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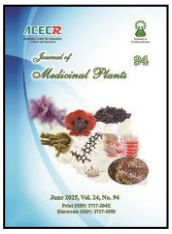

Research Article

Topical formulation of a skin-natural wound healing remedy based on Traditional Persian Medicine

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ABSTRACT

Background: Historical documents highlight various multifocal products for wound management, including the *Zaroor Arbaee* formulation, which comprises *Commiphora myrrha*, *Boswellia* sp., *Astragalus fasciculifolius*, and *Dracaena* sp. **Objective:** This study investigates a novel topical formulation for skin wound healing based on Traditional Persian Medicine, emphasizing these botanicals. **Methods:** A methanol extract was prepared, and an MTT assay was used to assess cytotoxicity on normal liver cells. Antimicrobial activity was evaluated using the microdilution method according to CLSI guidelines. A 5% topical cream was formulated, and total phenolic, flavonoid, and tannin contents were measured via spectrophotometry. High-performance thin-layer chromatography (HPTLC) was employed for phytochemical profiling, and essential oil extraction was performed to identify volatile constituents. Organoleptic and physicochemical tests were also conducted on the cream. **Results:** The MTT assay showed no cytotoxicity, and antimicrobial tests confirmed significant antifungal activity. Total phenolic, flavonoid, and tannin contents were 7.59 ± 0.31 mg, 0.54 ± 0.007 mg, and 2.07 ± 0.13 mg per 1.5 g of extract in a 30 g cream, respectively. Essential oil analysis identified *furanoeudesma-1,3-diene* (32.84%) and *curzerene* (14.94%) as major constituents. Additional assessments confirmed the cream's stability and spreadability. **Conclusion:** *Zaroor Arbaee* appears to be a promising traditional remedy for skin wound healing.

1. Introduction

Wound healing has been a critical facet of human health throughout history, and diverse cultures have contributed unique insights and formulations to expedite this natural restorative process [1]. Traditional Persian Medicine

Abbreviations: MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CLSI, Clinical and Laboratory Standards Institute; TLC, Thin-Layer Chromatography; HPTLC, High-Performance Thin-Layer Chromatography

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(TPM), deeply rooted in the historical fabric of the region, has long been recognized for its holistic approach to healthcare [2]. Encompassing a plethora of botanicals, minerals, and herbs, Traditional Persian Medicine emphasizes the equilibrium of bodily humors and the inherent healing potential of nature's bounty [3]. In this paper, we explore a novel skin wound healing topical formulation derived from the foundations of Traditional Persian Medicine, spotlighting key botanicals such as *Commiphora myrrha* (Nees) Engl**.** *(Myrrh)* [4], *Boswellia* sp. [5], *Astragalus sarcocolla* Dymock [6], and *Dracaena* sp. [7].

The quest for effective wound-healing remedies has persisted across cultures, with traditional medicinal systems offering unique perspectives on the amalgamation of nature's gifts for therapeutic purposes. The utilization of plant-derived compounds in wound care has gained substantial attention due to their multifaceted properties, including antimicrobial, anti-inflammatory, and regenerative capabilities [8]. The inclusion of *Commiphora Myrrha*, commonly known as *Myrrh*, and *Boswellia*, recognized as *Frankincense* aligns seamlessly with the rich tapestry of Persian pharmacopeia, known for its profound historical significance and medicinal efficacy [9].

Myrrh, a resin-producing tree indigenous to the Arabian Peninsula and northeastern Africa, has been revered for centuries for its medicinal attributes. The resin, extracted from the bark, contains bioactive compounds that demonstrate anti-inflammatory, antimicrobial, and tissue-regenerative properties [10]. Similarly, *Boswellia*, another resinous tree native to the arid regions of Africa and the Middle East, has been an integral component of traditional medicine. Its resin, commonly referred to as *Frankincense*, possesses anti-inflammatory and tissue-regenerative properties, making it an enticing candidate for inclusion in wound healing formulations [11]. *Astragalus*, a perennial herb native to arid regions of Asia, has been traditionally employed for its immunomodulatory and wound-healing properties. The unique blend of phytochemicals in *Astragalus*, including flavonoids and saponins, contributes to its therapeutic potential [12]. *Dracaena*, a genus of flowering plants with historical roots in traditional medicine, adds another layer of complexity to our formulation. Recognized for its possible anti-inflammatory and antioxidative benefits, *Dracaena* aligns with the holistic approach of Traditional Persian Medicine in addressing wounds at multiple levels [13].

