Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 20/07/2022;
Date Revised: 20/08/2022;
Date Published Online: 31/10/2022;

Authors' Affiliation:
1. Determinants of Medicinal Chemistry, School of Pharmacy, Mazandaran University of Medical Sciences, Sari - Iran
2. Department of Traditional Persian Medicine, School of Medicine, Mazandaran University of Medical Sciences, Sari - Iran
3. Traditional and Complementary Medicine Research Centre, Mazandaran University of Medical Sciences, Sari - Iran
4. Department of Biostatistics, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran-Iran.
5. Health Management and social Development Research Centre, Golestan University of Medical Sciences, Gorgan - Iran

***Corresponding Author:**
Seyde Sedighe Yosefi
Email:
s.yousefi@mazums.ac.ir

How to Cite:
Ebrahimzadeh MA, Yosefi SS, Pahlevanzadeh B, Mozafari A, (2022). Evaluation of predictive Investigating Potential Therapeutic Efficacy of 5% Topical Ointment of *Sambucus ebulus* Fruit Extract in Treatment of Cutaneous Leishmaniasis. Adv. Life Sci. 9(3): 334-339.

Keywords:
Leishmaniasis; Cutaneous Leishmaniasis; Sambucus ebulus; Medicinal plant; Treatment

Open Access

Investigating Potential Therapeutic Efficacy of 5% Topical Ointment of *Sambucus ebulus* Fruit Extract in Treatment of Cutaneous Leishmaniasis

Mohammad Ali Ebrahimzadeh[1], Seyde Sedighe Yosefi[2,3]*, Bagher Pahlevanzadeh[4], Arezoo Mozafari[5]

Abstract

Background: Leishmaniasis is a vector-borne zoonotic disease that remains as a major public health problem around the globe, with a higher burden in poor or developing countries. Pentavalent antimonials such as glucantime are currently being used as the first-line treatment for cutaneous form of leishmaniasis, but they are associated with significant side effects. Evidence suggests that extracts from different parts of *Sambucus ebulus* may have analgesic, anti-inflammatory and favorable skin healing effects. In this study, we investigated the potential therapeutic efficacy of *S. ebulus* fruit extract in treatment of cutaneous leishmaniasis.

Methods: This was a randomized, double-blind, placebo controlled clinical trial that was carried out on 95 patients with confirmed cutaneous leishmaniasis. The subjects were assigned to an intervention and a control group. All patients received the standard treatment for leishmaniasis. In addition, the intervention group received a 5% ointment prepared from the methanolic extract of *S. ebulus* fruit, while the control group received a placebo. Healing rate and clinical characteristics of the lesions were assessed before the intervention and once weekly until complete epithelialization of the lesions (or up to 12 weeks).

Results: The 5% topical ointment of *S. ebulus* fruit had no significant effect on healing probability, healing rate or treatment outcome, but it significantly reduced the lesion size.

Conclusion: Combination therapy with pentavalent antimonials and the *S. ebulus* fruit ointment could significantly reduce the lesion size but has no effect on the treatment outcome.

Introduction

Cutaneous leishmaniasis is a vector-borne zoonotic disease caused by the protozoans of the genus Leishmania [1-3]. The disease is widely distributed around the globe. According to the WHO's report, about 1.3 million new cases are reported annually [4-8]. Despite recent advances in the prevention, diagnosis and treatment of leishmaniasis, the disease still remains a major public health problem with a higher burden in poor countries [9].

Pentavalent antimonials are currently being used as the first-line treatment for leishmaniasis, but these chemical compounds are associated with significant side effects. The need for multiple painful injections, emergence of drug resistant parasites, lack of an effective vaccine and the high cost of treatment [10-15] highlight the urgent need for development of novel therapeutic agents and strategies [16, 17]. A wide range of compounds from plants and other natural sources with potential antileishmanial properties have been recently investigated as alternatives to the current chemical-based drugs [18].

Sambucus ebulus plant has been a plant for several years with green, ear-shaped, cross-combed leaves, with about 5 to 11 toothed leaflets. The length of each leaf is 5 to 30 cm, with small white or cream colored flowers. Its flowers turn into red to black fruits [19-21]

In Persian medicine (Iranian traditional medicine), rhizomes, leaf and fruit of *S. ebulus* have been used for topical treatment of inflammatory arthritis, such as rheumatoid arthritis, inflammation caused by insect bites, and sore throat [19-21]. Various studies have demonstrated that extracts from different parts of *S. ebulus* can exert analgesic and anti-inflammatory effects in a dose-dependent manner. This plant contains anthocyanin, flavonoid and phenolic compounds and compounds such as rutin and quercetin [21, 22].

