




## RESEARCH ARTICLE

# *Nigella sativa* L. seed extracts promote wound healing progress by activating VEGF and PDGF signaling pathways: An *in vitro* and *in silico* study [version 1; peer review: 2 approved, 1 approved with reservations]

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## Abstract




**Background:** A significant area of clinical research is the development of natural wound healing products and the management of chronic wounds. Healing wounds with medicinal plants has been a practice of ancient civilizations for centuries. *Nigella sativa* L. (*N. sativa*) is a medicinal plant that has several pharmacological properties.

**Methods:** The present study evaluated the wound healing properties of *Nigella sativa* L. (*N. sativa*) seed extracts using normal cell lines such as normal human dermal fibroblasts (NHDFs) and human umbilical vein endothelial cells (HUVECs). The expression levels of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) were analyzed through western blot analysis. Furthermore, computational analyses were carried out to screen the potential bioactive compounds for wound healing applications.

**Results:** The results of the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay revealed that, all the tested solvent extracts of *N. sativa* seeds (including ethanol, ethyl acetate, chloroform, and petroleum ether) did not exert any cytotoxic effects at the tested concentrations. Furthermore, the western blot analysis showed elevated levels of VEGF and PDGF upon treatment with *N. sativa* seed extracts. Gas chromatography-mass spectrometry (GC-MS) analysis of *N. sativa* extracts identified 268 phytochemicals. Molecular docking studies revealed that three phytochemicals of *N. sativa* extracts, including tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-, adaphostin

## Open Peer Review

Approval Status   

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and obeticholic acid had strong binding affinity with wound healing-related target proteins, showing docking scores ranging from -5.5 to -10.9 Kcal/mol. These compounds had acceptable Absorption, Distribution, Metabolism, and Excretion (ADME) properties.

**Conclusions:** Based on these results, *N. sativa* seed extracts might possess potential wound healing properties owing to the presence of a wide range of bioactive components.

### Keywords

Nigella sativa L., Wound healing, Vascular endothelial growth factor, Platelet-derived growth factor



This article is included in the **Bioinformatics** gateway.



This article is included in the **Plant Science** gateway.

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## Introduction

The management of wounds, especially extensive and full-thickness wounds, has long been a concern in the field of medicine.<sup>1</sup> Infection by pathogenic bacteria delay the healing process and increase health risks to the general public. Clinicians exploring effective ways to promote wound healing is a hot topic in research.<sup>2</sup> Vigorous development of advanced wound dressings is imperative for accelerating wound healing and achieving closure of wounds quickly.<sup>3</sup> Hemostasis, inflammation, proliferation, and tissue remodeling are sequential and timed processes involved in wound healing.<sup>4</sup> These complex processes are mediated by released cytokines, chemokines, and growth factors, which are released by neutrophils, macrophages, keratinocytes, and endothelial cells.<sup>5</sup> It is important to manage wounds in a timely and comfortable manner in order to facilitate a quick healing process.<sup>6</sup> The wound care industry has developed a number of products that are designed to treat wounds (for example MEBO, Calmoseptine<sup>®</sup>, Boroline). A variety of wound healing techniques have been developed over the years, including traditional (especially herbal) and modern methods.<sup>1,7</sup> Traditional herbal wound-healing therapies remain popular among rural populations in developing countries in part due to their availability and affordability, and they have been demonstrated to be effective, clinically accepted, and have few or no side effects.<sup>8</sup>

There has been a growing awareness in recent years that many phytochemicals possess medicinal properties that are effective in treating diseases and in healing wounds.<sup>9</sup> A chemical scaffold can provide a framework for developing synthetic and/or semi-synthetic analogues of drugs, which can be used in drug development for disease treatment in a wide range of settings.<sup>10</sup> As a result of the advent of modern techniques like molecular biology, metabolomics, phytochemical analysis, and drug discovery, natural products chemists have been able to unravel the ancient therapeutic hypotheses and mechanisms of herbal medicines.<sup>11–14</sup> It is common to find these types of treatments used in Ayurveda, Traditional Chinese medicine, and Traditional Thai medicine.<sup>15</sup>

*Nigella sativa* L. (*N. sativa*) (Family Ranunculaceae) seeds, commonly known as black cumin or black seeds, have a long history of being used as a treatment for a variety of ailments by traditional healers throughout the world, in regions like South-eastern Asia, the Middle East, Africa, and many areas of the Mediterranean.<sup>16,17</sup> It is also notable to point out that *N. sativa* possess a plethora of pharmacological properties. A variety of health-related conditions have been treated with this herb throughout history, including respiratory and digestive disorders, and kidney, liver, and cardiovascular diseases.<sup>18</sup> The most important pharmacological effects of *N. sativa* seeds can be attributed to thymoquinone, according to a previous study.<sup>19</sup> The extracts of *N. sativa* seeds also contain alkaloids, saponins, steroids, terpenoids, p-cymene, limonene, and fatty acids as well as proteins, carbohydrates, vitamins, trace minerals (like iron and zinc) and crude fiber.<sup>20</sup>

