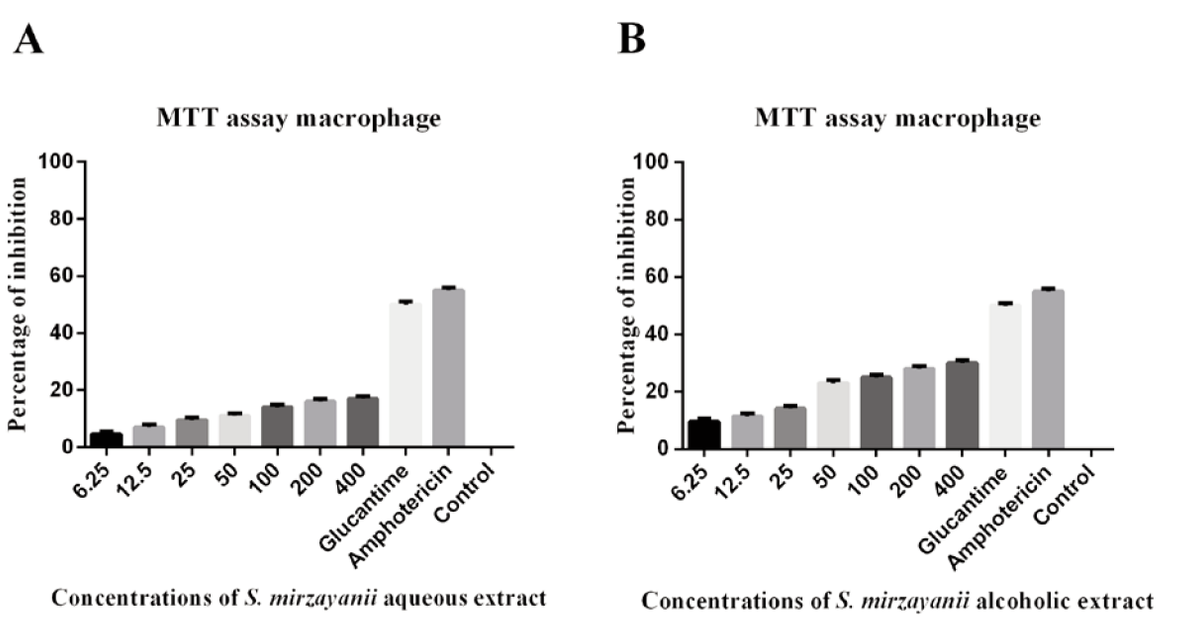

Figure 3. A.B. Mean and SD of the percentage of macrophage inhibition exposed to different concentrations of aqueous and alcoholic extracts of *S. mirzayanii* after 72 h. There is a statistical difference with the control group ($P < 0.05$).

