Development of *Lepidium sativum* Extracts/PVA Electrospun Nanofibers as Wound Healing Dressing

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**ABSTRACT:** *Lepidium sativum* L. (Garden cress/Hab El Rashad) (Ls), family Brassicaceae, has considerable importance in traditional medicine worldwide because of its antioxidant and anti-inflammatory activities. Ls fruits were used in Ayurvedic medicines as a useful drug for injuries, skin, and eye diseases. The aim of this study was to examine the effectiveness of the total ethanol extract (TEE) and polysaccharide (Poly) of Ls seeds loaded on poly(vinyl alcohol) (PVA) nanofibers (NFs) as a wound healing dressing and to correlate the activity with the constituents of each. TEE and Poly were phytochemically analyzed qualitatively and quantitatively. Qualitative analysis proved the presence of phenolic acids, flavonoids, tannins, sterols, triterpenes, and mucilage. Meanwhile, quantitative determinations were carried out spectrophotometrically for total phenolic and total flavonoid contents. High-performance liquid chromatography (HPLC) for TEE identified 15 phenolic acids and flavonoid compounds, with gallic acid and catechin as the majors. Separation, purification, and identification of the major compounds were achieved through a Puriflash system, column Sephadex LH20, and spectroscopic data (1H, 13C NMR, and UV). Eight compounds (gallic acid, catechin, rutin, kaempferol-3-O-rutinoside, quercetin-3-O-rhamnose, kaempferol-3-O-rhamnoside, quercetin, and kaempferol) were obtained. Gas–liquid chromatography (GLC) analysis for Poly identified 11 compounds, with galactose being the main. The antioxidant activity for both extracts was measured by three different methods based on different mechanisms: 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP), and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS). TEE has the highest effectiveness as an antioxidant agent with IC$_{50}$ 82.6 ± 8.35 μg/mL for DPPH and 772.47 and 758.92 μM Trolox equivalent/mg extract for FRAP and ABTS, respectively. The PVA nanofibers (NFs) for each sample were fabricated by electrospinning. The fabricated NFs were characterized by SEM and Fourier transform infrared spectroscopy (FTIR); the results revealed successful encapsulation of TEE and Poly in the prepared NFs. Moreover, the swelling index of TEE in the prepared NFs shows that it is the most appropriate for use as a wound dressing. Cytotoxicity studies indicated a high cell viability with IC$_{50}$ 216 μg/mL and 1750 μg/mL for TEE and Poly, respectively. Moreover, the results revealed that nano-fibers possess higher cell viability compared to solutions with the same sample quantities: 9-folds for TEE and 4-folds for Poly of amount 400 μg. The in vitro wound healing test showed that the TEE nanofibers performed better than Poly nanofibers in accelerating wound healing, with 90% for TEE, more than that for the Poly extract (82%), after 48 h. These findings implied that the incorporation of TEE in PVA nanofibers was more efficient than incorporation of Poly in improving the biological activity in wound healing. In conclusion, the TEE and polysaccharides of *L. sativum* L seed are ideal candidates for nanofibrous wound dressings. Furthermore, the contents of phenolic acids and flavonoids in TEE, which have potential antioxidant activity, make the TEE of *L. sativum* more favorable for wound healing dressing.

**INTRODUCTION**

The skin is the largest organ in the body and serves as one of the body’s first lines of defense against pathogens.$^1$ However, the skin can be injured by chemical and physical factors, and certain diseases (including diabetes).$^2$ Wound healing is a complex process, and it aims to restore the normal anatomic structure and function of the skin. Although the skin can regenerate spontaneously, the healing process is slow for some wounds.$^3$ To address this problem, many researchers have recently focused on developing wound dressings by combining medicinal plant extracts with natural polymer-based electrospun nanofibers (NFs).$^4$ Such dressings can be made by electrospinning a plant extract into polymeric nanofibers.$^5$ Poly(vinyl alcohol) (PVA) is one of the most popular synthetic polymers that is used because of its electrospinning ability and formation of excellent nanofibers (NFs).$^6$ PVA is very

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beneficial for biomedical applications, in particular, wound dressing and tissue engineering, owing to its biocompatibility, biodegradability, and nontoxicity. The electrospun nanofibers have unique characteristics, which include a large surface area to volume, high air permeability, and high absorption of secretions from the wound, as well as the possibility of releasing gradually the drug agents loaded on nanofibers. All of these make nanofibers good candidates that mimic the morphology of the extracellular matrix of the damaged tissue. This study is based on Lepidium sativum (Ls) seeds, also commonly known as garden cress; they are also called “Hab rchad” in Egypt. They belong to the Brassicaceae family. The seeds contain 35–54% carbohydrates, 27% protein, 14–26% fat, and 8% crude fiber. The carbohydrates of Ls seeds include 90% non-starch polysaccharides (Poly) and 10% starch. Phytochemical study of the plant extract reveals the presence of secondary metabolites such as flavonoids, tannins, glycosides, polyphenols, lectin, and mucilage. The Ls seed was reported as a rich source of minerals such as potassium, zinc, phosphorus, and calcium, so it was considered to be an important nutraceutical seed for nutrient enrichment. It also contains a sufficient amount of vitamins, mainly thiamine, riboflavin, and niacin, which work as cofactors and help in body metabolism. The Ls seeds extract also contains natural antioxidants such as tocophersols, which represent major phenolic compounds in Morocco. Valuable folk medicine uses were reported for Ls as therapy for inflammatory diseases including arthritis, hepatitis, and diabetes mellitus. One of the traditional uses of L. sativum in Saudi Arabia and other Arabic parts was for accelerating bone fracture healing and as an alternative to prescribed supplements. Nanofibers containing the extract can help in the management of patients with wound healing problems by the production of the wound dressing. So, the present work aims to examine the effectiveness of electrospun PVA/Ls seed total ethanol extract (TEE) and Poly as a wound healing dressing. The spinning conditions of NFs were optimized in detail, and both extracts and NFs were subjected to a panel of bio-evaluation assays, including antioxidant, cytotoxicity, and wound scratching. The constituents of each extract were also examined.

## MATERIALS AND METHODS

**Materials and Reagents.** Plant Material. Seeds of L. sativum L. were purchased from a local store, Sinai, Egypt. They were identified and authenticated by Dr. Therese Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and Ex-director of the Orman Botanical Garden, Giza, Egypt. Seeds were shade-dried and ground to a fine state. Two samples, namely total ethanol extract (70%) (TEE) and polysaccharide (Poly), were prepared from the powdered seeds.

