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In vitro Proliferation and Wound Healing Effects of *Narcissus tazetta* L. Bulb on Primary Human Dermal Fibroblasts

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Authors' contributions

This work was carried out in collaboration between all authors. Author FS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MM and MR managed the analyses of the study. Authors AP and AF managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: *Narcissus tazetta* L. bulbs have been used in Traditional Persian medicine (TPM), as "Zaroor" for wound healing. Toward quality assurance of the plant, pharmacognostic and phytochemical studies, physicochemical characterization, and healing effects of "Zaroor" were studied.
Study Design: Original Research Article.

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Place and Duration of Study: The study took place in herbal and traditional medicines research center at Faculty of Pharmacy and stem cell research center at Faculty of Allied Medical Sciences of Kerman, Iran from March 2016 to September 2017.
Methodology: Total flavonoid content (TFC) of the plant was measured and high-performance thin-layer chromatography (HPTLC) fingerprint profiling was planned for qualitative assessment of the plant with reference to rutin as the marker compound. Antioxidant activity was studied using DPPH and FRAP assay. Proliferation and wound healing effect of the plant was evaluated on the primary human dermal fibroblast by neutral red and scratch assay.
Results: The pharmacognostic studies and physicochemical characteristics indicated characters, which are of diagnostic value for plant standardization and quality control. HPTLC chromatogram of the plant extract confirmed the presence of rutin in comparison to Rf value of the standard. Maximum inhibition of DPPH radical and IC50 value was estimated at 10000 µg/ml (99.89%) and 2379.82±37.59 µg/ml respectively. In FRAP test, the antioxidant value was estimated 0.29±0.02 mM/mg SO4Fe. Plant extract exhibited no significant effects on cell proliferation in HDF cells after 48 hr treatment using neutral red assay. The greatest reduction in gap width was considered after 48 hr at 1.562- 6.25 µg/ml. This activity was significantly different from untreated cells as a control ($p<0.01$).
Conclusion: *N. tazetta* would be effective for wound healing through different mechanisms such as anti-inflammatory and antioxidant effect, which needs to be studied in more details.

Keywords: Narcissus tazetta; wound healing; scratch assay; human dermal fibroblast; antioxidant; HPTLC.

ABBREVIATIONS

HDFs : Primary human dermal fibroblasts
NR : Neutral red
PEN : Percolated extract of N. tazetta
TPM : Traditional Persian medicine
SC : Stratum corneum
TFC : Total flavonoid content

1. INTRODUCTION

Skin is one of the most important organs, which can protect the body against a variety of external and internal harmful agents such as microbes, toxins, temperature, UV radiation, and especially water retention. Stratum corneum (SC), the outer layer of skin, is composed of different parts such as cholesterol, proteins, fatty acids, and ceramides [1]. Alteration in SC integrity causes major skin dysfunction. External factors such as abrasion, inflammation, wound, and burn can damage skin and severely distress human life and health [2]. There are different types of the wounds like pressure ulcer, bedsore wounds and diabetic foot ulcer that affect importantly lifestyle of patients. Such wounds cause induction of pain, deformity, disability, and dependence on others and even osteomyelitis and death [3]. Considering the limitations and unresponsiveness of current drugs, the use of traditional treatments and medicinal plants is desired.

In TPM (Traditional Persian Medicine), different natural treatments have been suggested for wound healing. *Narcissus tazetta* L. bulb from Amaryllidaceae family with the common name of “Narjes” has been confirmed in several sources of TPM [4-8]. This ornamental plant has a white and yellow flower that appear from January to April and have desired odor [9]. Amaryllidaceae plants are predominantly tropical or subtropical plants, while *N. tazetta* occurs primarily in Southwestern Europe and Mediterranean region, with a center of diversity in the Iberian Peninsula (Spain and Portugal) as well as in Iran and Kashmir [10].

Presence of alkaloids such as galantamine [11], lycorine [12], flavonoids like rutin, quercetin and kaempferol, phenolic acids [13] and a glycoprotein called lectin [14] has been reported in the plant bulbs.

According to TPM: "the bulb of *Narcissus* is hot and dry in the third and has been proposed as a purifier, absorbent, and anthelmintic". The plant purifies the uterus and improves the internal and external ulcers. In TPM sources, a preparation of *N. tazetta* bulb powder known as "Zaroor" is recommended for wound healing. "Zaroor" has an astringent effect and can stop bleeding in topical uses. In TPM, the term "Zaroor" includes the fine powder of dried plants that sprinkled on organ for treatment aims [4,5,7]. A combination of "Zaroor" with honey and /or vinegar has been recommended for burns, nerve injury, and internal ulcers. Moreover, "Zaroor" can be used to remove skin stain [6,8].