The synthesis of these botanical components into a cohesive topical formulation represents a convergence of ancient wisdom and modern scientific understanding. The formulation aims not only to accelerate wound closure but also to mitigate inflammation, prevent infections, and promote tissue regeneration. As we embark on this scientific exploration, it is imperative to acknowledge the historical and cultural factors that have influenced the application of these botanicals in Persian Traditional Medicine, thereby paving the way for their integration into contemporary wound care practices.

This study aims to prepare a topical formula as a cream from the extracted powder of *Myrrh*, *Frankincense*, *Astragalus*, and *Dracaena*. Also, the plant materials, antifungal effects, and cytotoxicity or non-toxicity of the methanol extract of these compounds were investigated and the topical formula was assessed in terms of physical stability and some pharmaceutical tests based on three non-specific markers including phenol, tannin, and flavonoids.

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2. Materials and methods

The verification of the specimens was conducted by the botanist (Ms. Sedigheh Khademian) at the Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy. The voucher specimens were archived in the herbarium of the School of Pharmacy with the following codes:

Commiphora Myrrha (Nees) Engl. (Herbarium Code: HMP-678)

Boswellia sp. (Herbarium Code: HMP-945)

Astragalus fasciculifolius (Herbarium Code: HMP-312)

Dracaena sp. (Herbarium Code: HMP-507)

A combination of the four plant components was processed into both essential oil and extract.

2.1. Preparation of herbal powders

All plant materials were obtained from the Shiraz herbal market. The identity and quality of the samples were authenticated and approved by Dr. Zahra Sobhani, Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

The dried samples were turned into powder using a 40-mesh sieve. To prepare the extract, 25 g of each plant's powder was mixed and the final powder was dissolved in 100 ml of methanol (100% MeOH) (Merck, Germany) at 40°C for 30 minutes. This content was filtered by filter paper and an evaporator (Heidolph, Germany) was used to concentrate the extract. The concentrated extract was evaporated under 45°C and 25 mmHg conditions.

The Clevenger Apparatus (Pars Analiz, Iran) was used to extract essential oils. For this procedure, distilled water (500 mL) was added to the distillation flask. The water level should be below the level of the plant material. Then the dried plant material (100 g) was added to the distillation flask and the distillation flask was heated. The essential oil was separated from the water and collected in the graduated collection tube of the Clevenger apparatus. The separated content was stored in a cool (-20°C), dark place to preserve the quality of the essential oil.

2.2. Measurement of phenol, flavonoid, and tannin content of extracts

The total phenolic content of plant extracts was quantified through a spectrophotometric method utilizing the Folin-Ciocalteu technique [14], with Gallic acid serving as the calibration standard. A range of Gallic acid standards at known concentrations (6.25, 12.5, 25, 50, 100, 200 mg/L) was prepared. The extract or standard (0.5 mL) was combined with Folin-Ciocalteu reagent (2.5 mL, diluted 1:10) (Merck, Germany) and 2 mL of sodium carbonate solution (75 g/L) (Merck, Germany). The absorbance was measured at 765 nm using a spectrophotometer (PG Instruments Limited, UK).

The total flavonoid content in the extract was measured using the Dowd method, also known as the Aluminum chloride colorimetric technique [15]. A set of quercetin standards with predetermined concentrations (5, 20, 50, and 80 mg/L) was prepared. Subsequently, 2 mL of each standard solution or plant extract was mixed with 2 mL of 2% Aluminum chloride (Merck, Germany). The absorbance was recorded at 415 nm using a spectrophotometer (PG Instruments Limited, UK).Folin-Denis reagent [16] (Merck, Germany) was used to determine the amount of total tannin. For this purpose, 0.5 ml of tannic acid was mixed with 0.5 ml of reagent and sodium carbonate (Merck, Germany) (1 ml/mol). The resulting mixture was homogenized and the absorption of samples at 775 nm wavelength was done using a

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spectrophotometer (PG Instruments Limited, UK). A calibration curve was prepared from the tannic acid standard solutions (3.125, 6.25, 12.5, 25, 50, 100 mg/L).

2.3. Formulation of a topical preparation

Mineral oil (liquid paraffin), white beeswax (Raha Paraffin, Iran), and isopropyl myristate (Merck, Germany) were heated at 75°C. In another container, borax (sodium borate) (Merck, Germany) and preservatives were dissolved in water and heated at 75°C (water phase). This water phase was slowly assigned to the mineral oil-beeswax phase. After cooling at 35°C, the perfume was added. The extract was dissolved in water and added to the cold cream (0.5% active ingredient).