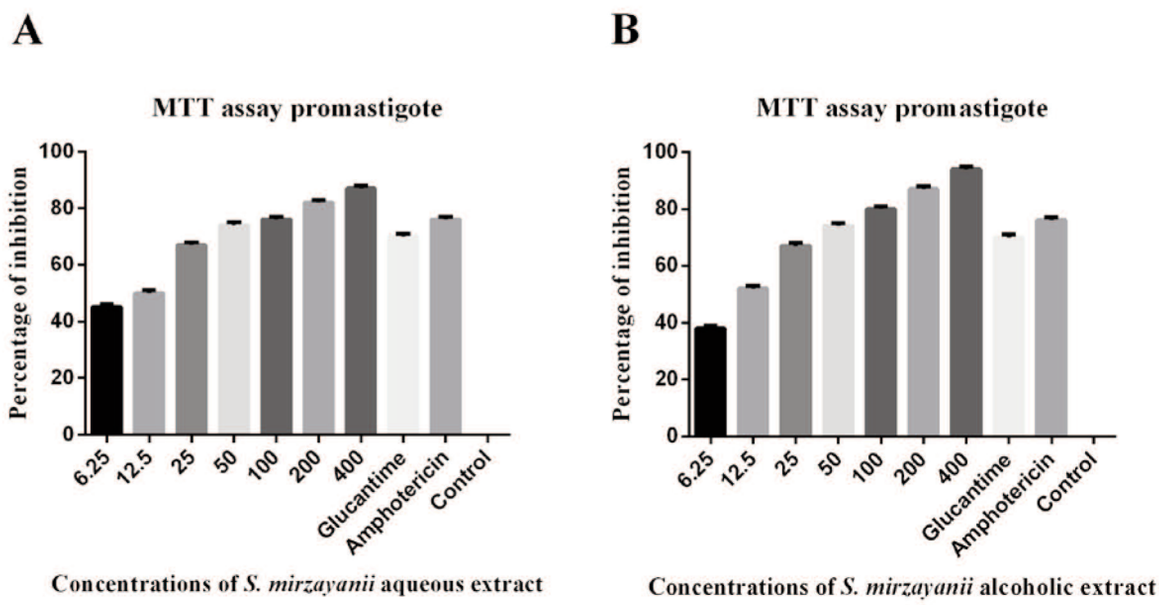

Figure 4. A,B. Mean and SD of the percentage of promastigotes inhibition exposed to different concentrations of aqueous and alcoholic extracts of *S. mirzayanii* after 72 h. There is a statistical difference with the control group ($P < 0.05$).

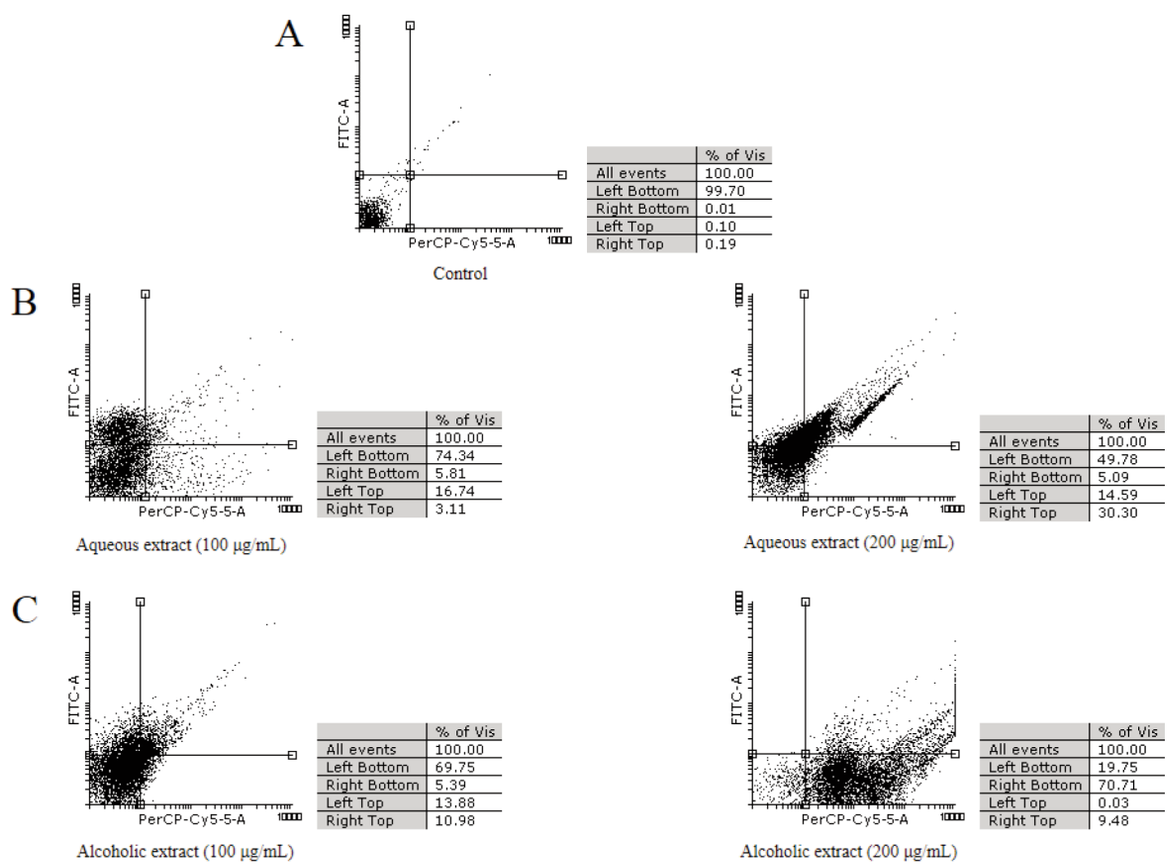

Figure 5. A–C. The results obtained from flow cytometry analysis on *L. major* promastigotes after treatment with 100 and 200 µg/ml concentrations of aqueous (B), alcoholic (C) extracts of *S. mirzayanii*, and control (A) after 72 h, *in vitro*