*N. sativa* seeds are reported to have several pharmacological effects, including analgesic, appetizer, anti-diabetic, antioxidant, anti-inflammatory, and antimicrobial properties.<sup>21</sup> Despite extensive research on the phytochemical pharmacological properties of *N. sativa* seeds, *N. sativa* seed extracts are not yet completely characterized chemically.<sup>22</sup> As people become more aware that natural products can have potential therapeutic effects on wound healing properties and at present do not have any known toxic effects, it is becoming clear that they are seeking out natural products that can work in this regard. As well as providing information regarding the compositional profile and evaluating the medicinal effects of herbal extracts and/or oils, there is a need to re-evaluate their therapeutic properties in addition to providing information regarding their compositional profile.<sup>23</sup> Consequently, the primary objective of the present study was to investigate the effects of *N. sativa* seed extracts and their phytochemicals on wound healing. Normal human dermal fibroblasts (NHDFs) and human umbilical vein endothelial cells (HUVECs) were used as primary cell lines in the present study to study these issues *in vitro*. The phytochemicals were also docked with multiple wound healing-related proteins (Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), transforming growth factor beta receptor 1 (TGFBR1) kinase, interleukin-1 beta (IL-1 $\beta$ ), protein kinase C (PKC)- $\beta$ II, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)).

## Methods

This study was performed at Chulalongkorn University (Thailand) and Saveetha University (India).

### Chemicals

Bovine serum albumin (BSA) (cat. no. 23209), HUVECs (cat. no. C0035C) and NHDFs (cat. no. C0135C) used in this study were purchased from Thermo Fisher Scientific Inc. Dulbecco's Modified Eagle Medium (DMEM) (cat. no. D6429), TRIS-buffered saline (TBS) (cat. no. SRE0071) and fetal bovine serum (FBS) (cat. no. F7524) were purchased from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from BioVision Inc. (cat. no. 2808). Anti-VEGF mouse monoclonal antibody was procured from Santa Cruz Biotechnology, Inc., (cat. no. sc-53462). Anti-PDGF Receptor  $\beta$  polyclonal antibody (produced in rabbit) was purchased from Sigma-Aldrich (cat. no. SAB4502149). Anti- $\beta$ -actin mouse monoclonal antibody was purchased from Santa Cruz Biotechnology, Inc. (cat. no. sc-69879). Enhanced Chemiluminescence Detection (ECL) kit was obtained from Amersham BioSciences UK Ltd (cat. no. RPN2209).

## Collection of materials

*N. sativa* seeds were purchased from a local herbal shop in Bangkok, Thailand. After being cleaned with tap water, they were dried under shade conditions, powdered, and air-tight packaged in a container.

## Extract preparation

In order to determine the yield on the plant material, 3,000 mL petroleum ether, chloroform, ethyl acetate, and ethanol were continuously shocked with 600 g plant material in a conical flask for 72 h (during the cold percolation process). After the extracts were collected and filtered using Whatman No. 1 filter paper, a rotary evaporator set at 40°C was used to dry them. In order to preserve the dried extracts until further use, they were stored at 4°C until use.

## GC-MS analysis and phytochemicals identification

Gas Chromatography-Mass Spectrometer Model Shimadzu GCMS-QP2020 NX (Shimadzu, Japan) equipped with 5 Sil MS 5% diphenyl/95% dimethyl polysiloxane capillary column (measuring 30 mm wide, 0.25 mm diameter, and 0.25 mm thick) was used to analyze the extracts of *N. sativa* seeds. Then, 100 µL of solvent extracts were diluted using 1,400 µL dimethyl sulfoxide (DMSO). Next, 1 µL diluted sample (100/1,400, V/V in DMSO) was injected in the split mode with a split ratio 1:10. Electron impact ionization was used for GC-MS detection with an ionization energy of 70 eV. A low flow rate of 1.0 mL per min of helium at a low pressure was used as the carrier gas in the column. Before the injector temperature was set at 250°C, 60°C was set for 15 min before gradually increasing to 280°C over 3 min. It was conducted at 70 eV with a scanning distance of 0.5 s as well as fragment sizes ranging between 50 Da and 650 Da for the MS analysis, 40 min were spent on the GC operation. Acquisition mode scan ranged from 35 m/z to 500 m/z with scan speed 2,500. Extracts were analyzed for their percentage composition of compounds. [NIST20R](#) and [Wiley libraries](#) were used to interpret and compare GC-MS data as well as compare retention indices.<sup>24</sup>

## *In vitro* cell line study

### *Cell lines and culture*

A humidified atmosphere containing 5% CO<sub>2</sub> was used for HUVEC and NHDF cell lines during the experiment to maintain 37°C for the duration. DMEM supplemented with 10% FBS and 1% antibiotics (100 U/mL penicillin and 100 g/mL streptomycin) was used to grow the cells in T-25 flasks. Trypsinization and passage were performed once the cells reached 70% confluency.