Materials for Nanofiber. Poly(vinyl alcohol) (PVA, M<sub>W</sub> = 72,000 g/mol; 86% hydrolyzed) was obtained from Lobache- 

Preparation of Polysaccharides from L. sativum Seeds. The precipitated polysaccharides (Poly) were submitted to a gel formation test to detect their nature. The precipitate formation or the color intensity was used as the analytical response to these tests.

**Quantitative Estimation of the Total Phenolic and Flavonoid Contents of TEE (70%).** All assays were performed spectrophotometrically using the microplate reader FluoStar Omega relating to pre-established standard calibration curves. Total phenolic content was determined using the Folin-Ciocalteau method, and the flavonoid content was determined by measuring the intensity of the color developed when flavonoids were complexed with aluminum chloride reagent. The results were expressed as gallic acid (GAE) and rutin (RE) equivalents, respectively, as described by Attard.

**High-Performance Liquid Chromatography (HPLC) Analysis of TEE of the L. sativum Seed.** High-performance liquid chromatography (HPLC) analysis of TEE was carried out using an Agilent 1260 series (Agilent Technologies, Wald- 

Separation and Identification of Compounds of TEE. The TEE of L. sativum seeds (3 g) was subjected to preparative...
separation by a PuriFlash 4100 system, Interchim Software 5.0 (Interchim; Montluçon, France), with a PDA-UV-Vis detector at 190–840 nm. The separation was carried out using a C18-HP column (30 μm). The mobile phase consisting of 1% formic acid (A) and acetonitrile (B) was programmed in a gradient elution. The process led to 130 fractions, which were inspected by paper chromatography 1MM (PC 1MM) and using butanol/acetic acid/water (BAW) 4:1:5 and acetic acid (HOAc) 15% as the running system. The similar fractions were combined to obtain eight substantial fractions (sub-fractions A−H). These fractions were subjected to different chromatographic techniques, including 3MM preparative paper chromatography and repeated Sephadex LH-20 column using eluents of different polarities, which led to the isolation and purification of eight compounds. The isolated compounds were structurally elucidated through different investigations: physical, chemical, chromatographic, and spectral data (UV, nuclear magnetic resonance (NMR), and mass spectroscopy (MS)).24–26

Gas−Liquid Chromatography (GLC) Analysis for the Polysaccharide of L. sativum. Preparation of the Sample.

The polysaccharide powder was subjected to acid hydrolysis according to the reported method by Chrums and Stephen.27 Briefly, the powder (100 mg) was heated in sulfuric acid in a sealed tube (2 mL, 0.5 M, 20 h) in a boiling water bath. At the end of hydrolysis, a precipitate was noticed. This was filtered off and the filtrate was freed of sulfate ion (SO₄²⁻) by precipitation with barium carbonate.

Part of the hydrolyzate polysaccharide was silylated according to the reported method.28 Briefly, the hydrolyzate solution (0.5 mL) was evaporated in small screw-topped septum vials to dryness under a stream of nitrogen at 40 °C. When almost dry, isopropanol (0.5 mL) was added and the drying was completed under the stream of nitrogen until a dry solid residue was obtained. Hydroxylamine hydrochloride in pyridine (0.5 mL, 2.5%) was added, mixed, heated (30 min, 80 °C), and allowed to cool. The silylating reagent (trimethylchlorosilane: N,O-bis-(trimethylsilyl) acetamide, 1:5 by volume) (1 mL) was added, mixed, and heated (30 min, 80 °C).

GLC Analysis. Silylated polysaccharide hydrolyzate (1 μL) was analyzed using the GLC apparatus (HP 6890) under the following conditions: column: ZB-1701, 30 m × 0.25 m × 0.25 μm; stationary phase: 14% cyanopropyl phenyl methyl polysiloxane; carrier gas: helium (with flow rate: 1.2 mL/min, pressure: 10.6 psi, and velocity: 41 cm/s); injector chamber temperature: 250 °C; back inlet with split ratio: 1:10, split flow: 11.9 mL/min, total flow: 18.7 mL/min, gas saver flow: 120 mL/min, and average time: 20 min; oven with 150 °C as initial temp., 2 min as initial time, 7 °C/min rate, and 200 °C as final temp., 20 min as the final time; and an FID detector (temp.: 270 °C, air flow: 450 mL/min, and H₂ flow: 40 mL/min).

Antioxidant Activity. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay. Evaluation of the radical scavenging activity of TEE and Poly of L. sativum was carried out by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method of Boly et al.29 Briefly, a freshly prepared DPPH reagent (0.1% in methanol, 100 μL) was added to each sample (100 μL) in a 96-well plate (n = 3), and the reaction was incubated at room temperature for 30 min in the dark. At the end of the incubation time, the resulting reduction in DPPH color intensity was measured at 540 nm.30 Data are represented as means ± standard deviation (SD) according to the following equation

\[
\text{percentage of inhibition} (\%) = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where \(A_0\) is the absorbance of the blank and \(A_1\) is the absorbance of the extract.

The IC₅₀ value is defined as the concentration of the extract or standard that allows a 50% reduction of DPPH. Lower IC₅₀ values indicate greater effectiveness of the antioxidant power of the extract. The samples were analyzed in triplicate.

Reducing Power (FRAP Assay, Ferric Reducing Antioxidant Power). The ferric reducing ability assay was carried out for the TEE and Poly of L. sativum according to the method of Benzie et al.,31 with minor modifications carried out in microplates. It is based on the rapid reduction of ferric-triprydiltriazine (Fe³⁺-TPTZ) by the antioxidants present in the samples forming ferrous-tripyridyltriazine (Fe²⁺-TPTZ). Briefly, the TPTZ reagent (300 mM acetate buffer (pH 3.6), 10 mM TPTZ, in 40 mM HCl, and 20 mM FeCl₃₀ in the ratio of 10:1:1 v/v/v, respectively) was freshly prepared. A volume of 190 μL of the TPTZ reagent was mixed with 10 μL of the sample in a 96-well plate (n = 3); the reaction was incubated (30 min) at room temperature in the dark. The resulting blue color at the end of the incubation time was measured at 593 nm using the microplate reader FluorStar Omega.30 The data were represented as means ± SD. The increase in absorbance of the reaction medium indicates the increase in iron reduction. The ferric reducing ability of the samples was presented as μM TE/mg sample (Trolox equivalent per milligram sample) using the linear regression equation extracted from the Trolox calibration curve (linear dose−response curve of Trolox).