As principal constituents of *S. ebulus* extracts, flavonoids can exert anti-lipid peroxidation effects and increase the bioavailability and stability of collagen fibers, thus accelerating and promoting the wound healing process [22]. Despite the beneficial effects of *S. ebulus* extract, no study has yet investigated the potential effects of *S. ebulus* fruit extract on the wound healing process in patients with cutaneous leishmaniasis. Given the favorable skin healing effects [23], anti-Giardia activity [24] and anti-hydatidosis effects [25] of *S. ebulus*, in this study, we investigated effectiveness of the plant's fruit extract for treatment of leishmaniasis.

Methods

Study design

This research was carried out as a randomized, double-blind, placebo controlled clinical trial in accordance with the proposed methodology of the World Health Organization for clinical trials on cutaneous leishmaniasis treatment interventions [26].

Ethical considerations

The study has been registered at the Iranian Registry of Clinical Trials (registration code: IRCT20171028037044N4). Written informed consent was taken from all participants prior to sampling and intervention. This study was performed according to the principles specified in the Declaration of Helsinki. Approval was obtained from the Ethics Committee of the Mazandaran University of Medical Sciences (approval No. IR.MAZUMS.REC.1396.2150).

Sample size and study population

The study was performed on patients with cutaneous leishmaniasis who were referred to the Leishmaniasis Treatment Center in Gonbad-e Kavus (northeast of Iran) from November 1, 2017 to March 30, 2019.

Diagnosis was confirmed via microscopic detection of Leishman bodies within the macrophages in a slit-skin smear from the edge of lesions that lasted more than 14 days. Patients were enrolled in the study after explaining the research objectives and obtaining written informed consent. Exclusion criteria were non-localized leishmaniasis, pregnancy, known hypersensitivity/allergy to pentavalent antimonials, having lesions aged more than three months, receiving any type of leishmaniasis treatment within the past month, presence of lesions on eyelids and nose or in 1 cm proximity of lips and eyes, and unwillingness to continue participation in the study. For patients with more than one lesion, the uppermost (preferably non-facial), ulcerative, parasite-positive lesion was chosen as the index lesion.

Preparation of the extract

The drug and the placebo were prepared at the Faculty of Pharmacy of Mazandaran University of Medical Sciences, Iran. The fruit of *S. ebulus* was collected from rural areas of Sari (Mazandaran Province, Iran), dried and then powdered at room temperature, in the dark. The extract was prepared by soaking the fruit powder in methanol for three days [19].

Interventions and follow up

Of 892 patients referred to the Leishmaniasis Treatment Center in Gonbad-e Kavus, 95 patients (male:60; female:35) were enrolled in the study and then randomly assigned into an intervention (n=45) and a control (n=50) group. Demographic information and baseline morphological characteristics of the lesions (number, location and size) were recorded. Lesion size was determined by measuring the largest diameter of the

lesion. All patients were treated according to the national guideline for the management of cutaneous leishmaniasis in Iran [27]. In addition to the standard treatment, the intervention group was treated with the 5% paraffin- and vaseline-based *S. ebulus* ointment. The control group received a placebo ointment without the *S. ebulus* extract. The ointment was applied twice daily until complete re-epithelialization of the lesions.

Outcome measures

The healing process and clinical features of the lesions (lesion size, induration diameter, and degree of epithelialization) were assessed and recorded by trained personnel (unaware of subject allocation) before the intervention and once weekly until complete re-epithelialization of the lesions (or up to 12 weeks). Treatment success was verified by observing complete re-epithelialization of the ulcer on day 42; otherwise, the treatment outcome was recorded as failure. The degree of recovery was determined as completely healed (complete epithelialization of the lesion), moderately healed (≥50% reduction of the lesion size), slightly healed (<50 reduction of the lesion size and worsened (increased lesion size or deterioration of its clinical status) at the end of the intervention.