2.4. Stability and organoleptic assessment

The color and smell of the product from the same production series were compared at the end of specific periods (end of the first, third, and sixth months) after the production of the product.

To check the pH, a prepared sample was diluted 1:10 in deionized water and assessed by a pH meter at room temperature.

The mechanical stability of the cream was controlled via a centrifuge. For this purpose, 5–10 g of cream was investigated by centrifugation between 2000–5000 rpm. The possible separation of phases was checked at different times (between five minutes and one hour). Additionally, 1 g of cream was centrifuged at 10,000 and 15,000 rpm for 30 minutes.

To test homogeneity, the content was dispensed by partial pressure, and the uniformity and presence of bubbles were checked.

To test spreadability, 0.5 g of the product was placed between two glass plates under the force of 42 g, 542 g, and 1042 g for 3 minutes, and the surface covered was measured in square millimeters. Ideally, the surface covered by the 42 g weight should be more than 300 square millimeters, the 242 g weight should be more than 700 square millimeters, and the 542 g weight should be more than 1000 square millimeters.

To check the physical stability, the product was transferred to 3 separate test tubes and stored under different conditions: refrigerated storage at 4°C, room temperature storage at 25°C, and accelerated stability testing at 40°C, all for 6 months. The samples were examined and compared in terms of appearance, color, smell, rough feeling when applied to the skin, uniformity, phase separation, changes in product consistency, and the time of occurrence of any of these changes.

The rheological property of the cream was checked 24 hours after preparation. A cone-and-plate rheometer (RC/S, Brookfield, USA) with spindle C50-1 was used for this purpose.

2.5. Toxicity assessment

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to evaluate the cytotoxicity of the cream on HepG2 cells. HepG2 cells (Pasteur Institute, Iran) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, USA) and 1% Penicillin-Streptomycin solution (Biowest, France), at 37°C in a humidified environment containing 5% CO2. Cells were subcultured upon reaching 80-90% confluence through trypsinization. The cells were plated in well plates and exposed to different cream concentrations (1%, 3%, 5%, 10%, 15%, and 20%), with untreated cells as the control group. Incubation was performed at 37°C in a CO2 incubator for 24, 48, and 72 hours. Post-incubation, a 5 mg/mL MTT stock solution was

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prepared in sterile phosphate-buffered saline (PBS) (Sigma-Aldrich, Germany), and MTT solution at a final concentration of 0.5 mg/mL was added to each well. After 3-4 hours of incubation, the medium was gently aspirated, and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany) was used to dissolve the formazan crystals. Absorbance was measured at 570 nm with a microplate reader. Cell viability was determined using the formula: Viability (%) = (Absorbance of treated sample / Absorbance of control) × 100. All experiments were conducted in triplicate to ensure statistical robustness [17, 18].

2.6. Antimicrobial susceptibility assessment

The antimicrobial efficacy of the extract was evaluated using the microdilution technique in accordance with Clinical & Laboratory Standards Institute (CLSI) guidelines [19]. The tested microorganisms included *Candida albicans, C. glabrata, C. krusei, C. tropicalis, C. dubliniensis, C. parapsilosis, Aspergillus flavus, A. fumigatus, A. clavatus, Pseudallescheria boydii, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis*, and *Pseudomonas aeruginosa*. Serial dilutions of the extract (ranging from 0.06 to 32.0 µg/mL) were prepared in 96-well plates using Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco BRL, France) buffered with 3-(N-morpholino)propanesulfonic acid (MOPS) (Sigma, St. Louis, USA) to assess antifungal activity. For antibacterial activity, dilutions of the extract (0.25 to 128 µg/mL) were prepared in Mueller-Hinton agar (Merck, Germany) and tryptic soy broth (Merck, Germany). Yeast or bacterial suspensions were adjusted to a specific cell density using spectrophotometry at 530 nm. A 0.1 mL aliquot of the suspension was added to the 96-well plates, followed by incubation at 32°C for 24-48 hours for yeasts and at 37°C for 24 hours for bacteria in a humid environment. Sterile controls (200 µL of germ-free culture medium) and growth controls (culture medium with microbial suspension but without extract) were included. Growth in each well was compared to the growth control. All experiments were conducted in triplicate. Additionally, wells containing fungal suspensions with minimal growth were subcultured on Sabouraud dextrose agar, and those with bacterial suspensions showing limited growth were subcultured on tryptic soy agar to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). MFC and MBC represent the lowest concentrations of an antimicrobial agent required to eliminate fungi or bacteria, respectively.