3-Ethylbenzothiazoline-6-sulfonic Acid (ABTS) Assay. The assay was carried out according to the method of Armao et al.,32 with minor modifications carried out in microplates. The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay measures the relative ability of antioxidants to scavenge the ABTS generated in the aqueous phase, as compared with the Trolox (water-soluble vitamin E analogue) standard. Briefly, ABTS (192 mg) was dissolved in distilled water and transferred to a volumetric flask (50 mL); then the volume was completed with distilled water. One milliliter of the prepared solution was added to 140 mM potassium persulphate (17 μL) and the mixture was kept for 24 h in the dark. After that, 1 mL of the reaction mixture was diluted to 50 mL with methanol to obtain the final ABTS dilution used in the assay. A volume of 190 μL of the freshly prepared ABTS reagent was mixed with the sample (10 μL) in a 96-well plate (n = 6); the reaction was incubated for 120 min at room temperature in the dark. The decrease in ABTS color intensity at the end of the incubation time was measured at 734 nm.30 The data are stated as means ± SD according to the following equation:

\[
\text{percentage inhibition} = \left( \frac{\text{avg blank absorbance} - \text{avg test absorbance}}{\text{avg blank absorbance}} \right) \times 100
\]

where ‘avg’ is average. The results of ABTS⁺ radical assays were presented as μM TE/mg sample [Trolox equivalent
antioxidant capacity (TEAC)) using Trolox as a standard reference.

**Statistical Analysis.** Microsoft Excel was used as the software for data analyses, whereas IC\textsubscript{50} values were calculated using Graph pad Prism version 5.**33**

**Preparation of Nanofibers. Optimization of PVA/TEE and PVA/Poly Nanofibers.** The TEE and Poly of \( L. \text{ sativum} \) (0.3, 0.6, and 1%, w/v) were dispersed separately in PVA solution (10%, w/v) with continuous stirring for 6 and 18 h, respectively, at 50 °C in a closed vial to enhance the homogeneity of the mixture solution. Citric acid (CA) (1.5%, w/v) was added to the solution, which was electrospun into the NFs by an electrospriner (NANO-N-01A, MECC, Japan). The produced NFs were thermally treated at 80 °C for 18 h, then at 100 °C for 6 h.

**Instrumental Characterization of PVA/TEE and PVA/Poly Nanofibers.** SEM: the successful formation of the uniform and appropriate surface morphology of NFs was investigated by SEM (FS SEM, Quattro S, Thermo Scientific). FTIR: the nature of binding among the nanofibrous scaffold compositions was revealed by FTIR (Bruker Vertex 70, Germany), at wavenumbers ranging between 400 and 4000 cm\(^{-1}\). ζ- Measurements: the surface charge of the prepared nanofibrous scaffolds was measured by a nano-zetasizer apparatus (Malvern Instruments, U.K.).

**Physicochemical Characterization of PVA/TEE and PVA/ Poly Nanofibers. Roughness.** The swelling ratios of both types of nanofibers were studied in distilled water at 37 °C and the swelling % of NFs was calculated by the equation

\[
\text{swelling (%) } = \frac{W_s - W_c}{W_o} \times 100
\]

where \( W_s \) is the weight of the swollen nanofiber and \( W_c \) is the weight of nanofibers after immersion.

**In Vitro Hydrolytic Degradation.** The weight loss pattern of the prepared nanofiber samples was evaluated by investigating the in vitro hydrolytic degradation in PBS solution. Dried nanofiber samples were weighed and immersed in 10 mL of phosphate-buffered saline solution (PBS) (pH 7.4, 37 °C). At specific time intervals, samples were removed, wiped, and gently dried at ambient temperature, then reweighed.

\[
\text{weight loss (%) } = \frac{(W_0 - W_f)/W_0} \times 100
\]

where \( W_0 \) is the original weight of the nanofiber sample, and \( W_f \) is the weight of nanofibers after a specific incubation time.

**In Vitro Bio-Evaluation Tests.** Cell Culture. The adherent Vero cells (normal African green monkey kidney epithelial cells) originated from ATCC CCL-81 were grown and maintained in Dulbecco’s modified Eagle’s medium (DMEM). High Glucose (4500 mg/L \( \beta \)-Glucose) was enriched with 200 units/mL penicillin, 200 \( \mu \)g/mL streptomycin, fetal bovine serum (FBS) (10%), L-glutamine (2 mM final concentration), and sodium pyruvate (1 mM final concentration). The cells were maintained in monolayer culture in a 5% \( \text{CO}_2\)-humidified incubator at 37 °C. Cells were subcultured twice a week.

**In Vitro Cell Viability.** TEE and Poly were tested for cytotoxicity using Vero cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylenetetrazolium bromide (MTT) assay.**36** Firstly, Vero cells were seeded in 96-well tissue culture plates at a density of \( 1 \times 10^4 \) cells/well and incubated under 5% \( \text{CO}_2 \) at 37 °C. After 24 h, the media was replaced with fresh media containing serial 2-fold dilutions of TEE and Poly starting from 200 to 0.39 \( \mu \)g/mL. After 48 h of incubation, the cells were treated with MTT dye for 4 h and the formazan crystals were solubilized in dimethyl sulfoxide. The absorbance was read at 570 nm using a multimode microplate reader (CLARIOStar Plus, BMG LABTECH, Germany). The cytotoxicity of the prepared nanofibers (PVA, PVA-TEE and PVA-Poly) was determined with the same assay. The nanofibers were cut into circular shapes and sterilized under UV for 2 h. The relative cell viability was determined by the formula

\[
\text{relative cell viability (%) } = \frac{\text{the mean optical density of sample}}{\text{the mean optical density of control}} \times 100
\]

**In Vitro Scratch Wound Assay.** The wound-healing effect of the prepared nanofibers was tested in vitro based on the previously described method.**37** Cells were seeded into a 12-well tissue culture plate at a density of \( 2 \times 10^5 \) cells/well. After the cells formed a confluent monolayer (70–80%), a scratch was applied using a 200 \( \mu \)L pipette tip on the cell sheet to form a cell-free area. After scratching, the wells were gently washed with PBS twice to remove any cell debris. The prepared NF scaffolds were then immersed directly in the wells and the rate of wound closure was monitored after 0, 24, and 48 h by an inverted fluorescence microscope (Axio observer S, Carl Zeiss, Germany). The closure rate was determined by measuring the wound gap area according to the formula

\[
\text{wound closure (%) } = \frac{\text{wound area } x \text{ h}}{\text{wound area } 0 \text{ h}} \times 100
\]

**RESULTS AND DISCUSSION**

**Phytochemical Analysis.** The healthy properties of such edible seeds are due to the presence of a variety of phytoconstituents such as polysaccharides, flavonoids, glycosides, phenolics, saponins, tannins etc.**38** The preliminary screening tests were useful in the detection of these bioactive constituents and in subsequently facilitating their quantitative estimation.