Statistical analysis

The relationship between qualitative variables was assessed using the Chi-square test and the Fisher's exact test. The Mantel-Hansel method was used to modify the confounding variables, and multiple logistics regression was used when required. The Cochran test was used for repeated measurements of the qualitative dichotomous variable. The Mann–Whitney U test was used to evaluate the effect of the intervention on ranked qualitative variables or abnormal quantitative variables in both groups. Quantitative data were described using mean and standard deviation. Normal distribution of quantitative variables was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The homogeneity of the variances was tested using the Loon test. Independent t-test was used to compare the mean of these traits between the two groups. Quantitative was assessed repeated measures ANOVA. Survival analysis by the Kaplan-Meier method was applied to compare the recovery time. To adjust the confounding variables, covariance analysis and multiple regression analyses were used. All statistical analyses were performed using IBM SPSS Statistics 18 at significance level of 0.05.

Results

There was no significant difference between the two groups in terms of mean age, mean weight, gender, mean lesion age, the mean lesion diameter at baseline, and location of the lesions (Table 1). We found no significant difference between the two study groups in terms of outcome, healing rate and degree of recovery (Table 2).

The Kaplan-Meier curve was plotted to compare the likelihood of healing between the two groups (Figure 1). Comparison of the recovery probability between the two groups did not differ significantly between the two groups using the log-rank test (df =1, P-value=0.18). Figure 2 shows the mean changes in the diameter of lesions (mm) in the two groups over the treatment period. The reduction of mean diameter of lesions in the intervention group was more significant compared to that of in the control group.

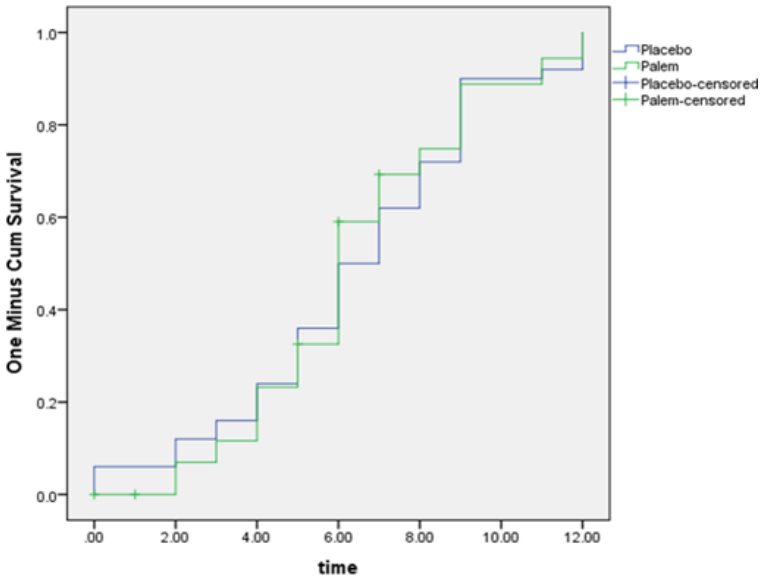

Figure 1: The Kaplan-Meier curve representing the probability of lesion healing in the two groups

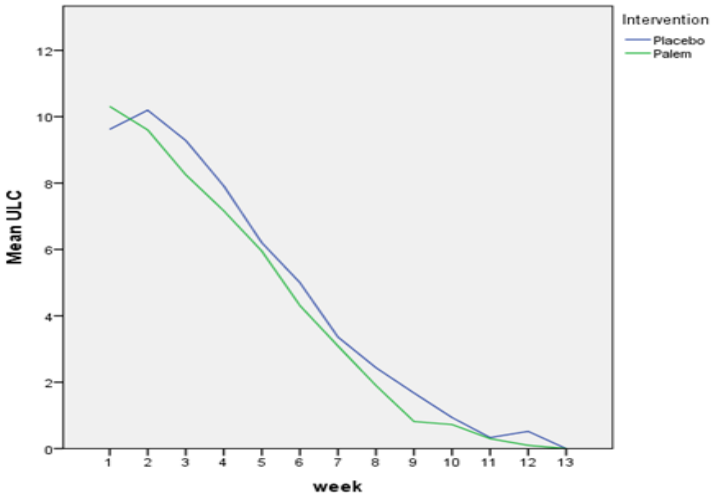

Figure 2: Reduction of the mean diameter of lesions in the two groups

Criteria	Subgroup	Control	Intervention (%)	P-value
Age means (years)	-	26.94(19.28)	27.44(17.37)	0.74
Weight mean(kg)	-	55.33(27.74)	54.71(30.5)	0.7
Size of Lesion mean(mm)	-	9.38(4.4)	10.33(3.51)	0.42
Size of Induration mean(mm)	-	16.64(6.67)	15.11(5.32)	0.18
Lesion age mean(day)	-	30.96(16.91)	36.82(20.43)	0.19
Location	Leg, Arm Trunk& Head	20 26 4	16 23 6	0.66
Gender	Male Female	31 19	29 16	0.83