2.7. High-Performance thin layer chromatography (HPTLC)

The best solvent system was selected for (HPTLC). To prepare the spectrum of thin layer chromatography (TLC) chromatogram (CAMAG, Switzerland), 3 mg/mL of the plant extract and final formulation was prepared and 10 microliters of each of them were loaded on a silica gel plate 60F254 (Merck, Germany) with the dimensions 10 x 20 cm. The loading condition of the samples was chosen linearly with a bandwidth of 6 mm and the distance from the X and Y axis was set to 15 mm. Spots were planted in a volume of 10 microliters at a certain distance of 1 cm. The volume of the mobile phase was chosen to be 10 ml and the distance of its movement to the solvent front was set to 80 mm. The drying time was also chosen to be one minute. The selected solvent system (mobile phase) includes a mixture of 10 ml of ethyl acetate (Merck, Germany), 20 ml of

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toluene (Merck, Germany), and 20 ml of methanol (Merck, Germany). This selected solvent system was then transferred into the mobile phase compartment of the device. The plate was then dried, the plate was examined in visible light and ultraviolet light with wavelengths of 254 and 366 nm and an image was prepared. Subsequently, the spots of the present compounds appeared on the HPTLC plate with sulfuric acid-anisaldehyde (Merck, Germany) reagent by heating. For this purpose, sulfuric acid-anisaldehyde reagent was freshly prepared and poured into the TLC tank (Fluka, Germany). The HPTLC plate was immersed in the reagent solution inside the tank and removed from it. After drying the plate at room temperature under the hood, it was heated to 110°C by a hot gun and the spots appeared on it.

2.8. GC-MS analysis

The gas chromatography (GC) analysis was performed using a Shimadzu GC system (model 9-A) fitted with a flame ionization detector (FID). Quantification of the identified compounds was carried out using Euro Chrom 2000 software from KNAUER, applying the area normalization method. The relative composition of the compounds was calculated based on the peak areas obtained from the GC (9A-Shimadzu). Compounds were identified by comparing their retention indices and mass spectra with those of authentic reference standards. A DB-5 fused-silica column (30 m × 0.25 mm, film thickness 0.25 μm) was used, with a temperature gradient programmed from 40 to 250 °C at a rate of 4 °C/min. The injector was maintained at 250 °C, and the detector was set at 265 °C. Helium (99.99% purity) was employed as the carrier gas. The GC/mass spectrometry (MS) analysis was conducted using a Varian-3400 gas chromatograph interfaced with a Saturn II ion trap detector, utilizing the same DB-5 fused-silica column and temperature program as in the GC analysis. The mass spectrometer operated with an ionization potential of 70 eV and an electron multiplier energy of 2000 V. Kovats Indices (KI) were applied to characterize the retention behavior of the compounds in GC.

3. Results

3.1. Phenol, flavonoid, and tannin content of the product

In this study, 5% cream is filled in a tube weighing 30 g. Since the cream was 5%, the amount of extract in this tube was 1.5 g. Since the amount of total phenol, total flavonoid and total tannin in 1 gram of the extract was equivalent to 7.59 ± 0.31 mg GAE (gallic acid equivalent), 0.54 ± 0.007 mg Q (quercetin), and 2.07 ± 0.13 mg tannic acid, respectively, these amounts were equivalent to 11.385 ± 0.465 mg GAE, 0.81± 0.0105 mg Q, and 3.105 ± 0.195 mg tannic acid in a 30-gram tube of cream, respectively.

Yield of Extraction

Commiphora: 12%

Boswellia: 10%

Astragalus: 8%

Dracaena: 15%

Organoleptic properties of the product

The color (white) and smell of the product from the same production series were compared at the end of specific periods (end of the first day, second day, third day, first, second, and third week, and the first, third, and sixth month) after the production and any change were observed. The pH of the cream was 6.3 at the time of production, 3 months after production was equal to 6.5, and 6 months after production was 6.8.

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3.2. Stability, homogeneity, spreadability, and rheological properties of the product

Phase separation was not observed in the centrifuge test indicating the mechanical stability of the product. The cream was dispensed from the tube completely uniformly with no air bubbles indicating the homogeneity of the product (Figure 1). The spreadability evaluation showed that the covered surfaces with weights of 42 g, 242 g, and 542 g were 153.86, 254.34, and 314.25 square millimeters, respectively. From the point of view of physical stability, the sample did not crystallize at refrigerator temperature. Therefore, the temperature of the refrigerator seems suitable for storing these products. During 6 months of storage at 25°C and 40°C, the product showed acceptable stability, and phase separation was not observed. The consistency of the product remaining unchanged, the consistent smell of the formulations over time, and the absence of a rough feeling on the skin are all positive indicators regarding the stability and quality of the product.