The results of phytochemical screening listed in Table 1 revealed the presence of a wide array of chemicals, including carbohydrates, steroids, flavonoids, phenolics, tannins, proteins/amino acids, and alkaloids in the TEE. These results are

| phytochemical constituents       | results |
|----------------------------------|---------|
| carbohydrates (reducing sugars)  | +++     |
| steroids and/or terpenoids       | +       |
| flavonoids                       | +       |
| phenolics                        | ++      |
| tannins                          | ±       |
| cardiac glycosides               | −       |
| proteins/amino acids             | +       |
| alkaloids                        | ±       |
| anthraquinones                   | −       |
| saponins                         | −       |
| +++ abundant                     | ± traces|
| ± absent                         |         |

Table 1. Preliminary Phytochemical Screening Tests in the Total Ethanol Extract (70%) (TEE) of \( L. \text{ sativum} \) Seeds
Yield, Total Phenolic, and Total Flavonoid Contents of the TEE of L. sativum Seeds. The yield of TEE and poly was 20/100 g seeds and 30/100 g seeds, respectively. The amounts of total phenols and total flavonoids were measured in the TEE of Ls seeds. The measurements were done using the linear regression equation of the calibration curve, using gallic acid and rutin as standards. The total phenolic and total flavonoid contents were 54.83 mg GAE/g extract and 10.01 mg RE/g extract, respectively. These results were compatible with those reported by Chatou et al.41 Phenolics have a broad spectrum of biological activities, including radical scavenging, antiallergic, and antimicrobial.42

HPLC Analysis of TEE. The HPLC analysis of TEE revealed the identification of fifteen compounds: eleven phenolic acids and four flavonoids (Table 2). The main compounds detected were gallic acid (14.8227%) and caffeic acid (10.4147%) as phenolic acids and catechin (10.1689%) and rutin (2.4268%) as flavonoid compounds. The obtained results were similar to those reported by Abd El-Salam et al. and Panwar et al. as gallic acid and catechin were the main phenolic acid and flavonoid, respectively.53,54

| no | R₁ | compounds | area % | concn (μg/g) |
|----|----|-----------|-------|--------------|
| 1  | 3.133 | gallic acid | 14.8227 | 11712.47     |
| 2  | 3.807 | chlorogenic acid | 0.2030 | 138.89       |
| 3  | 4.108 | catechin | 10.1689 | 12672.16     |
| 4  | 4.566 | protocatechuic acid | 6.2717 | 682.15       |
| 5  | 5.111 | methyl gallate | 0.1614 | 21.00        |
| 6  | 5.350 | caffeic acid | 10.4147 | 12672.16     |
| 7  | 6.524 | ellagic acid | 2.7754 | 2889.52      |
| 8  | 6.930 | syringic acid | 0.7206 | 73.63        |
| 9  | 7.264 | rutin | 2.4268 | 2889.52      |
| 10 | 8.281 | coumaric acid | 0.1106 | 9.29         |
| 11 | 9.358 | vanillic acid | 2.7620 | 393.50       |
| 12 | 9.467 | ferulic acid | 2.8781 | 368.53       |
| 13 | 9.927 | naringenin | 0.0394 | 20.06        |
| 14 | 12.229 | taxifolin | 1.2344 | 752.37       |
| 15 | 13.423 | cinnamic acid | 0.1451 | 13.28        |

Structural Elucidation of the Isolated Compounds from TEE. Chromatographic investigation of the TEE of L. sativum seeds led to the isolation and identification of eight compounds: gallic acid (1), catechin (2), quercetin-3-O-α-rhamnopyranosyl (1‴ → 6‴)-β-glucoside (rutin) (3), kaempferol-3-O-rutinoside (4), quercetin-3-O-rhamnoside (5), kaempferol-3-O-rhamnose (6), quercetin (7), and kaempferol (8); these compounds were reported previously in the plant.55,56

The identification of each compound was done according to their R₁ values, color reactions, acid hydrolysis, UV spectrophotometry using chemical shifts (aluminum chloride (AlCl₃), hydrochloric acid (HCl), sodium methoxide solution (NaOMe), sodium acetate (NaOAc), and boric acid (H₃BO₃)), and electron ionization mass spectrometry (EI/MS).1H and C13 NMR and Co-PC were done with the reference samples; then, comparison of their spectroscopic data was done with previously reported values.54,56

Gallic Acid. Off-white amorphous powder, melting point (mp) 254–256 °C. It appears as a blue light fluorescence spot under UV on PC, which turned to dark blue when sprayed with FeCl₃ solution; EI/MS showed a molecular ion peak [M − H]₁⁺ at m/z 169; the UV at λ max nm (MeOH) (270) confirmed a phenolic acid skeleton.26 The 1H NMR spectrum (Acetone-d₆, 400 MHz) revealed the presence of two equivalent aromatic protons (H₂–H₆) at δ 7 ppm.