The aim of the present study is the pharmacognostic study, preparation, standardization and quality control of "Zaroor" and studying its healing effects on primary human dermal fibroblast (HDF) migration and proliferation by scratch wound assay.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Rutin, ethyl acetate, formic acid, acid acetic glacial, and Dimethyl sulfoxide (DMSO) were purchased from Merck (Germany). 1, 1-Diphenyl-2-picrylhydrazyl (DPPH); butylated hydroxytoluene (BHT); 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) and phosphate buffered saline (PBS) were supplied by Sigma–Aldrich. Dulbecco's modified eagle's medium (DMEM) and fetal bovine serum (FBS) were used of grade Gibco (Germany). Penicillin-G, streptomycin and amphotericin B were purchased from Biostar (Argentina). Primary human dermal fibroblasts (HDF) were prepared from Afzalipour School of Medicine in Kerman. The other chemicals were of analytical grade.

2.2 Plant Materials

Fresh bulbs were purchased from a local market in Kerman (Iran) in December of 2015. The bulbs were skinned, chopped, and dried in the shade at room temperature, milled, and passed through a sieve (mesh 60).

2.3 Authentication

For authentication of *N. tazetta*, the bulbs were planted in December 2015 in the Botanic garden of the school of Pharmacy, Kerman University of Medical Sciences, Iran. After flowering in March 2016, herbarium specimen was prepared and a voucher specimen was deposited at Herbarium center of faculty (KF1624).

2.4 Different Types of Extraction

Determination of the extract yield by different methods of extraction is one of the methods for quality control of medicinal plants [15]. Different extracts were prepared by maceration, percolation, sonication, 1 hr warm extraction, 2 hr warm extraction and 4 hr warm extraction from "Zaroor" [16]. Each experiment was performed in triplicate.

2.4.1 Maceration

The plant materials (30 g) were macerated in methanol 80% for 72 hr. The extract was replaced with fresh solvent every 24 hr [16]. Finally, it was filtered, pooled, concentrated under vacuum, dried, and weighed.

2.4.2 Percolation

Plant materials (85 g), were mixed with 80% methanol, transferred to a percolator, and allowed to stand for 24 hr, then to which the same solvent was added (totally 9 liters). Percolation was set to a flow rate of 10 drops/min [16]. Extraction was continued until the positive test of an eluted extract of alkaloid reagent.

2.4.3 Sonication

In this step, 10 g of dried powder was mixed with 150 ml of methanol 80% and was put in bath ultrasonic for half an hour, three times. The extract was filtered through Whatman filter paper and dried in an oven at 50 °C after concentrating the extract under vacuum.

2.4.4 Warm extraction

This method was carried out for 1, 2 and 4 hours extraction. An amount of 5 gr of dried powder was mixed with 50 ml methanol 80% separately and was put in Mary Bath respectively for 1, 2 and 4 hours. The extracts were filtered through Whatman filter paper, dried in oven 50℃ after concentrating the extract by rotary and weighed.

2.5 Total Flavonoid Content

Total flavonoid content was determined by colorimetric assay [17]. Rutin standard solution (25 to 200 µg/ml) in methanol 60% was used to make the calibration curve by measuring the absorbance of the dilutions at 510 nm with Eliza reader (Biotek, USA). Each experiment was performed in triplicate and the results were shown as Mean±SD.

2.6 Phytochemical Screening

Phytochemical screening of the plant bulb was performed for confirmation of the presence of tannins, alkaloids, saponins, cardiac glycosides, steroids, anthraquinones, terpenoids, and flavonoids [18,19].

2.7 High-Performance Thin-Layer Chromatography Analyses (HPTLC)

Several concentrations of rutin (10-200 µg/ml) and plant extract were prepared. Each concentration was spotted (20 µl) two times in the bandwidth of 6 mm using an automatic syringe with airflow on HPTLC Silica gel 60 F254 pre-coated. Thin Layer Chromatography (TLC) glass plate 20×10 cm of Merck was prewashed by distilled methanol and activated at 110°C for 1 hr prior to chromatography.

The plate was placed in the glass tank with the mobile phase of ethyl acetate: formic acid: glacial acetic acid: water (100:11: 11: 26 v/v/v/v) to move 70 mm from the bottom at room temperature. After drying, the plate was scanned at 254 nm using Camag automatic TLC sampler 4, equipped with Wincats-4 software.