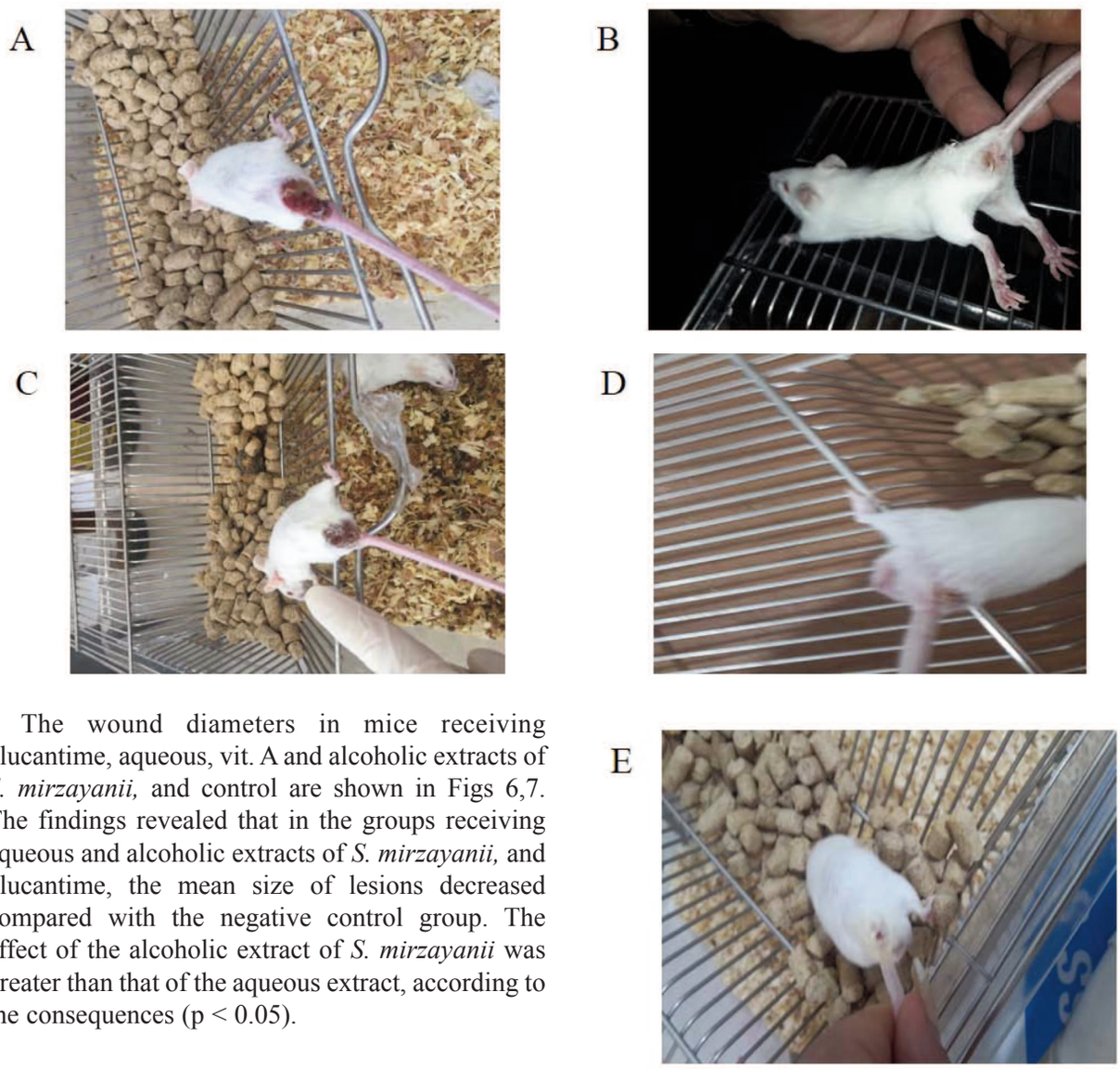

Figure 6. The figures of lesions in control (A), treated mice with glucantime (B), Vit A (C), aqueous extract of *S. mirzayanii* (D), and alcoholic extract of *S. mirzayanii* (E) 28 days after finishing treatment