Catechin. Off-white amorphous powder, soluble in methanol, dark in color under UV light (λ 254 nm), and converted to faint yellow after exposure to ammonia vapor. It turned to pink to purple color on using a vanillin sulfuric acid spray reagent. The UV spectrum data exhibited one UV maxima at 280 nm in the MeOH spectrum. The 1H NMR spectrum showed signals of aromatic proton at different chemical shifts δ (ppm). Three protons of ring B, H₂*, appeared at δ 6.89 (J (H₂-H₆): = 2 Hz), as a doublet, due to meta-coupling with H6. H6* appeared at δ 6.77, as a doublet of doublet due to meta-coupling with H2' (J(H₂', H₆): H2) = 2 Hz) and ortho-coupling with H5' (J(H₅', H₆): 8.05 Hz), which appeared at δ 6.81 as a doublet (J(H₅', H₆): = 8.05 Hz). The two protons related to ring A, H-6 and H-8, appeared at δ 5.91 and 5.99, respectively, that they are meta-coupled. The protons at δ 4.56 appeared as a doublet with J(H-2, H-3a): 7.8 Hz for H-2, while at δ 4.00, as a multiplet for H-3; peaks appeared at δ 2.53 and 2.89 each for one proton, (H-4a) and (H-4e), and appeared as a doublet of doublet with J(H-4a, H-3a): 8.50 Hz, J(H-4a, H-4e):16.10 Hz, J (H-4e, H-3a): 5.5 Hz, and J (H-4e, H-4a): 16.1. The 13C NMR spectrum showed peaks at δ 28.5 for (C-4), 68 for (C-3), 82.1 for (C-2), 94.5 for (C-6), 95.8 for (C-8), 101 for (C-2'), 115.3 for (C-3'), and 116.1 for (C-6') and other aromatic peaks at δ 131.4, 144.9, 145.7, 156.8, 157.9, and 158.1. The UV spectrum agreed with that published for catechin (Flavan-3-OH).37 1H NMR and 13C NMR spectral data were similar with those published for Catechin.

Quercetin-3-O-α-rhamnosyl (1‴ → 6‴)-β-glucoside (Rutin). Yellowish green powder, soluble in methanol, deep purple spot on PC under UV light, turned to fluorescent yellow when fumed with NH₃ or spraying with AlCl₃ solution; EI/MS showed a molecular ion peak [M + H]⁺ at m/z 301, 358, and 420; AlCl₃: 275, 305 sh, 420; AlCl₃/HCl: 268, 301 sh, 358, 400; NaOAc: 271, 324 sh, 380; NaOAc/H₂O: 262, 379. 1H NMR (500 MHz, DMSO-d₆): δ ppm 7.55 (2H, m H-2’/6’), 6.85 (1H, d, J = 9 Hz, H-5’), 6.40 (1H, d, J = 1.50 Hz, H-8), 6.20 (1H, d, J = 1.50 Hz, H-6), 5.35 (1H, d, J = 7.04 Hz, H-1’), 4.39 (1H, s, H-1’), 3.90–3.20 (m, remaining sugar protons), 0.99 (3H, d, J = 6.2 Hz, H-6’).

Kaempferol-3-O-Rutinoside. Yellow powder, 1H NMR (MeOD, 400 MHz): aromatic protons at δ ppm 8.04 (2H, d, J = 8.6, H-2’,6’), 6.88 (2H, d, J = 8.6, H-3’,5’); 6.21 (1H, d, J = 2.0, H-6), 6.41 (1H, d, J = 2.0, H-8), 5.14 (1H, d, J = 7.4, β sugar), 4.51 (1H, s, rhamnose, H-1), 0.88 (3H, d, J = 6.2 Hz, rhamnosyl CH₃), 3.25–3.82 (sugar protons).13C NMR (MeOD, 100 MHz): δ 159.56 (C-2), 135.66 (C-3), 179.55 (C-4), 163.15 (C-5), 100.12 (C-6), 166.15 (C-7), 95.05 (C-8), 158.70 (C-9), 108.84 (C-10), 121.00 (C-1’, 132.50 (C-2’), 116.30 (C-3’), 161.61 (C-4’), 116.30 (C-5’), 132.50 (C-6’), 104.76 (C-1’), 74.06 (C-2’), 78.31 (H-3’), 71.61 (H-4’), 78.04 (H-5’), 68.73 (H-6’), 102.56 (H-1’), 72.24 (H-2’), 72.49 (H-3’), 73.61 (H-4’), 69.88 (H-5’), 18.03 (H-6’), 18.03 (CH₃).
Quercetin-3-O-Rhamnoside. Yellow powder, purple spot on PC under UV light, turned to fluorescent yellow when sprayed with NH₃ vapor or spraying with AlCl₃. Complete acid hydrolysis yields quercetin as an aglycone in the organic layer and rhamnose as a sugar moiety in the aqueous layer (Co-PC hydrolysis yields quercetin as an aglycone in the organic layer and rhamnose as a sugar moiety in the aqueous layer). Spectral data in methanol: λ max nm MeOH: 256, 265 sh, 352; NaOMe: 272, 324 sh, 390; AlCl₃: 275, 300 sh, 428; AlCl₃/HCl: 270, 300 sh, 354, 402; NaOAc: 270, 320 sh, 370; NaOAc/H₂BO₃: 260, 368. ¹H NMR aglycone moiety: δ (ppm) 7.4 (2H, m, H-2′ and H-6′), 6.82 (1H, d, J = 8.4 Hz, H-5′), 6.35 (1H, d, J = 1.5 Hz, H-8), 6.20 (1H, d, J = 1.5 Hz, H-6). Sugar moiety: δ (ppm) 5.40 (1H, s, H-1″), 3.5–3.2 (m, sugar protons), 0.98 (3H, d, J = 6.2 Hz, rhamnosyl CH₃).

Kämpferol-3-O-Rhamnoside. Yellow amorphous powder, ¹H NMR (400 MHz, MeOD) (δ ppm): δ 7.84 (2H, dd, J = 8.6, 2.5 Hz, H-2′ and H-6′), 7.00 (2H, dd, J = 8.6, 2.5 Hz, H-3′ and H-5′), 6.45 (1H, d, J = 2.3 Hz, H-8), 6.25 (1H, d, J = 1.8 Hz, H-6), 5.52 (1H, d, J = 1.4 Hz, H-1″), 4.22 (1H, d, J = 1.4 Hz, H-2″), 3.70, 3.30 (remaining sugar protons), and 0.90 (3H, d, J = 6.0 Hz, Me-6″). ¹³C NMR (100 MHz, MeOD), δ ppm: 178.5 (C-4′), 164.2 (C-7′), 162.2 (C-5′), 160.1 (C-9′), 157.6 (C-4′), 157.2 (C-2′).