2.8 DPPH Assay

Antioxidant activity of percolate extract of *N. tazetta* (PEN) was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay [20]. A volume of 50 µL of various concentrations of the extract was added to 150 µL of diphenyl-picrylhydrazyl (0.004%) and incubated at room temperature. The absorbance was measured at 517 nm using Elisa reader (Biotek, USA) and the percentage of inhibition activity was calculated using the following equation.

$$I\% = ((A0-A1)/A0) \times 100$$

Where A0 = is absorbance of the control, and A1 = is absorbance of the extract/standard.

BHT and methanol 50% were used as a reference and negative control, respectively.

IC_{50} values (concentration that inhibits 50% of DPPH radical) were determined for extract and BHT [21].

2.9 Ferric Reducing Antioxidant Power (FRAP) Assay

Total antioxidant capacity (TAC) of the extract was determined by Ferric Reducing Antioxidant Power **(**FRAP) method [22]. This method is based on the ability of antioxidant compounds to reduce complex Fe3+ to Fe2+, which gives a blue color with maximum absorbance at 593 nm. In this process, 5 µl of PEN (4 mg/ml) was added to 295 µl of freshly prepared FRAP reagent [combined of 10 mM of 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, 20 mM FeCl3 solution and 300 mM acetate buffer (pH= 3.6) in a ratio of 1:1:10]. An aqueous solution of ferrous sulfate was used to prepare a standard curve. The solvent without extract (methanol 50%) with FRAP solution was used as a control. Absorbance was determined at 593 nm. FRAP values were expressed as mmol of Fe (II)/mg dry weight of the extract. Each measurement was taken in triplicate and the results reported as mean ± SD.

2.10 Microscopic Analyses

Microscopic analysis was used for ensuring the purity, adulteration identifying, and description of physical characteristics of each plant (10×40X, NIKON, Japan).

2.11 Physicochemical Studies

Physicochemical analyses were carried out using standard procedures [23].

2.11.1 Moisture content

A definite amount of dry and fresh chopped bulb of *N. tazetta* was taken in a carefully weighed dry petri dish. The samples were spread in uniform thickness and reweighed. The set was placed in a dryer oven and heated at 105°C until the difference between two successive weighting did not exceed 5 mg.

2.11.2 Ash values

2.11.2.1 Total ash value

About 2 g of air-dried powder *N. tazetta* bulb was taken in two silica crucibles and was spread in a uniform thickness. The sample was incinerated at 500-600°C until its color turned to white due to carbon removal. Then it was cooled and weighted and the percentage of ash was calculated in the total dried bulb of *N. tazetta* bulb.

2.11.2.2 Water-soluble and water-insoluble ash values

A volume of 25 ml distilled water was added to total ash and was boiled for 5 min. The insoluble matter was collected in an ash-less filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450°C. Then, it was cooled and weighed to determine water insoluble

ash content from the air-dried material. The water-soluble ash was calculated by subtracting the water-insoluble ash from the total ash.

2.11.2.3 Acid-insoluble ash value

The total ash obtained from second above silica crucibles was boiled for 5 min with 25 ml of hydrochloric acid TS (about 70g/lit) and then filtered through ash-less filter paper (Whatman). The filter paper was ignited and cooled in a silica crucible. Acid soluble ash was calculated by weighting.

2.12 Cell Culture and Condition

Primary human dermal fibroblasts (HDFs) isolated from the adult human skin of a single donor was prepared from Kerman Afzalipour medicine school. HDFs were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 20% fetal bovine serum (FBS), and 100 U/ml penicillin-G, 100 µg/ml streptomycin, and 1 µg/ml amphotericin B and incubated at 37°C in 5% CO2.

2.12.1 Fibroblast proliferation assay

Proliferative activity of PEN was determined by neutral red (NR) method [24]. HDF cells (about 1×104 cells) were seeded on a 96-well microtiter plate. After 1 day of incubation, the growth medium was replaced with exposure medium and various concentrations of PEN were added to each well. Untreated cells were used as a control. NR solution was added (10% of the culture medium volume) after 48 h and then incubated for 4 hr. After incubation, the medium was carefully removed and the cells quickly rinsed with NR assay fixator. The fixator was removed and the incorporated dye was then solubilized in a volume of NR assay solubilization solution equal to the original volume of culture medium. Absorbance was measured at 540 nm with Eliza reader (Biotech, USA). The assay was made in triplicate.