The wound diameters in mice receiving glucantime, aqueous, vit. A and alcoholic extracts of *S. mirzayanii,* and control are shown in Figs 6,7. The findings revealed that in the groups receiving aqueous and alcoholic extracts of *S. mirzayanii,* and glucantime, the mean size of lesions decreased compared with the negative control group. The effect of the alcoholic extract of *S. mirzayanii* was greater than that of the aqueous extract, according to the consequences ($p < 0.05$).

Discussion

Being prevalent in around 98 countries, leishmaniosis is known to be one of the most significant health issues in the world [29,30]. Anti-leishmaniosis medications, including glucantime, amphotericin B, and sodium stibogluconate (pentostam), have been used as first-line therapies for the disease in recent years. Due to drugs' side effects, high cost and drug resistance [31], many researchers have focused on finding new drugs that are cheaper, safer, and with less side effects. To this end, natural compounds extracted from plants are good sources for new drugs [32]. In the same vein, this study was designed to comparatively evaluate the anti-leishmaniosis effects of aqueous and alcoholic extracts of *S. mirzayanii* on *L. major* both under *in vitro* and *in vivo* conditions.

In previous research, a wide variety of biological functions, such as antioxidant, anti-inflammatory, cytotoxic, insecticidal, anti-diabetic, and anti-microbial, have been attributed to essential oils, crude extracts, and purified compounds from different sections of the *Salvia* genus [33–36].

However, until this study, no research has been conducted to examine the anti-leishmaniosis effect of *S. mirzayanii*. Our findings revealed that promastigotes and amastigotes of *L. major* were susceptible to different concentrations of aqueous and alcoholic *S. mirzayanii* extracts. A study by Zomorodian et al. [37] shows that *S. mirzayanii* essential oil has a wide range of antibacterial and

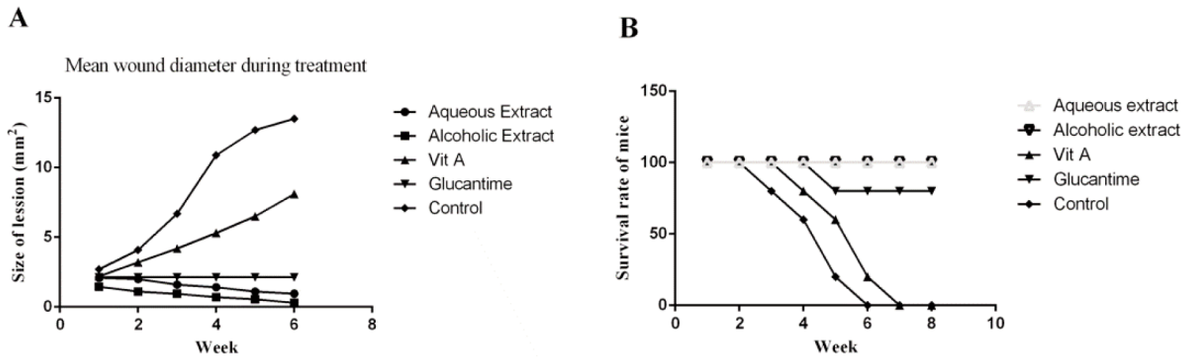

Figure 7. Mean and SD of the wound diameter (A) and survival rate (B) of mice

antifungal effects. In the present research, aqueous and alcoholic extracts of *S. mirzayanii* have lowered the survival rate of promastigotes, suggesting that this plant has anti-leishmaniosis efficacy.