Quercetin. Light yellow spot on PC intensifies on exposure to NH₃ or spraying with AlCl₃ reagent. The UV spectrum data in methanol: λ max nm MeOH: 352; NaOMe: 272, 324 sh, 390; AlCl₃: 275, 300 sh, 428; AlCl₃/HCl: 270, 300 sh, 354, 402; NaOAc: 270, 320 sh, 371; NaOAc/H₂BO₃: 262, 369.

Kämpferol. Yellow powder, soluble in methanol, mp 276–278 °C. It showed a deep purple color in UV light (λ 365 nm), converted to yellowish green on exposure to ammonia vapor, and intensifies after spraying with a 5% AlCl₃ reagent. The UV spectral data in methanol: λ max nm MeOH: 376, 297, 267, 255 sh. NaOMe: λ416, 323, 277. AlCl₃/MeOH: 395, 349, 300, 274. AlCl₃/HCl/MeOH: 398, 352, 303 sh, 274. NaOAc/MeOH: 395, 302, 273. NaOAc/H₂BO₃/MeOH: 353, 320 sh, 290 sh, 266. NMR: δ 8.00 (2H, d, J = 8.84 Hz, H-2′/6′), 6.91 (1H, d, J = 8.84 Hz, H-3′/5′), 6.42 (1H, d, J = 1.84 Hz, H-8), 6.15 (1H, d, J = 1.84 Hz, H-6), δ 12.47 (1H, s, H-5).

GLC Analysis of Polysaccharide. Precipitation and purification of the polysaccharide content of L. sativum seeds (Table 3) revealed the identification of eleven sugars with galactose (21.884%) as the major one, followed by arabinose (20.476%), glucose (17.226%), galacturonic acid (11.039%), rhamnose (8.875%), mannose (4.316%), glucuronic acid (3.121%), xylose (1.527%), sorbitol (0.928%), mannitol (0.576%), and ribose (0.416%). These results were approximately similar to those of Abd El-Aziz et al.⁵⁸ as Ls mucilage contains l-arabinose, d-xylose, l-galactose, l-rhamnose, and d-galacturonic acid as the major constituents with d-glucose and mannose as trace components. The Ls mucilage is widely used in traditional medicinal preparations in Saudi Arabia as a cough syrup. It also has anti-hyperglycemic properties, which help in diabetes.⁶⁹

Antioxidant Activity; DPPH, FRAP, and ABTS. Free radicals are generated normally in the body during vital processes. Usually there is a balance between the liberated free radicals and naturally occurring scavengers in the body such as glutathione; with the increase in age this situation becomes out of balance, leading to a higher percentage of liberated free radicals that target many organs in the body, causing harmful effects and various diseases. The TEE of L. sativum seeds was rich in polyphenol constituents, which could exhibit a higher antioxidant activity and prevent these diseases.⁶⁰

Physical injuries of the skin lead to tissue damage or cut, and as a result, a series of biochemical reactions occur, which involve inflammation, proliferation, and migration of different types of immune system cells. Wound healing aims to restore the disrupted skin through contraction and closure of the wound to restore the skin as a functional barrier.⁵¹ The wound healing process may be restrained by the presence of reactive oxygen species (ROS), which can damage the wound’s surrounding cells and facilitate microbial infection.⁵²

Free radical scavengers are cytoprotective substances that have an essential role in the deactivation and removal of ROS, thus regulating the wound healing process.⁵³

Plant-derived antioxidants such as tannins, phenolic acids, flavones, flavonols, catechins, and other compounds are natural free radical scavengers that help in protecting vital cells from the harmful effects of ROS. In the present study, the antioxidant activities of L. sativum seeds were found to be relatively high in TEE, which was rich in flavonoids and phenolic constituents; these constituents were responsible for the process of wound healing.⁵⁴

Franke and Meyer⁵⁵ and Huang et al.⁵⁶ mentioned that a single method is not adequate for evaluating the antioxidant capacity of extracts. Different methods can yield widely diverging results, so several methods with different mechanisms must be used. Evaluation of the antioxidant activities of both the TEE and Poly of Ls seeds was carried out by three methods (DPPH, FRAP, and ABTS), as shown in Table 4.

The IC₅₀ of TEE and Poly in the DPPH radical scavenging assay was 82.6 ± 8.35 and 100.0 ± 15.2 µg/mL, respectively. A low IC₅₀ value indicates a high antioxidant activity. The reducing power (FRAP) of TEE and polysaccharide was

| Table 3. Results of Polysaccharide Hydrolysate Analysis of L. sativum L. Seeds Determined by GLC |
|-----------------------------------|-------------------------------|
| name                              | retention time | area % |
|-----------------------------------|-----------------|-------|
| 1                                 | 19.994          | 1.527 |
| 2                                 | 20.249          | 1.411 |
| 3                                 | 20.753          | 0.416 |
| 4                                 | 21.990          | 8.875 |
| 5                                 | 23.410          | 0.576 |
| 6                                 | 24.855          | 0.928 |
| 7                                 | 26.209          | 21.884|
| 8                                 | 26.522          | 17.226|
| 9                                 | 26.960          | 11.039|
| 10                                | 27.646          | 3.121 |
| 11                                | 28.977          | 4.315 |

| Table 4. Antioxidant Activity of the Lepidium sativum Seeds’ TEE and Poly Using DPPH, FRAP, and ABTS Assays |
|-----------------------------------|-----------------|-------------------------------|
| antioxidant activity              | free radical scavenging activity (DPPH) IC₅₀ (µg/mL) | iron reducing power (FRAP) (µM Trolox equivalent/mg extract) | ABTS |
|-----------------------------------|-----------------|-------------------------------|-------|
| TEE                               | 82.6 ± 8.35     | 772.47                        | 758.92|
| Poly                              | 100.0 ± 15.2    | 33.70                         | 57.14 |
| Trolox                            | 42.42 ± 0.87    |                               |       |
772.47 and 33.70 μM Trolox equivalent/mg extract, respectively; thus, the extract showed a higher FRAP ability. The antioxidant ability to reduce the ABTS generated in the aqueous phase resulting in decreasing the color was 758.92 and 57.14 μM Trolox equivalent/mg extract for the total ethanol extract (70%) and polysaccharide of L. sativum seeds, respectively. Major flavonoids (catechin and quercetin) showed excellent radical scavenging activity,57 and the high radical scavenging activity of TEE compared to poly might be attributed to the presence of these compounds.