2.12.2 Scratch assay

The wound was created on a confluent HDF monolayer on a 6-well dish by making a horizontal and vertical scratch with a sterile 100-µL plastic pipette tip. Wound margins were photographed immediately after wounding. Then, the cells were treated with various concentrations of PEN in DMEM containing 5% FBS, incubated at 37 °C with 5% CO2 and the same fields of the wound margin were photographed after 24-hr and 48-hr time points. Untreated cells were used as the control. The average extent of wound closure was evaluated by measuring the width of the wound using ImageJ software [25].

2.13 Statistical Evaluation

The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-test and students t-test (SPSS version 16 and Prism 5).

3. RESULTS

3.1 Extraction Methods and Total Flavonoid Content (TFC)

According to the results, the yield of extraction in different methods revealed that (Table 1) the highest yield of extraction is due to sonication and percolation methods (26.2±0.3 and 26.5±0.8 %w/w respectively). The order of the other methods was as follows: cold maceration > 4 hr warm maceration > 2 hr warm maceration > 1 hr warm maceration. In statistical comparison, percolation and sonication methods display more significant difference yield of extract than the other methods (P= 0.00).

As shown in (Table 1), total flavonoid content of different extracts exhibits a wide variation.

Table 1. Effect of different extraction methods on extract yield and total flavonoids content (TFC) of *N. tazetta* bulb

Method of extraction	Amount of total flavonoid (mg g−1)	Yield of extraction (%)
Percolation	27.04±0.93*	26.5*
Sonication	24.80±1.25*	26.2*
Cold maceration	21.71±1.19	18.2
4 hr w. maceration	17.14±0.80	17
2 hr w. maceration	14.97±0.57	16.4
1 hr w. maceration	15.01±0.74	16

**Results are the mean±SD of three experiments and differences with P = 0.00 considered significant. W.: warm*

Although a significant difference was not observed in flavonoid content of percolation and sonication methods (P >0.05), they were significantly different from the other used methods (P = 0.00). Therefore, the results of determination of total flavonoids confirmed that the percolation method was the best one for *N. tazetta* bulb. In this sense, percolated extract of *N. tazetta* (PEN) we used through the experiment. The flavonoid content was calculated for different extractions using the following linear equation regarding the calibration curve.

A=0.0032B-0.0085, R2=0.9998

Where

A is the absorbance
B is the flavonoid content in µg g-1

3.2 Phytochemical Screening

Results of phytochemical screening of *N. tazetta* bulb confirmed the presence of flavonoids, alkaloids, saponins and tannins in the crude extract of the plant (Data not shown).

3.3 HPTLC Fingerprinting

The calibration curve of rutin as standard equivalent was linear in the range of 10–100 µg/mL (Fig. 1). Rutin appeared in R_f 0.45 and 0.46 in the standard spot and PEN sample respectively (Fig. 2). The results indicated that rutin was estimated 0.29 % w/w in a plant sample.

3.4 DPPH Assay

As shown in (Table 2), both PEN and BHT inhibited DPPH radical in a dose-dependent manner. IC_{50} for DPPH activity was 2379.82±37.59 µg/ml and 48.40±2.88 µg/ml for PEN and BHT respectively.

3.5 Frap Assay

In this assay, the ability of extracts was estimated about 0.29±0.02 mM/mg to reduce iron (III) to iron (II) considering ferrous calibration curve (y=0.358x+0.099).

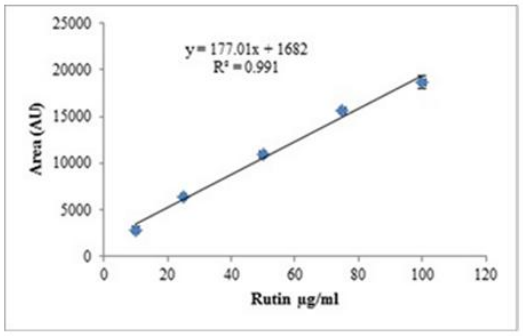

Fig. 1. Calibration curve of rutin at a maximum wavelength, (λmax=254 nm)

3.6 Microscopic Analyses

Microscopic examination of *N. tazetta* bulb indicated the presence of stomata in bud part, vena in scale, epidermis cells and granularity starch in the bulb of the plant (Fig. 3).

3.7 Physicochemical Studies

As shown in (Table 3), the moisture content of the *N. tazetta* bulb ranged from 74.13±1.16 w/w % in the fresh bulb to 9.9±0.2 w/w % in dried one which is significantly different (P = 0.00).