Table 1: Demographic information and lesion characteristics in the study groups

Variable	-	Control	Intervention	P value
Treatment method	Observe Systematic glucantime Subcutaneous glucantime Cryotherapy Cryotherapy & topical Fluconazole	0 18 0 19 12 1	1 12 2 14 12 4	0.27
Treatment outcome	Success Failure	24 26	25 19	0.3
Degree of recovery	Completely healed Moderately healed Slightly healed Worsened	7 9 10 24	7 7 6 24	0.87
Average treatment duration (day)		31.18(19.05)	34.48(21.42)	0.45

Table 2: Clinical information and treatment outcomes in the two study groups

Discussion

Although leishmaniasis generally self-heals within 4-12 months, it can lead to scarring, particularly on the face, which requires medical care. Pentavalent antimonials such as glucantime are currently used as the standard therapy for leishmaniasis [30]. However, these chemicals are associated with numerous limitations including limited availability, toxicity, severe side effects, and drug resistance [11-15].

Given the potential of traditional and complementary medicine, increased tendency of people towards non-chemical therapies, and the emphasis of the World Health Organization on utilization and appropriate integration of traditional medical knowledge into health services [28], various studies have exploited the effectiveness of herbal compounds for treatment of leishmaniasis. Jaffary et al. reported that combination therapy with Cassia fistula fruit gel and intralesional meglumine antimoniate injection can significantly increase healing rate in patients with cutaneous leishmaniasis [29].

Shirani-Bidabadi et al. investigated the therapeutic efficacy of thyme, yarrow and propolis herbal extracts and systemic glucantime for treatment of cutaneous leishmaniasis in Balb/c murine model. They reported that application of the alchoholic extracts of thyme and yarrow twice daily for six weeks significantly reduced mean lesion diameter compared to treatment with glucantime [30]. Another study claimed that a local drug containing white and black alum, butter, turmeric and copper sulphate could be used as an inexpensive and effective alternative to amphotericin B for accelerating the lesion healing process in patients with cutaneous leishmaniasis [31]. In another study by Jafari et al., topical administration of Achilles millefolium 5% gel (containing 5% polyphenol) twice daily for four weeks along with intralesional injection of glucantime had no significant effect on the treatment of treatment of acute cutaneous leishmanial lesions. This is inconsistent with the previous reports on the healing properties of Achilles millefolium for treatment of leishmaniasis. However, administration of the gel at higher concentrations (24-40%) might result in a favorable outcome [32].

Ali et al. evaluated the efficacy of twice daily administration of 5% trichloroacetic acid cream for eight weeks in the treatment of cutaneous leishmaniasis lesions. They demonstrated that the cream could have beneficial effects on treatment of leishmaniasis scars, suggesting that the cream could be considered as a cheap and safe alternative to chemical drugs [33].

Today, the use of medicinal plants in the treatment of diseases is very widespread [34-37]. One of the most important reasons for using medicinal plants is their less side effects compared to chemical drugs [38-40]. Plant antioxidants have found special use in many industries such as pharmaceutical, health, medicine and food industries [41-47].

In our study, although the 5% topical ointment of *S. ebulus* fruit had no effect on healing probability, healing rate, degree of recovery or treatment outcome, it significantly reduced the lesion size. Considering the lack of any notable side effects following application of the ointment, it is suggested to evaluate effects of extracts from the fruit or other parts of *S. ebulus* at higher concentrations on the wound healing process in treatment of cutaneous leishmaniasis.

Competing Interests

The authors declare that there is no conflict of interests regarding publication of this study.

Author Contributions

All the authors have contributed dedicatedly in terms of giving their technical expertise to give a tenable shape to this manuscript.