Preparation and Characterization of Nanofibers. Spinning Condition Optimization of PVA/TEE and PVA/Polysaccharides (NFs). The formation of morphologically accepted NFs of both samples was found at a concentration of about 0.6% (wt/v %). The results revealed that the homogeneous and beads/droplets-free PVA and beads/droplets-free total ethanol L. sativum extract NFs have been produced with spinning conditions of voltage 27 kV, distance 15 cm, and feeding rate 0.1 mL/h, while PVA/polysaccharide NFs have been formed at voltage 30 kV, distance 15 cm, and feeding rate 0.2 mL/h. Thereafter, these electrospinning conditions allowed the formation of a Taylor cone that is essential for the formation of nanofibers.58

Instrumental Characterization of PVA/TEE and PVA/Polysaccharides. SEM. The morphologies of PVA, PVA/TEE-NFs, and PVA/Polysaccharide NFs are shown in Figure 1. SEM images of the three samples indicated the successful formation of uniform, non-woven, randomly oriented round-shaped with smooth surface, and continuous NFs.

Interestingly, it was noted that the addition of TEE and Poly to PVA NFs decreased the average diameter of the nanofibers from 230 ± 40 to 140 ± 60 and 200 ± 50 nm, respectively. This reduction has arisen from the anionic nature of both extracts caused by carboxyl and hydroxyl groups.59 Such high charge density could decrease the diameter of nanofibers by increasing the electric conductivity and the ionic strength of the spinning solution, which in turn increases the elongation of the jet produced by the electrical field.60

Furthermore, some thick parts appeared in PVA/TEE-NFs and PVA/Poly NFs, indicating that both types of extracts are encapsulated in NFs. Similar results were previously
FTIR. FTIR spectra were studied to reveal the nature of the interaction between NF compositions and confirm the successful cross-linking reaction by CA. Figure 2 shows the FTIR spectra of pure PVA, cross-linked PVA/TEE L. sativum NFs, and cross-linked PVA/poly NFs. The PVA spectrum presents the characteristic bands detected at ν 3289, 2903, and 1713 cm⁻¹ corresponding to hydroxyl, alkyl, and acetyl groups, respectively.62 Both the FTIR spectra of TEE and polysaccharides showed bands attributed to free hydroxyl groups and the bonded O—H of carboxylic acid at around 3297 and 3261 cm⁻¹, respectively. Additionally, the appearance of strong bands around 2924 and 2854 cm⁻¹ were attributed to the -aliphatic CH stretching and bending vibrations.63 In the spectra of TEE L. sativum, characteristic bands of flavonoids appeared at 1602 and 1455 cm⁻¹ due to the stretching vibration of C==C, C==O, CH₃, CH₂, and aromatic rings.63 Moreover, the two distinctive bands of hydroxyl flavonoids at 1513 cm⁻¹ and 1272 cm⁻¹ would be related to the N—H bending vibration and C—O.63,64 In the polysaccharides’ spectra (Poly), the fingerprint bands for polysaccharides at 1399, 879, 1043, and 1083 cm⁻¹ were clearly observed, which could be related to the C—H bending vibration, C—C stretching vibration, glycosidic, and C—O—H bonds, respectively. Furthermore, the presence of a band at 1594 cm⁻¹ can be assigned to the COO— stretching vibration in galacturonic and glucuronic acid.65

In the FTIR spectra of the uncross-linked PVA/TEE L. sativum and PVA/poly NFs, a change in intensities, brooding, and shifting of the O—H stretching vibration band from 3297 and 3261 to 3333 and 3317 cm⁻¹ was recorded, respectively. These noted changes could be explained as a result of the formation of intermolecular/intramolecular hydrogen bonds between PVA and the two types of L. sativum extracts. This observable brooding was consistent with a previously published work of doping PVA with the l. sativum extract.64

Previously, Fahami et al.59 reported that there was an occurrence of physical interaction among the components of L. sativum poly/PVA nanofiber, and there was no chemical interaction.59,61

Upon addition of citric acid as a cross-linker to PVA/TEE and PVA/Poly, a new band appears at 1721 and 1711 cm⁻¹, respectively. These new bands were related to the C—O of the ester group. This band confirms a successful cross-linking reaction between the —COOH group of citric acid and the —OH of PVA.66 In further, CA might react with the —OH present in two L. sativum extracts.

ζ-Potential Measurements. ζ-Potential distribution values of the ethanol extract (TEE), Poly, and the prepared NFs are shown in Table 5. ζ-Potential measurements revealed the anionic nature of TEE and Poly, which indicated that the negative surface charges were −23.9 and −13.6 mV, respectively. This result was consistent with the previous estimated ζ-potential of biopolymers extracted from L. sativum, which was 16 mV.62 It was found that the ζ-potential values of PVA, PVA/TEE, and PVA/poly NFs were −0.62, −22.7, and −5.9 mV, respectively. It was clearly observed that PVA/TEE and PVA/poly NFs have more negative ζ-potential than PVA NFs alone. Such ζ-potential would produce the electrostatic repulsion force between similarly charged adjacent particles, which has an important role in the stability of the colloidal suspension of the prepared NFs by making the solution resistant to aggregation.

Physicochemical Characterization of PVA/TEE and PVA/Poly L. sativum Nanofibers. Swelling Study. Since the swelling behavior of dressings should be studied for investigating their ability to absorb wound exudates during the wound healing process, the swelling % values of three tested NFs are shown in Figure 3A. Generally, it was observed that the incorporation of the two extracts (TEE L. sativum and Poly) into PVA NFs decreased the swelling ratio, since the swelling ratio of PVA NFs, PVA/TEE L. sativum NFs, and PVA/Poly NFs recorded 477.6, 421.1, and 289.6% after 1 h of swelling. This reduction in swelling % could be due to the intramolecular hydrogen bond interaction between PVA and the two types of L. sativum extracts causing a decrease in the swelling capacity of the NFs.
Meanwhile, the effect of polysaccharides incorporated on decreasing the swelling ratio was sharply compared to L. sativum TEE, owing to its constant slow swelling rate. This might be attributed to a high content of mucilaginous substance in L. sativum TEE that can absorb water and produce a large amount of hydrocolloids with high molecular weight.64 In addition, the CA cross-linker might increase the surface hydrophobicity by obstructing the hydrophilic hydroxyl groups of polysaccharides through the formation of a diester bond with the carboxylic groups of CA.68