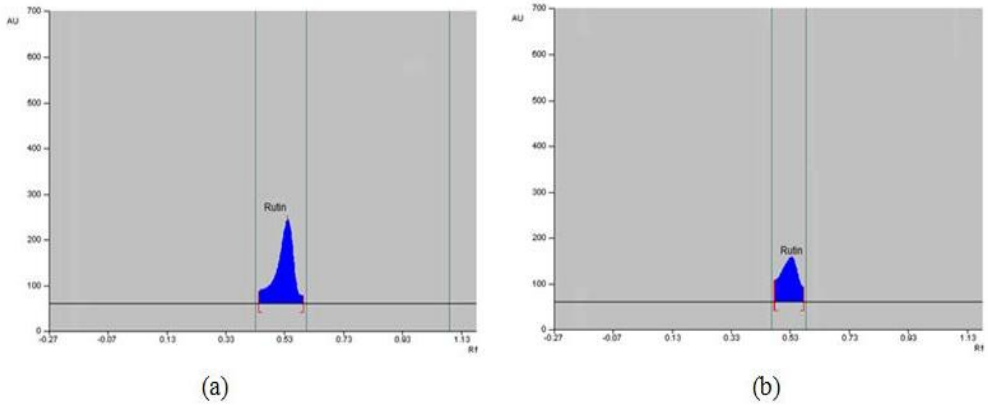

Fig. 2. Chromatogram: HPTLC of (a) reference rutin and (b) percolated extract of *N. tazetta* (PEN). (Resolution at 254 nm)

Table 2. IC_{50} value and percentage of DPPH radical scavenging activity of different concentrations of a percolated extract of *N. tazetta* (PEN) in comparison to BHT as positive reference

IC_{50} (µg/ml)	Inhibition activity %	Concentration (µg/ml)	Sample
2379.82±37.59	7.85±0.47	100	PEN
	27.39±4.02	1000	
	49.88±2.62	2000	
	79.95±2.99	4000	
	94.64±1.58	6000	
	98.37±1.52	8000	
	99.89±0.14	10000	
48.40±2.88	8.90±3.96	0.1	BHT
	17.81±3.13	1	
	39.75±2.24	10	
	80.54±2.00	100	

Each experiment was done in triplicate and the results were expressed as Mean ±SD

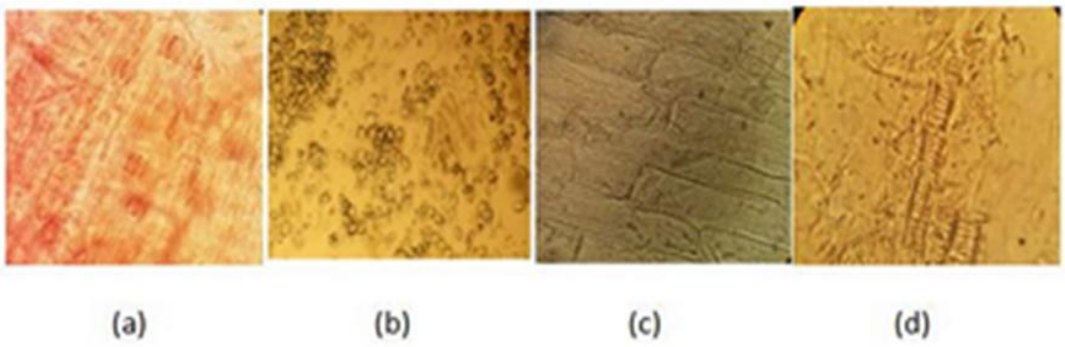

Fig. 3. A microscopic observation of bulb section of *N. tazetta* showing (40X). a) stomata in bud part; b) granularity starch; c) epidermis cells; d) vena in scale

Table 3. The mean of ash values and moisture content of fresh and dried *N. tazetta* bulb

Type of plant	Moisture content (%)	Total Ash (%)	Water-soluble ash (%)	Water-insoluble ash (%)	Acid-insoluble ash (%)
Fresh bulb	74.13±1.16	-	-	-	-
Dried bulb	9.90±0.20	4.90±0.20	2.46±0.04	2.41±0.20	0.10±0.00

The result of ash value determination of *N. tazetta* was shown in (Table 3) Total ash of the plant was estimated to be 4.9±0.2 w/w% of which 2.46/4.9 ×100 was water soluble and 0.1/4.9 ×100 was acid insoluble ash. Results are the mean of three independent experiments.