The rheological properties of the formulation were assessed. It was observed that the formulation exhibited semi-plastic behavior in terms of flowability. Thus, the cream has a viscosity that decreases with increased shear stress or agitation. The rheogram (Figure 2-4) illustrated clear thixotropic behavior in the formulation. The rheogram showed distinct back-and-forth curves, indicating the thixotropic nature of the cream. The appearance and expandability of the formulation were evaluated and deemed optimal. The cream possesses good rheological properties, however, it provides a pleasing sensory experience, possibly in terms of texture and spreadability.

3.3. Cell viability

The amount of cell viability in HepG2 cells under the influence of different concentrations of methanol extract at 24, 48, and 72 hours is shown in Figure 5. As it is known, increasing the concentration of methanolic extract increases the percentage of cell viability. In each concentration, increasing the cells' treatment time with the extract is associated with growth in cell viability.

Fig. 1. Homogeneity test

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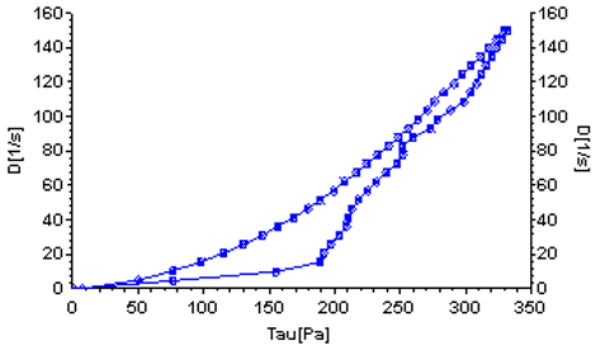
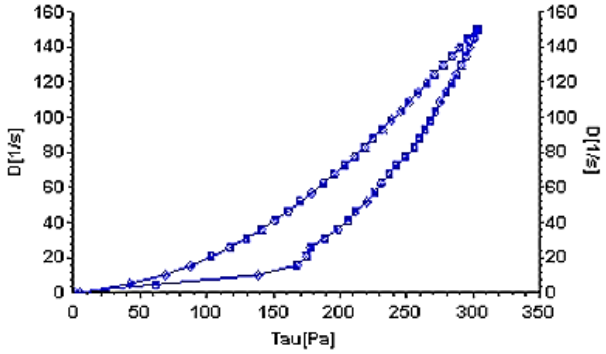


Thixotropy: 4422.441 [Pa/s]

Mean of viscosity =2.4999[Pas]

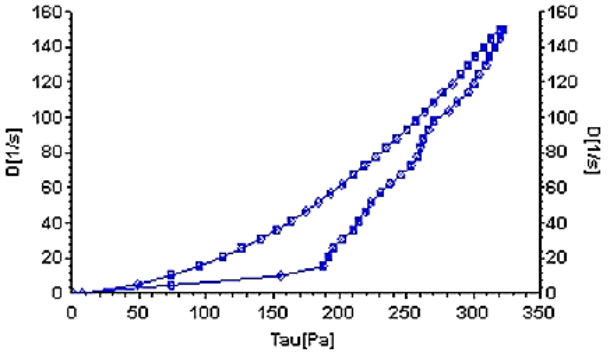
Fig. 2. Rheogram of cold cream formulation



Thixotropy: 5483.698 [Pa/s]

Mean of viscosity =2.2761[Pas]

Fig. 3. Rheogram of cold cream + plant extract formulation



Thixotropy: 5058.541 [Pa/s]

Mean of viscosity =2.4325[Pas]