From the swelling results, it was suggested that PVA/TEE L. sativum NFs could act as a suitable wound dressing scaffold since they have a proper swelling ratio that meets the requirements of wound healing, such as retaining wound exudates and nutrients, while PVA/polysaccharides NFs were not recommended owing to an inadequate and low swelling ratio that would result in insufficient nutrient supply to achieve the process of wound healing.69

**Hydrolytic Degradation.** Hydrolytic degradation of the tested nanofibers was performed as a function of weight loss (%) and evaluated by immersion of the three nanofibers for 14 days, as shown in Figure 3B. The results revealed that PVA NFs represent a higher hydrolytic degradation rate than other NFs, since there was about 52% of PVA NFs that were degraded after incubation for 14 days. However, PVA NFs had a constant degradation rate resulting from the secession of cross-linker segments that bind between the PVA chains, leading to degraded polymers with a low molecular weight.7

Conversely, as PVA/L. sativum TEE-NFs have a more stable hydrolytic degradation rate, they reach a hydrolytic degradation value of around ~25% after 14 days of the incubation period. Meanwhile, PVA/polysaccharide NFs show resistance against hydrolytic degradation compared to other nanofibers as they reach hydrolytic degradation (~3%) after 14 days of immersion time. This slowest hydrolytic degradation is probably owing to the high cross-linking density resulted from the formation of hydrogen and diester bonds.70,71

**In Vitro Bio-Evaluation Tests.** **Cell Viability.** To investigate the cytotoxicity of the L. sativum total ethanol extract (TEE) and polysaccharide, the MTT colorimetric assay was performed on Vero fibroblast cell lines with different extract concentrations (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 μg/mL). After 48 h of incubation with the polysaccharide extract, the cells showed high viability, as 216 μg/mL of Poly extract kills less than 50% of the incubated cells compared to the control group (cells were treated with only culture medium; Figure 4A). However, there seemed to be a marked difference between the polysaccharides and the TEE of L. sativum in terms of cell viability, demonstrating an excellent and almost 8-fold efficacy to that of the ethanol extract with an IC50 value of 1.75 mg/mL. This marked difference between the polysaccharides and TEE may suggest that TEE was enriched with other important components that improved the cell viability (Figure 4B). The cytotoxicity of the prepared nanofibers was also analyzed by the MTT assay. The results supported the data obtained by MTT assay for the extracts in solution form. As shown in Figure 4C, the PVA nanofibers doped with TEE had a higher cell viability compared to the polysaccharides-doped and bare PVA nanofibers. The bare PVA showed a good cell viability of about 90% of the control. The result was expected since PVA is known to be nontoxic and biocompatible.9,72 It is also worth noting that the cell
viability of the TEE-doped nanofibers was higher than that of the positive control, highlighting its potential as an anti-inflammatory agent.

Another MTT test was conducted to compare the cell viability of extracts in solution and nanofiber forms. After calculating the quantity of the extract in each sample, the cells were incubated with the same amount of extracts and observed after 48 h. As shown in Figure 4D,E, the nanofibers possess higher cell viability compared to solutions with the same extract quantities. The ratio of cell viabilities between both forms increased as the quantity increased, highlighting the low exposure rate of materials from nanofibers, which was beneficial for safe and long-term treatment.

In Vitro Wound Healing. The scratch wound healing assay was performed to understand the effect of the prepared nanofibers in accelerating or decelerating the healing of wounds in vitro. The scratch assay results obtained from the images using the scratch test are given in Figure 5. After 24 h of treatment with the nanofibers, about 35% of the scratched area was healed in control (cells treated only with medium). A relatively higher trend was observed in the PVA and PVA-poly nanofibers, achieving about 45% wound closure. The incorporation of TEE in the nanofibers increased the gap closure efficiency and healing capability to about 63% in 24 h. After 48 h, the wound closure percent reached 63, 69, 82, and 90% for the control, bare PVA, PVA-polysaccharide, and PVA-total ethanol extract, respectively (Table 6). Overall, it had been observed that incorporating Poly and TEE into the nanofibers improved the cell migration for the in vitro wound model. However, TEE showed a higher healing capability compared to the polysaccharides. The results revealed their potential as anti-inflammatory agents for wound dressing.

Table 6. In Vitro Wound Healing Data Obtained by Analyzing the Bright-Field Images after 0, 24, and 48 h of Incubation

|       | width 1 (mm) | width 2 (mm) | width 3 (mm) | average width (mm) | wound closure % | relative scratch gap |
|-------|--------------|--------------|--------------|--------------------|----------------|---------------------|
| 0 h   |              |              |              |                    |                |                     |
| control | 615          | 621          | 608          | 615                | 0              | 1.00                |
| PVA   | 563          | 568          | 549          | 560                | 0              | 1.00                |
| polysaccharide | 579        | 571          | 552          | 567                | 0              | 1.00                |
| extract | 605          | 590          | 588          | 594                | 0              | 1.00                |
| 24 h  |              |              |              |                    |                |                     |
| control | 360          | 467          | 375          | 401                | 35             | 0.65                |
| PVA   | 312          | 321          | 304          | 312                | 44             | 0.56                |
| polysaccharide | 316        | 302          | 316          | 311                | 45             | 0.55                |
| extract | 221          | 175          | 256          | 217                | 63             | 0.37                |
| 48 h  |              |              |              |                    |                |                     |
| control | 235          | 229          | 216          | 227                | 63             | 0.37                |
| PVA   | 183          | 152          | 183          | 173                | 69             | 0.31                |
| polysaccharide | 123        | 94           | 84           | 100                | 82             | 0.18                |
| extract | 68           | 31           | 87           | 62                 | 90             | 0.10                |

*Table 6: In Vitro Wound Healing Data Obtained by Analyzing the Bright-Field Images after 0, 24, and 48 h of Incubation*

*Extract = TEE.*
CONCLUSIONS

*L. sativum* is a rich medicinal plant with phytoconstituents, phenolic acids and flavonoids, which are responsible for antioxidant and anti-inflammatory activities. Owing to these activities, the total ethanol extract and polysaccharide show a promising wound-healing effect. Furthermore, total ethanol extract is more active due to their being enriched in phenolic acids and flavonoids, which show scavenging activity and accelerate the healing process. So, extracts derived from *L. sativum* seeds were suggested as an ideal candidate for nanofibrous wound dressings, which help in healing wounds after surgery or in diabetic patients.

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Notes

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