3.8 Proliferation and Scratch Assay

NR assay was carried out to determine the proliferation effect of various concentrations of PEN on HDFs. 48 hr after cells treated, PEN hasn't shown any significant effect (P>0.05) on the proliferation of fibroblast cells compared to untreated cells (Fig. 4) and IC_{50}= 35.19. The effect of various concentrations of PEN was studied on the migration of HDFs.

The results of fibroblast migration show that PEN increases cell migrations at low concentrations (1.562 and 3.125 µg/ml) while there was no migration at higher concentrations (Fig. 5A). As shown in (Fig. 5B), gap width decreased significantly at 1.562 and 3.125 µg/ml in compared to untreated cells (***P<0.01) (respectively 33.08% and 32.85%) after 24 hr. Furthermore, PEN caused significant decrease in gap width at 6.25 µg/ml (***P<0.01) and 10 and 12.5 µg/ml (*P<0.05) (38.08%, 26.65% and 23.69% reduction respectively) after 48 hr in comparison to untreated cells.

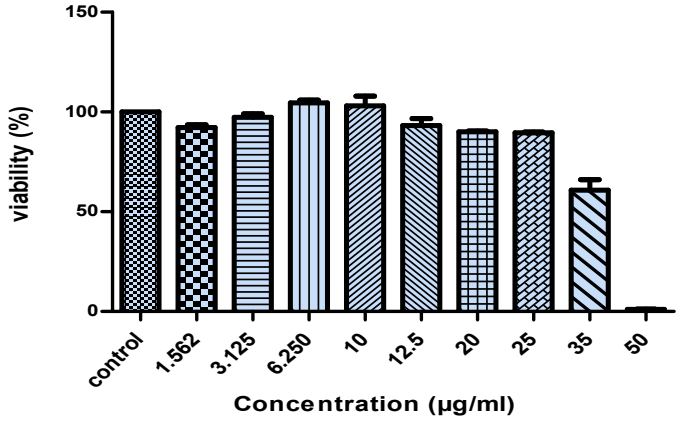

Fig. 4. Proliferating effect of different concentrations of a percolated extract of *N. tazetta* (PEN) on human dermal fibroblasts (HDFs) using neutral red assay. The value from the baseline control group was set at 100%. The results were expressed as mean ± S.D

4. DISCUSSION

In TPM, different parts of *Narcissus* are recommended for treatment of a variety of disorders and diseases such as wound healing [4,5,8]. In traditional medicine of Turkey, China, and Jordan, the plant bulb is used to treat wounds and inflammation [26,27], but the wound-healing properties of *Narcissus* bulb have not been investigated so far. Given the application of "Zaroor" form of *Narcissus* bulb in TPM, various extracts of *Narcissus* bulb were prepared in the present study through percolation, sonication, maceration and 1, 2, and 4 hr warm extraction. Next, their percentages were compared in order to investigate the physicochemical properties of *Narcissus*. The amounts of the extract obtained in sonication and percolation methods were significantly higher than the other methods ($P<0.05$) (Fig. 1). Regarding the phytochemical studies of *Narcissus* bulb, the presence of alkaloids, flavonoids, saponins, and tannins was confirmed, which is in accordance with other studies in this field [28-31]. Various factors interfere with the wound healing process, for example, oxidants are an inhibitor of wound healing, and inflammation and infection can delay wound healing, so antioxidant, anti-inflammatory and antibacterial agents can play an important role in wound healing [32]. We have reported antioxidant effects of some medicinal plants which was related to the other pharmacological and or biological activities of the plants [21,33,34]. On the other hand, flavonoids are among that potent antioxidant [35], which has the capability to inhibit free radicals and chelates the metals. Flavonoids, mainly due to their astringent and antibacterial properties, which seems to be responsible for wound contraction and increasing the rate of epithelialization and contribute to the process of wound healing [36]. Tannins, in addition to the antioxidant properties and free radical scavenging effect, by increasing wound contraction and formation of capillaries and fibroblasts lead to wound healing [37]. Researches have shown that saponins accelerate the biological activity including hemolytic, antibacterial, antiviral antioxidant and surface activity [38]. In addition, saponins have anti-inflammatory activity, which can decrease edema and inflammation of the skin [39]. Many studies have proved antioxidant [40] and antimicrobial effects of alkaloids too [41]. The presence of these compounds in bulbs of *Narcissus Tazetta* can be responsible for wound healing and biological activity of the plant, which has reported in Traditional Persian Medicine.