Fig. 4. Rheogram of cold cream + plant extract formulation after heating cycles

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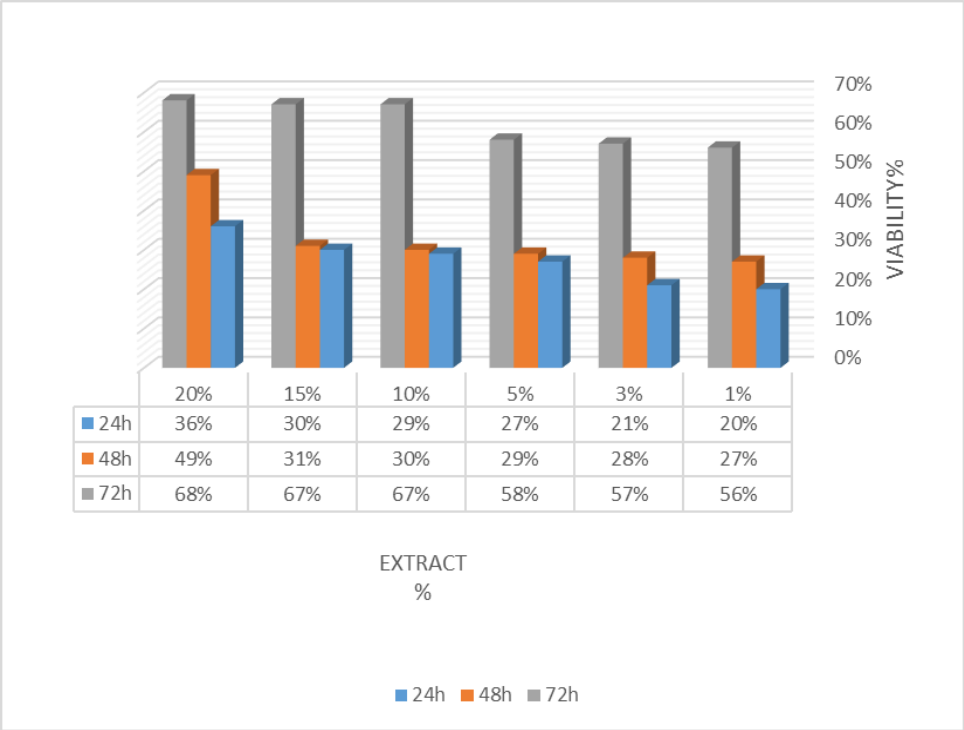

Fig. 5. Cell viability in different concentrations of methanol extract at 24, 48 and 72 hours

3.4. Anti-microbial properties of the product

The plant extract exhibited potent antifungal activity against all tested yeasts and fungi, with MIC values ranging from 0.032–4 µg/mL and 2–8 µg/mL (Table 1). However, it showed no inhibitory effects against Gram-positive or Gram-negative bacteria at concentrations up to 64 µg/mL. Thus, the extract from the combination of these four plants represents a promising resource for combating fungal infections.

3.5. Fingerprints of TLC and HPTLC

The extraction efficiency of the herbal composition was 9.5%. After extracting each component and the final formulation, a concentration (3 mg/ml) of each was prepared for loading on thin layer chromatography paper. Various solvent systems were used to determine the best for performing chromatography. and finally, according to the type of chromatogram pattern obtained from them, the solvent system containing toluene and ethyl acetate with a ratio of 80-20 (toluene-ethyl acetate) was selected. TLC and HPTLC documentation are shown in Figures 6 & 7.

3.6. Components of the products

Essential oil extraction was carried out using the distillation method with water and by the Clevenger Apparatus for 4 hours. Then, the amount of essential oil obtained and the efficiency of essential oil extraction were calculated. The efficiency of essential oil extraction was 1% and the extraction rate was 0.5 ml. The results of GC/MS analysis regarding compounds of produced extract are shown in the Table 2. If the KI reference was around ± 5 units compared to the KI calculated for the components, then identification was approved. Alpha-pinene, dl-Limonene, and *β*-cis-Ocimene were the 3 main components of essential oils.

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3.7. Purpose of Essential Oil Extraction

The purpose of extracting essential oils was to utilize their therapeutic properties. In this study, the essential oils extracted from the selected plants were aimed to identify the volatile active compounds and evaluate their antimicrobial and anti-inflammatory properties. However, according to the method description, only the extracts were added to the cream, and essential oils were not used in the formulation of the cream. The primary goal of extracting these oils was to identify their aromatic and active compounds for further analysis.

Table 1. The antimicrobial effect of the extract against yeast and fungi

Strain	ATCC/CBS	MIC(µg/mL)	MFC(µg/mL)
C. albicans	A1905	0.25	0.5
C. albicans	A5982	0.25	0.125
C. albicans	A1949	0.125	0.5
C. dubliniensis	C8501	0.25	0.5
C. dubliniensis	A7987	0.125	0.5
C. dubliniensis	A7988	0.25	0.25
C. dubliniensis	C8500	0.125	0.25
C. glabrata	A90030	0.125	0.25
C. glabrata	A863	0.25	0.5
C. glabrata	A6144	0.5	0.5
C.glabrata	A2192	0.125	0.25
C. glabrata	A2175	0.25	0.25
C. krusei	A6258	0.25	0.5
C. tropicalis	A750	0.5	0.5
C. neoformance	A9011	0.03	1