References

1. Soltani S, Foroutan M, Hezarian M, Afshari H, Kahvaz MS. Cutaneous leishmaniasis: an epidemiological study in southwest of Iran. Journal of Parasitic Diseases, (2019); 43(2): 190-7.
2. Falih B, Mohammed S, Mohammed N. Effects of the silver nanoparticle synthesis from the leaves of the Capparis spinosa plant on the liver of mice infected with visceral leishmaniasis. Caspian Journal of Environmental Sciences, (2022); 20(4): 785-791.
3. Agh-Atabay M, Sofizadeh A, Ozbaki G, Malaki-Ravasan N, Ghanbari M, Mozafari O. Ecoepidemiological characteristics of a hypoendemic focus of zoonotic cutaneous leishmaniasis in north Iran (southeast of Caspian Sea). Journal of Vector Borne Diseases, (2016); 53(3): 248.
4. Piroozi B, Moradi G, Alinia C, Mohamadi P, Gouya MM, Nabavi M, et al. Incidence, burden, and trend of cutaneous leishmaniasis over four decades in Iran. Iranian Journal of Public Health, (2019); 48(1): 28-35.
5. Khamesipour A, Ghoorchi MH, Khatami A, Eskandari SE, Javadi A, Zartab H, et al. Comparison of efficacy of intralesional injection of meglumine antimoniate once-weekly with twice-weekly in the treatment of anthroponotic cutaneous leishmaniasis in Mashhad: a randomized clinical trial. Dermatology and Cosmetic, (2011); 2(1): 1-8.
6. Organization WH. Leishmaniasis. Fact sheet, n. 375, February, (2013).
7. WHO CD. Sustaining the drive to overcome the global impact of neglected tropical diseases second WHO report on neglected tropical diseases. Geneva: WHO, (2013).
8. Alexandrino-Junior F, Freire MCLC, Lione VdOF, Cardoso EA, Marcelino HR, Genre J, et al. A Functional Wound Dressing as a Potential Treatment for Cutaneous Leishmaniasis. Pharmaceutics, (2019); 11(5): 200.
9. Mirzapour A, Spotin A, Behniafar H, Azizi H, Maleki B, Shahker aminia H, et al. Intra-Species Diversity of Leishmania major and L. tropica from Clinical Isolates of Cutaneous Leishmaniasis in Southwest Iran Inferred by ITS1-rDNA. Iranian Journal of Public Health, (2019); 48(5): 893-901.
10. Mozafari O, Shorofi SA, Yousefi SS. First Report on Infant Acute Urticaria after Mother's Parenteral Use of Meglumine Antimoniate (Glucantime): A Case Report. Iranian Journal of Public Health, (2016); 45(9): 1217.
11. Ebrahimi Y, Hasanvand A, Valibeik A, Ebrahimi F, Abbaszadeh S. Natural antioxidants and medicinal plants effective on hyperlipidemia. Res. J. Pharm. Technol. 2019 Mar 31;12:1457-62.
12. Ponte-Sucre A, Gamarro F, Dujardin J-C, Barrett MP, Lopez-Velez R, Garcia-Hernandez R, et al. Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. PLoS neglected Tropical Diseases, (2017); 11(12): 0006052.
13. Niapour A, Bohlooli S, Sharifi Pasandi M, Mohammadi-ghalehbin B. In vitro Anti Leishmanial Effect of Agrostemma githago Extract on Leishmania Major Promastigotes by Cell Count and MTT Assay. Journal of Mazandaran University of Medical Sciences, (2018); 28(165): 13-23.
14. Stevanovic S, Sencanski M, Danel M, Menendez C, Belguedj R, Bouraiou A, et al. Synthesis, In Silico, and In Vitro Evaluation of Anti-Leishmanial Activity of Oxadiazoles and Indolizine Containing Compounds Flagged against Anti-Targets. Molecules, (2019); 24(7): 1282-1289.
15. Rahman R, Goyal V, Haque R, Jamil K, Faiz A, Samad R, et al. Safety and efficacy of short course combination regimens with AmBisome, miltefosine and paromomycin for the treatment of visceral leishmaniasis (VL) in Bangladesh. PLoS Neglected Tropical Diseases, (2017); 11(5): e0005635.
16. Rajasekaran R, Chen Y-PP. Potential therapeutic targets and the role of technology in developing novel antileishmanial drugs. Drug Discovery Today, (2015); 20(8): 958-68.