For standardization, the TFC of different extracts of the plant was determined, the highest of which was observed in the percolated extract (27.04±0.93 mg/g). Although this test has not been reported so far for *Narcissus* species bulb, according to the study of Li et al., the amount of TFC varies in flowers of 15 different species of *Narcissus* (3.59-15.37 mg/g) [42]. Various studies have also been carried out on the bulb of the plants of Alliaceae family. In the bulb of the old and fresh garlic (*A. sativum*), the TFC was 5.78±0.09 mg/g and 4.16±0.03 mg/g, respectively [43]. In another study on *A. hookeri*, this amount in fresh bulb extract was reported to be between 5.0±0.6 mg/g and 16.7±5.2 mg/g

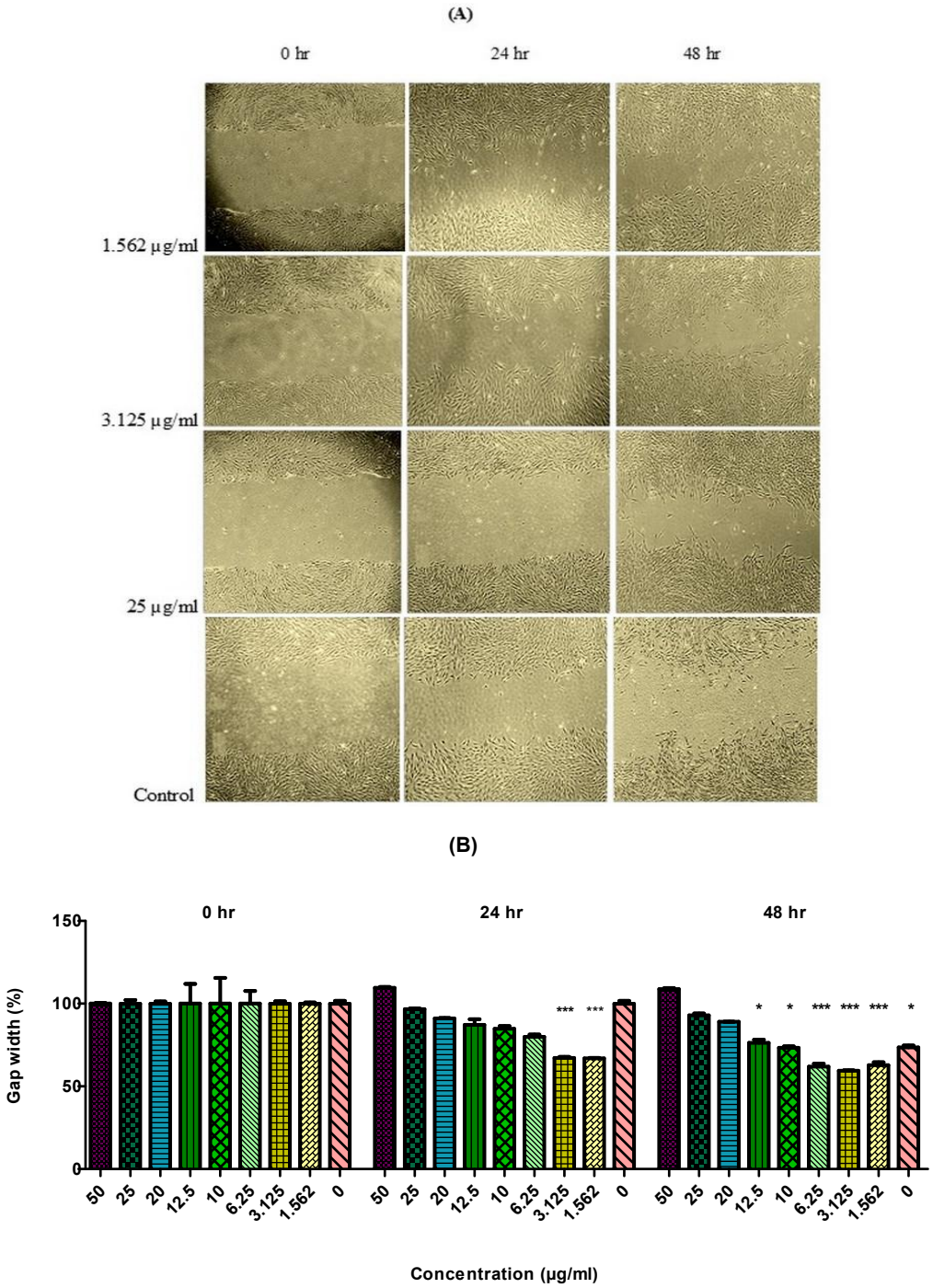

Fig. 5. (A) Wound healing effect of different concentrations of PEN using scratch assay on human dermal fibroblasts (HDFs). (B) Gap widths of scratch in comparison of untreated cells were analyzed with ImageJ software