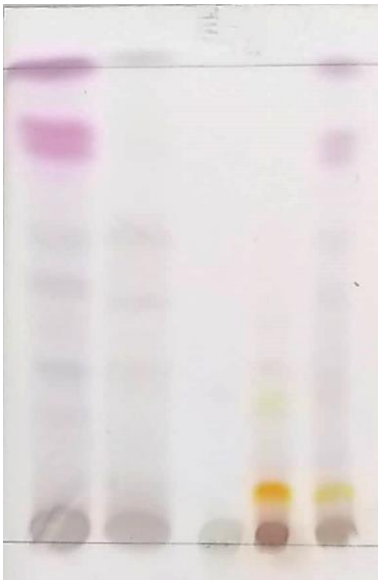

Fig. 6. TLC for methanolic extracts. From the left side: a) *Dracaena*, b) *Myrrh*, c) *Astragalus*, d) *Frankincense*, e) Final formulation

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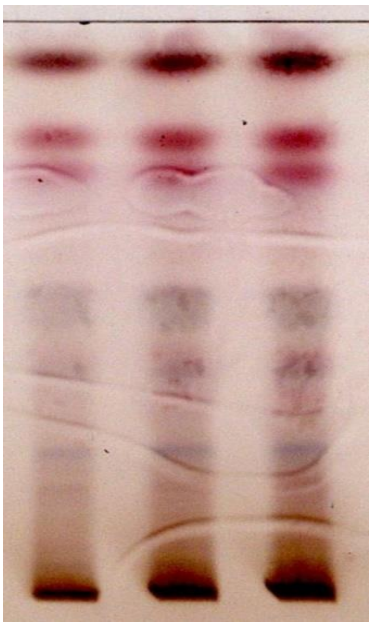

Fig. 7. HPTLC for methanolic extracts. From the left side: a) *Astragalus*, b) *Dracaena*, c) Final formulation

Table 2. Components of the essential oil

No	Components	Area	KI_{cal}	KI_{Ref}	ID	Ref.
1	α-Pinene	0.83	944	942	MS/KI	[45]
2	dl-Limonene	0.98	1032	1028	MS/KI	[46]
3	β-cis-Ocimene	0.74	1038	1034	MS/KI	[46]
4	γ-Terpinene	0.94	1068	1064	MS/KI	[47]
5	n-Octyl formate	1.39	1108	1104	MS/KI	[48]
6	Linalool	0.59	1115	1110	MS/KI	[49]
7	Octyl acetate	9.79	1215	1211	MS/KI	[50]
8	L-α-bornyl acetate	0.78	1294	1290	MS/KI	[51]
9	δ-Elemene	0.55	1343	1339	MS/KI	[52]
10	β-Elemene	3.92	1396	1392	MS/KI	[53]
11	trans-Caryophyllene	0.67	1425	1420	MS/KI	[53]
12	γ-Elemene	0.55	1439	1435	MS/KI	[54]
13	Germacrene-D	0.90	1478	1474	MS/KI	[46]
14	β-Selinene	0.93	1480	1476	MS/KI	[55]
15	Curzerene	14.94	1500	1495	MS/KI	[56]
16	δ-Cadinene	0.34	1528	1524	MS/KI	[54]
17	Spathulenol	1.71	1575	1571	MS/KI	[57]
18	Furanoeudesma-1,3-diene	32.84	1594	1590	MS/KI	[58]
19	Lindestrene	6.42	1656	1651	MS/KI	[59]
20	Cembrene A	2.88	1964	1960	MS/KI	[60]
	Identification			**79.81**		

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4. Discussion

The present study focused on the safety and efficacy of a formulation derived from documents of TPM in wound healing. The formulation, known as "*Zaroor*" or "*Zomād*" in TPM, was selected for its widespread use and popularity. The ingredients of this product include *Myrrh* (known as "Mor" in the Persian language), *Boswellia* (known as "Kondor" in the Persian language), *Astragalus* (known as "*Anzaroot*" in the Persian language), and *Dracaena* (known as "*Khune Siavash*" in the Persian language). The investigation included an analysis of volatile compounds in the essential oil of *Zoroor*, revealing the presence of 20 major compounds. Notably, Furanoeudesma-1,3-diene, Curzerene, Octyl acetate, and Lindestrene were identified as the main components, with Curzerene, Furanoeudesma-1,3-diene, and Lindestrene linked to *Myrrh* and Octyl acetate associated with *Frankincense*.