17. Machado-Silva A, Guimarães PPG, Tavares CAP, Sinisterra RD. New perspectives for leishmaniasis chemotherapy over current anti-leishmanial drugs: a patent landscape. Expert Opinion on Therapeutic Patents, (2015); 25(3): 247-60.
18. Mozafari O, Shorofi SA, Shirzadi MR, YOUSEFI SS. Treatment of Cutaneous Leishmaniasis in Persian Medicine. Iranian Journal of Public Health, (2017);46(10):1450-1.
19. Ebrahimzadeh MA, Rafati MR, Damchi M, Golpur M, Fathiazad F. Treatment of paederus dermatitis with *Sambucus ebulus* Lotion. Iranian journal of Pharmaceutical Research, (2014); 13(3): 1065.
20. Jiménez P, Tejero J, Cordoba-Diaz D, Quinto E, Garrosa M, Gayoso M, et al. Ebulin from dwarf elder (*Sambucus ebulus* L.): A mini-review. Toxins, (2015); 7(3): 648-58.
21. Ebrahimzadeh MA, Mahmoudi M, Saiednia S, Pourmorad F, Salimi E. Anti-inflammatory and anti-nociceptive properties of fractionated extracts in different parts of *Sambucus ebulus*. Journal of Mazandaran University of Medical Sciences, (2006); 16(54): 35-47.
22. Hasanvand A, Ebrahimi Y, Mohamadi A, Nazari A. Zingiber officinale Roscoe reduces chest pain on patients undergoing coronary angioplasty: a clinical trial. Journal of Herbmed Pharmacology. 2019 Jan 2;8(1):47-50.
23. Ghabaee DNZ, Ebrahimzadeh MA, Akbari J, Amiri FT. Wound healing activity of *Sambucus ebulus*. International Journal of Pharmaceutical Sciences and Research, (2017); 8(1): 132-149.
24. Rahimi-Esboei B, Ebrahimzadeh M, Gholami S, Falah-Omrani V. Anti-giardial activity of *Sambucus ebulus*. uropean Review for Medical and Pharmacological Sciences, (2013); 17(15): 2047-50.
25. Gholami S, Rahimi-Esboei B, Ebrahimzadeh M, Pourhajibagher M. In vitro effect of *Sambucus ebulus* on scolices of Hydatid cysts. European review for medical and pharmacological sciences, (2013); 17(13): 1760-5.
26. Olliaro P, Vaillant M, Arana B, Grogl M, Modabber F, Magill A, et al. Methodology of clinical trials aimed at assessing interventions for cutaneous leishmaniasis. PLoS neglected Tropical Diseases, (2020); 7(3): e2130.
27. Shirzadi MR. National Guideline for Leishmaniasis Surveillance in Iran. Tehran, Iran: Center for Diseases Management; (2012).
28. Organization WH. WHO traditional medicine strategy 2002-2005, (2002).
29. Jaffary F, Nilforoushzadeh MA, Ansari N, Rahimi M. Treatment of cutaneous leishmaniasis: cassia fistula fruit gel- intralesional glucantime Vs. placebo gel- intralesional glucantime combination. Tehran University Medical Journal, (2010); 67(10): 705-11.
30. Shirani-Bidabadi L MM, Saberi S, Zolfaghari-Baghbaderani A, Nilforoushzadeh MA, Abdoli H, Moatar F, Hejazi SH,. The effectiveness of mix extracts of Thyme, Yarrow and Propolis on Cutaneous Leishmaniasis: a comparative study in animal model (Balb/c). Tehran University Medical Journal, (2009); 66(11): 785-90.
31. Bahrami AM KA, Maspi N. Effects of Drug Combined of Black And White Alum on Cutaneous Leishmaniasis Lesion. HBI_Journals, (2010); 17(4): 53-60.
32. Jaffary F, Nilforoushzadeh MA, Tavakoli N, Zolfaghari B, Shahbazi F. The efficacy of Achilles millefolium topical gel along with intralesional injection of glucantime in the treatment of acute cutaneous leishmaniasis major. Advanced Biomedical Research, (2014); 3(5): 354-369.
33. Ali NM, Fariba J, Elaheh H, Ali N. The efficacy of 5% trichloroacetic acid cream in the treatment of cutaneous leishmaniasis lesions. Journal of Dermatological Treatment, (2012); 23(2): 136-9.
34. Solati K, Karimi M, Rafieian-Kopaei M, Abbasi N, Abbaszadeh S, Bahmani M. Phytotherapy for wound healing: The most important herbal plants in wound healing based on Iranian ethnobotanical documents. Mini-Reviews in Medicinal Chemistry, (2021); 21(4): 500-519.
35. Bahmani M., Jalilian A, Salimikia I, Shahsavari S, Abbasi N. Phytochemical screening of two Ilam native plants Ziziphus