Comparing the effects of aqueous and alcoholic *S. mirzayanii* extracts, we found that the anti-leishmaniosis efficacy of alcoholic extracts was higher than that of aqueous extracts for longer periods. The compounds responsible for anti-leishmaniosis activity of *S. mirzayanii* can also be proposed to be more soluble in ethanol than in water.

Recent research has shown that in *S. mirzayanii* plant, phenolic compounds demonstrate antioxidant function by inactivating free lipid radicals or preventing hydroperoxides' decomposition into free radicals [38]. The major constituents of *S. mirzayanii* essential oil considered as antibacterial agents are 5-neo-cedranol, alpha-terpinyl acetate, 1,8-cineol, bicyclogermacrene, δ-cadinene, globulol, alpha-cadinol, tau-cadinol, 7-epi-alpha-selinene, linalyl acetate, linalool, β-elemene, γ-cadinene and alpha-guaiene [39]. These phytochemicals found in *S. mirzayanii* may also have relevance to the anti-leishmaniosis effects of *S. mirzayanii*. To elucidate the key constituents of *S. mirzayanii* for its anti-leishmaniosis properties, more research is suggested to be done in the future.

The findings of this study revealed anti-leishmaniosis effects of *S. mirzayanii* against *L. major* promastigotes and amastigotes. Moreover, compared with the control group, the wound sizes of *L. major*-infected BALB-c mice which received treatments with the plant derivatives were reduced significantly, and their survival rate also improved. These results suggest that *S. mirzayanii* can be used alone or in combination with common anti-leishmanial drugs to treat leishmaniosis.

References

[1] Desjeux P. 2001. The increase in risk factors for leishmaniasis worldwide. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 95: 239-243. https://doi.org/10.1016/s0035-9203(01)90223-8

[2] Desjeux P. 2004. Leishmaniasis: current situation and new perspectives. *Comparative Immunology, Microbiology and Infectious Diseases* 27: 305-318. https://doi.org/10.1016/j.cimid.2004.03.004

[3] Murray H.W., Berman J.D., Davies C.R., Saravia N.G. 2005. Advances in leishmaniasis. *Lancet* 366: 1561-1577. https://doi.org/10.1016/s0140-6736(05)67629-5

[4] Wheeler R.J., Sunter J.D., Gull K. 2016. Flagellar pocket restructuring through the *Leishmania* life cycle involves a discrete flagellum attachment zone. *Journal of Cell Science* 129: 854-867. https://doi.org/10.1242/jcs.183152

[5] Pagheh A.S., Fakhar M., Mesgarian F., Rahimi-Esboei B., Badiee F. 2013. Incidence trend of rural cutaneous leishmaniasis in Gonbad-e-Qabus city, (Golestan, Iran) during 2009-2012. *Journal of Mazandaran University of Medical Sciences* 23: 27-33.

[6] Malmasi A., Ardestani B.Z., Mohebali M., Akhoundi B., Ziaieet S. al. 2014. Evaluation of a novel herbal immunomodulator drug (IMOD) in treatment of experimental canine visceral leishmaniasis. *Iranian Journal of Pharmaceutical Research* 13: 1357.

[7] Kumar R., Bumb R.A., Ansari N.A., Mehta R.D., Salotra P. 2007. Cutaneous leishmaniasis caused by *Leishmania tropica* in Bikaner, India: parasite identification and characterization using molecular and immunologic tools. *The American Journal of Tropical Medicine and Hygiene* 76: 896-901.

[8] Abamor E.S., Allahverdiyev A.M. 2016. A nano-technology based new approach for chemotherapy of cutaneous leishmaniasis: TIO2@ AG nanoparticles –*Nigella sativa* oil combinations. *Experimental Parasitology* 166: 150-163.