Frankincense, a crucial component of the formulation, is known for its anti-inflammatory [20], antioxidant [21], wound healing [22], and antimicrobial properties [23]. Similarly, *Myrrh*, another key component, exhibits anti-inflammatory [24], antimicrobial [25], antioxidant [26], and wound-healing effects [27]. *Dracaena* contributes anti-inflammatory, wound healing [28], antimicrobial, and antioxidant effects [29], while *Astragalus* is acknowledged for its anti-inflammatory and antioxidative characteristics [12]. The mechanisms behind these effects involve complex interactions between bioactive compounds present in these plants and various cellular and molecular pathways. *Frankincense* contains bioactive compounds known as boswellic acids [30]. These acids inhibit pro-inflammatory enzymes like 5-lipoxygenase, reducing the production of inflammatory leukotrienes [31]. Boswellic acids also interfere with the nuclear factor-kappa B (NF-κB) signaling pathway, a key regulator of inflammation [32]. *Frankincense* contains terpenes with potential antimicrobial properties [30]. These compounds may disrupt microbial cell membranes and inhibit the growth of bacteria and fungi [33]. Terpenoids found in *Frankincense* exhibit antioxidative capacity, mitigating free radicals and safeguarding cells against oxidative damage [34]. Some components in *Frankincense* may stimulate cell proliferation, aiding in tissue regeneration [35]. *Myrrh* contains sesquiterpenes with anti-inflammatory effects [36]. Similar to *Frankincense*, *Myrrh's* terpenoids contribute to its antimicrobial activity, suppressing the growth of pathogens [37]. *Myrrh* contains polyphenolic compounds that act as antioxidants, scavenging free radicals [38]. *Myrrh* may stimulate the activity of fibroblasts, promoting collagen synthesis and wound closure [39]. *Dracaena* may contain triterpenoids and phenolic compounds with anti-inflammatory, anti-microbial, and antioxidant properties [40]. *Astragalus* species, including *Astragalus fasciculifolius*, often contain anti-inflammatory polysaccharides that modulate immune responses. Saponins found in *Astragalus* species possess antioxidant activity, protecting cells from oxidative damage [41].

To identify *Myrrh* plant gum, functional groups in its compounds were investigated using GC/MS techniques [42]. Regarding *Frankincense*, GC/MS fingerprinting indicated the likely presence of *Boswellia carterii* species [43]. The gum of *Astragalus* was identified based on the appearance characteristics (almost spherical and small pieces, with a diameter of up to 2 mm in yellow, white, or red and transparent crystal shape, which often stick together to form larger pieces and are broken due to the least pressure). *Dracaena* was identified by comparing samples in the market, acknowledging this as a study limitation, and it is recommended that in future studies, the identification or preparation of fingerprints should be done with conventional device methods.

The antifungal effects of the extract were evaluated against various strains, demonstrating

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efficacy against Candida and Aspergillus species. The antifungal mechanisms of action of *Frankincense*, *Myrrh*, *Dracaena*, and *Astragalus* involve a combination of bioactive compounds that exhibit inhibitory effects on various stages of the fungal life cycle by disruption of cell membranes, inhibition of fungal enzymes, and anti-oxidant effects. These compounds include boswellic acid in *Frankincense* [30], terpenoids in *Frankincense* and *Myrrh* [44], and phenolic compounds in *Dracaena* [29]. Moreover, the study assessed the extract's safety using the MTT test and colorimetric methods, revealing its safety on human cells, and supporting its potential use in topical formulations. Given the previously established wound-healing effects of the plant extracts, it is hypothesized that the cream formulation will retain these beneficial properties. The study concludes that the topical formulation may offer therapeutic benefits for skin conditions, emphasizing the safety and efficacy of the tested extract in the cream.

5. Conclusion

Based on TPM principles, the formulated cream demonstrated stability, microbiological safety, and suitable rheological behavior. HPTLC fingerprinting emerged as a valuable method for quality control assessment, confirming the presence of characteristic compounds from each plant in the formulation. Considering the documented therapeutic effects of the constituent plants, the polyherbal paste shows potential as a valuable traditional treatment for wound healing. These results bolster the expanding research advocating for the incorporation of TPM into modern health and wellness formulations.

Author contribution

M. F.: Conceptualization, methodology, investigation, writing the original draft. Z. S.: Methodology, investigation, data analysis. K. Z.: Data curation, validation, and review. S. S. H.: Investigation and data collection. M. M. Z.: Supervision, project administration, writing, and review.

Conflicts of interest

The authors declare no conflict of interest.

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