nummularia (Burm.f.) Wight & Arn. And Ziziphus spina-christi (Mill.) Georgi using HS-SPME and GC-MS spectroscopy. Plant Science Today, (2020); 7(2): 275-280.
36. Abbasi N, Khalighi Z, Eftekhari Z, Bahmani M. Extraction and phytoanalysis of chemical compounds of Eucalyptus globulus leaf native to Dehloran, Ilam province, Iran by HS-SPME and GC-MS. Advances in Animal and Veterinary Sciences, (2020); 8(6): 647-652.
37. Abbasi N, Khosravi A, Aidy A, Shafiei M. Biphasic response to luteolin in MG-63 osteoblast-like cells under high glucose-induced oxidative stress. Iranian Journal of Medical Sciences, (2016); 41(2): 118-125.
38. Vladimirovna Demina, G., Borisovna Prokhorenko, N., Ravilevna Kadyrova, L. The influence of soil quality on the vitality of Trifolium pratense L. cenopopulations in the subzone of deciduous forests of Tatarstan, Russia. Caspian Journal of Environmental Sciences, (2020); 18(5): 411-419.
39. Ur Rehman I, Malik L, Zaheer S, Abdullah M. A Brief Study of Mass Spectrometry of Natural Products. Plant Biotechnology Persa, (2022); 4 (1):50-55
40. Obaid R, Kadhim Hindi N, Kadhum S, jafaar alwaeli L, Jalil A. Antibacterial activity, anti-adherence and anti-biofilm activities of plants extracts against Aggregatibacter actinomycetemcomitans: An in vitro study in Hilla City, Iraq. Caspian Journal of Environmental Sciences, (2022); 20(2): 367-372.
41. Wani NA, Khanday WI, Tirumale S. Phytochemical analysis and evaluation of antibacterial activity of different extracts of soil-isolated fungus chaetomium cupreum. Journal of Natural Science, Biology and Medicine, (2020); 11(1): 72-80.
42. Ebrahimi Y, Hasanvand A, Safarabadi AM, Sepahvand H, Moghadasi M, Abbaszadeh S. A review of the most important herbal drugs effective in chest pain due to cardiac disease. Anaesthesia, Pain & Intensive Care. 2019 Jul 3;23(1).
43. Zarei L, Pourjabali M, Naghdi N, Naji-Haddadi S, Bahmani E. A systematic review of the most important medicinal plants native to Iran effective on testicular morphology and hormonal testicular function. Journal of Pharmaceutical Sciences and Research. 2017 May 1; 9(5):562.
44. Bahmani M, Taherikalani M, Khaksarian M, Rafieian-Kopaei M, Ashrafi B, Nazer M, Soroush S, Abbasi N, Heydari R, Zarei L, Alizadeh M. Synthesis and evaluation of the antibacterial effect of titanium dioxide nanoparticles in comparison with ampicillin, colistin, and ertapenem on Staphylococcus aureus. Journal of Pharmaceutical Negative Results. 2019 Jan 1;10(1):16-20.
45. Sanaei N, Mohammadi R, Raisi A, Zarei L. Extract of *Berula angustifolia* (L.) Mertens Enhances Wound Healing in Streptozotocin-induced Diabetic Rats. Wounds: A Compendium of Clinical Research and Practice. 2018 Jun 25;30(8):242-8..
46. Rahmati M, Shokri S, Ahmadi M, Ahmadi M, Marvi Moghadam N, seyfi S, et al . Comparison of Pesticide Effect of Copper Oxide Nanoparticles Synthesized by Green Chemistry and Plant Extracts on Anopheles Stephensi Mosquitoes. Plant Biotechnol Persa 2022; 4 (1) :89-96
47. Naderi M A, Afkhami H, Ghaffarian F, Rahimi M, Sameni F, Khorshidi N et al . Investigation of Antibacterial Effect of Ferula macrocolea Extract and Quantity Determination of Inhibitory Effect on 4 Standard Strains of Gram Positive and Gram Negative Bacteria. Plant Biotechnol Persa 2022; 4 (1) :97-102