https://doi.org/10.1016/j.exppara.2016.04.008
[9] Salam N., Al-Shaqha W.M., Azzi A. 2014. Leishmaniasis in the Middle East: incidence and epidemiology. *PLoS Neglected Tropical Diseases* 8: e3208. https://dx.doi.org/10.1371/journal.pntd.0003208
[10] González, C., Wang O., Strutz S.E., González-Salazar C., Sánchez-Cordero V., Sarkar S. 2010. Climate change and risk of leishmaniasis in North America: predictions from ecological niche models of vector and reservoir species. *PLoS Neglected Tropical Diseases* 4: e585. https://doi.org/10.1371/journal.pntd.0000585
[11] Saroufim M., Charafeddine K., Issa G., Khalifeh H. et al. 2014. Ongoing epidemic of cutaneous leishmaniasis among Syrian refugees, Lebanon. *Emerging Infectious Diseases* 20: 1712-1715. https://doi.org/10.3201/eid2010.140288
[12] Monzote L. 2011. Antileishmanial patents anti - leishmanial current drugs and relevant patents. *Recent Patents on Anti-infective Drug Discovery* 6: 1-26. https://doi.org/10.2174/157489111794407868
[13] Davis A.J., Kedzierski L. 2005. Recent advances in antileishmanial drug development. *Current Opinion in Investigational Drugs (London, England: 2000)* 6: 163-169.
[14] Tiuman T.S., Santos A.O., Ueda-Nakamura T., Dias Filho B.P., Nakamura C.V. 2011. Recent advances in leishmaniasis treatment. *International Journal of Infectious Diseases* 15: e525-e532. https://doi.org/10.1016/j.ijid.2011.03.021
[15] Raeisi M., Mirkarimi K., Jannat B. et al. 2020. In-vitro effect of some medicinal plants on *Leishmania major* Strain MRHO/IR/75/ER. *Medical Laboratory Journal* 14: 46-52. http://dx.doi.org/10.29252/mlj.14.4.46
[16] Croft S.L., Seifert K., Yardley V. 2006. Current scenario of drug development for leishmaniasis. *The Indian Journal of Medical Research* 123: 399-410.
[17] Kumar S., Suresh P.K., Vijayababu M.R. et al. 2006. Anticancer effects of ethanolic neem leaf extract on prostate cancer cell line (PC-3). *Journal of Ethnopharmacology* 105: 246-250. https://doi.org/10.1016/j.jep.2005.11.006
[18] Soosaraei M., Fakhar M., Teshnizi S.H. et al. 2017. Medicinal plants with promising antileishmanial activity in Iran: a systematic review and meta-analysis. *Annals of Medicine and Surgery* 21: 63-80. https://doi.org/10.1016/j.amsu.2017.07.057
[19] Akbari M., Oryan A., Hatam G. 2017. Application of nanotechnology in treatment of leishmaniasis: a review. *Acta Tropica* 172: 86-90. doi:10.1016/j.actatropica.2017.04.029
[20] Foster S. 1999. Tyler's honest herbal: a sensible guide to the use of herbs and related remedies. Routledge.
[21] Hamidpour R., Hamidpour S., Hamidpour M., Shahlari M. 2013. Sage: The functional novel natural medicine for preventing and curing chronic illnesses. *International Journal of Case Reports and Images* (IJCRI), 4: 671-677. doi:10.5348/ijcri-2013-12-408-RA-2
[22] Chang J.Y., Chang C.J., Kuo C.C., Chen L.T. 2004. Salvinal, a novel microtubule inhibitor isolated from *Salvia miltiorrhizae* Bunge (Danshen), with antimitotic activity in multidrug-sensitive and-resistant human tumor cells. *Molecular Pharmacology* 65: 77-84. doi:10.1124/mol.65.1.77
[23] Amirghofran Z., Azadmehr A., Javidi K., Bahmani M. et al. 2010. Effect of *Salvia mirzayanii* on the immune system and induction of apoptosis in peripheral blood lymphocytes. *Natural Product Research* 24: 500-508. http://dx.doi.org/10.1080/14786410802267502
[24] Ghotbabadi F.S., Alizadeh A., Zadehbagheri M. et al. 2012. Phytochemical composition of the essential oil, total phenolic content, antioxidant and antimicrobial activity in Iranian *Satureja sahendica* Bornm. at different ontogenesis conditions. *Journal of Medicinal Plants Research* 6: 3525-3534. doi:10.5897/JMPR11.374
[25] Rowshana V., Tarakemehb A. 2013. Comparison of *Salvia mirzayanii* volatile compounds extracted by headspace extraction and hydrodistillation methods. *International Journal of Plant, Animal and Environmental Sciences* 3: 136-140.
[26] Zarshenas M.M., Krenn L. 2015. Phytochemical and pharmacological aspects of *Salvia mirzayanii* Rech. f. & Esfand. *Journal of Evidence-Based Complementary and Alternative Medicine* 20: 65-72. https://doi.org/10.1177/2156587214553938
[27] Ghaffarifar F., Heydari F.E., Dalimi A. et al. 2015. Evaluation of apoptotic and antileishmanial activities of Artemisinin on promastigotes and BALB/C mice infected with *Leishmania major*. *Iranian Journal of Parasitology* 10: 258.
[28] Foroutan-Rad M., Khademvatan S., Hashemitabar M., Saki J. 2016. *Holothuria leucospilota* extract induces apoptosis in *Leishmania major* promastigotes. *Iranian Journal of Parasitology* 11: 339.
[29] Alvar J., Vélez I.D., Bern C., Herrero M. et al. 2012. Leishmaniasis worldwide and global estimates of its incidence. *PloS One* **7**: e35671. doi:10.1371/journal.pone.0035671
[30] Postigo J.A.R. 2010. Leishmaniasis in the world health organization eastern mediterranean region. *International Journal of Antimicrobial Agents* 36 (Suppl. 1): S62-S65. https://doi.org/10.1016/j.ijantimicag.2010.06.023
[31] Chávez-Fumagalli M.A., Ribeiro T.G., Castilho R.O. et al. 2015. New delivery systems for amphotericin B applied to the improvement of leishmaniasis treatment. *Revista da Sociedade Brasileira de Medicina Tropical* 48: 235-242.