*Key: 0: untreated cells; ***: P<0.01; *: P<0.05*

[44]. In a study by Duan et al. on another species of onion (*A. cepa*), the amount of TFC was 43.33 ± 0.41 in 70% methanolic extract, 49.63 ± 0.55% in 70% ethanolic extract, and 27.57±0.11

in chloroform and methanol extract (2:1 v/v) [45]. In a study on anti-oxidant properties and minerals content of A. paradox, the TFC in the plant bulb in different forest areas of northern Iran was reported 1.3±0.0-7.7±1.2 mg/g by Khodadadi et al. [46]. As a result, it can be claimed that *Narcissus* bulb has a fairly large flavonoid content.

HPTLC is an inexpensive way to isolate, quantify, and semi-quantitative analyze of samples [47] and is referred as the fingerprint of plant compounds. HPTLC is used to control the quality, purity, stability, identification and validate the plant phytoconstituents [48]. Since rutin is a major flavonoid of Amaryllidaceae family [49], HPTLC was used as a reference. The Rf index in methanolic extract of *Narcissus* bulb was calculated to be 0.45 using ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26 v/v/v/v) as a mobile phase, which was approved in comparison to the rutin standard.

In the DPPH test, the radical inhibitory power of PEN (4000 µg/mL) was approximately equivalent to the effect of 100 µg/mL BHT, and in the FRAP test, the anti-oxidative activity of PEN was 0.29±0.02 mM/mg. In similar studies, DPPH scavenging effect of ethanolic extract of *N. broussonetii* bulb (10 and 20 mg/mL) was 82.5% and 79.3%, respectively [50]. According to Meriga et al., the anti-oxidant effect of 500 µg/mL methanolic extract of *A. sativum* bulb was determined as 80% [51].

The primary human fibroblasts were used to investigate the in vitro wound-healing effect of *Narcissus* bulb extract. The results of neutral red assay showed that by increasing the concentration, cellular growth increases slightly and a significant reduction was observed at > 20 µg/mL (IC_{50}=35.19) between the treated and untreated (control) cells. Regarding the healing effect of the extract (1.562 - 50 µg/mL concentrations) using the scratch test, a significant decrease was observed in the wound width at 1.562 µg/mL and 3.125 µg/mL compared to the controls (untreated cells) after 24 hr ($P<0.01$). The effectiveness of the extract decreased at higher concentrations. After 48 hr, a significant decrease was observed in the wound width at 1.562, 3.125, 6.25 µg/mL ($P<0.01$), 10 and 12.5 µg/mL ($P<0.05$), while the effectiveness decreased at higher concentrations. In the control cells, the wound width did not change after 24 hr but significantly decreased after 48 hr ($P<0.05$).

Kern et al. indicated that cosmetic cream 1% and 3% comprising processed *N. tazetta* bulb extract reduced the cell proliferation and inhibited cell growth of human fibroblasts and keratinocytes primary cultures [52]. As a study by Ding et al. hexane and ethylacetate fractions of Calendula officinalis flowers exhibited no effect on cell proliferation on human fibroblasts, but the hydro-alcoholic extract and its aqueous fraction significantly decreased the gap width in the migration test at 100 µg/ml. Calendula is a medicinal herb highly used to treat and heal skin lesions, ulcers, burns, and duodenal ulcers [25]. The result of a study on Chamomile showed that this plant has no effect on fibroblasts proliferation [53]. It is worth mentioning that Chamomile was also used from far past as an anti-inflammatory and antiseptic drug and is effective in the treatment of gastrointestinal ulcers, oral ulcers, hemorrhoids, and acnes [54]. *Narcissus* bulb has also relatively good antiviral [10], antibacterial, anti-inflammatory [31], and anti-oxidant effects [52]. It seems that these effects might be responsible for their impact on the ulcer. Plants are effective in wound healing, especially through helping to disinfect, remove of damaged tissue, and create a damp environment to help improve the recovery process. Overall, these properties could be attributed to different plant constituents [55].

5. CONCLUSION

The present study showed that Narcissus bulb could be effective in wound healing. Wound healing occurs through various mechanisms such as anti-inflammatory, antioxidant, angiogenesis, and anti-bacterial effects, which also control the repair process of the plant. In this regard, a higher number of animal tests are needed to find the main mechanism and also ensure the safety of *Narcissus* bulb. In this regard, clinical studies are also recommended to evaluate its efficacy in wound healing.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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