https://doi.org/10.1590/0037-8682-0138-2015
[32] Albakhit S., Khademeyatan S., Doudi M., Foroutan-Rad M. 2016. Antileishmanial activity of date (*Phoenix dactylifera L*) fruit and pit extracts *in vitro*. *Journal of Evidence-Based Complementary and Alternative Medicine* 21: NP98-NP102. https://doi.org/10.1177/2156587216651031
[33] Bahadori M., Mirzaei M. 2015. Cytotoxicity, antioxidant activity, total flavonoid and phenolic contents of *Salvia urmiensis* Bunge and *Salvia hydrangea* DC. ex Benth. *Research Journal of Pharmacognosy* 2: 27-32.
[34] Firuzi O., Miri R., Asadollahi M., Eslami S., Jassbi A.R. 2013. Cytotoxic, antioxidant and antimicrobial activities and phenolic contents of eleven *Salvia* species from Iran. *Iranian Journal of Pharmaceutical Research: IJPR* 12: 801-810.
[35] Jassbi A., Miri R., Alizadeh M., Asadollahi M., Massrorbabanari M., Baldwin I.T. 2014. Quantification of phenolic diterpenoids and rosmarinic acid in *Salvia eremophila* and *Salvia santolinifolia* by LC-DAD-MS. *Austin Chromatography* 1: 5.
[36] Tavakkoli M., Miri R., Jassbi A.R., Erfani N., Asadollahi M., Ghasemi M., Saso L., Firuzi O. 2014. *Carthamus, Salvia* and *Stachys* species protect neuronal cells against oxidative stress-induced apoptosis. *Pharmaceutical Biology* 52: 1550-1557. https://doi.org/10.3109/13880209.2014.908394
[37] Zomorodian K., Moein M., Pakshir K., Karami F., Sabahi Z. 2017. Chemical composition and antimicrobial activities of the essential oil from *Salvia mirzayanii* leaves. *Journal of Evidence-Based Complementary and Alternative Medicine* 22: 770-776. https://dx.doi.org/10.1177/2156587217717414
[38] Valifard M., Mohsenzadeh S., Kholdebarin B., Rowshan V. 2014. Effects of salt stress on volatile compounds, total phenolic content and antioxidant activities of *Salvia mirzayanii. South African Journal of Botany* 93: 92-97. https://doi.org/10.1016/j.sajb.2014.04.002
[39] Haghighat M.H., Alizadeh A., Nouroznejadfard M.J. 2012. Essential oil composition and antimicrobial activity in Iranian *Salvia mirzayanii* Rech. & Esfand. *Advances in Environmental Biology* 6:1985-1990.

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