### *Cell viability analysis*

In culture media, 10 mg/mL stock solutions of plant extracts were diluted in DMSO. To determine cell viability, cells were seeded into 96 well plates at a density of 5x10<sup>3</sup> cells per well and incubated at 37°C and 5% CO<sub>2</sub> for 24 h. Fresh DMEM supplemented with various solvent extracts of *N. sativa* seeds (petroleum ether, chloroform, ethyl acetate and ethanol) (0, 10, 20, 50, 100 g/mL) was added, and incubation was carried out for 24 h. After the incubation with extracts, cells were incubated for 2 h in growth media (DMEM) containing 20% MTS solution to assess viability. Microplate readers were used to measure the absorbance of formazan at 490 nm. The crude extracts were dissolved in 0.5% DMSO, which represents the highest concentration of DMSO used in the vehicle culture medium.

### *Protein expression analysis by western blotting*

Laemmli (1970) described sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a method for separating proteins.<sup>25</sup> Using equal volumes (50 µg) of samples and buffer, sample mixtures were heated at 95°C for 4 min, then cooled on ice. The dye front was reached at the bottom of the running gel after separating proteins with a Bio-Rad mini slab gel apparatus at a constant voltage of 100 V. In this experiment, polyvinylidene difluoride (PVDF) membranes were charged at a constant voltage of 100 V for 1 h in order to transfer protein bands. Incubation with primary antibodies (anti-VEGF (mouse monoclonal antibody 200 µg/ml) and anti-PDGF Receptor β (rabbit polyclonal antibody 100 µg/ml); β-actin (mouse monoclonal antibody 100 µg/ml) was used as the control) at appropriate dilutions followed by blocking with 5% BSA blocking solution at room temperature for 1 h. A secondary antibody (goat anti-mouse monoclonal antibody 400 µg/ml), purchased from Santa Cruz Biotechnology, Inc., (cat. no. sc-2005; 1:10,000) was incubated for 1 h after primary antibody incubation. Incubation with secondary antibody was followed by two washes (5 min each) with Tris-buffered saline, Tween (TBS-T) and placement on Saran Wrap™ (protein-side up). After adding detection reagent mixture to the blot, blots were incubated for 30-60 sec and we drained off excess reagent (ECL). [Quantity One 1-D Analysis Software](#) (RRID:SCR\_014280) (Bio-Rad) was used to quantify the immunoblot signals. Using a probe consisting of β-actin, similar amounts of proteins were loaded onto the membranes.

## In silico analysis

### Selection and preparation of ligands

Through GC-MS analysis, a total of 268 phytochemicals were identified in four different extracts of *N. sativa*. The 3D structures of all the identified compounds were extracted from the [PubChem](#) database (RRID:SCR\_004284).<sup>26</sup> A list of phytochemicals identified are provided in Tables 1-4 as *Underlying data*.<sup>42</sup> Using [PyRx software](#) (RRID:SCR\_018548) with default parameters, energy minimization of each ligand was performed using universal force fields, followed by Gasteiger charges to achieve a good structural conformation for docking.

### Selection and preparation of receptors

As part of this study, six different proteins such as TNF $\alpha$ , TGFBR1 kinase, IL-1 $\beta$ , PKC- $\beta$ II, VEGF and PDGF that participate in wound healing were selected, and their crystal structures were retrieved from [Protein Data Bank](#) (PDB). Using [Chimera](#) 1.16 (RRID:SCR\_002959), any missing residues in the selected target proteins were modelled, nonstandard hetero atoms were removed, polar hydrogens and Gasteiger charges were added, and then energy minimization of each protein performed with 100 steepest descent gradient steps using amber force field (Amber ff14SB). Finally, the energy minimized protein was converted into pdbqt format for molecular docking.

### Protein-ligand docking

The [Autodock Vina](#) (RRID:SCR\_011958) was used for the molecular docking of phytochemicals of *N. sativa* with selected wound healing target proteins. If the ligand binding site is represented, it will be located at the center of the grid box. A value of eight is set for the exhaustiveness of the model. A configuration file was created based on the dimensions of the XYZ axis determined by Discovery studio's visualizer. In Autodock Vina 1.1.2, this configuration file was used for docking using the command line. To dock ligands with a degree of flexibility, Autodock Vina uses the Monte Carlo algorithm. Monte Carlo algorithm used in Autodock Vina is relatively faster than other docking programs.<sup>27</sup> In addition to the results file, the binding modes were generated as a single file (PDBQT format) in a log format. [BIOVIA Discovery Studio](#) (RRID:SCR\_015651) visualizer was used to analyze the binding interactions between best docked ligands and receptors. Strong hydrogen bonds (2.2 to 2.5), moderate hydrogen bonds (2.5 to 3.2), and weak hydrogen bonds (up to 3.6) were measured with respect to the hydrogen atom of the heavy atom.

### ADME properties prediction

[QikProp](#) (RRID:SCR\_014906) module was used to predict ADME properties (Schrodinger Suite 2022). To determine a ligand's pharmacokinetics and pharmacodynamics, the QikProp module analyses its properties, which are resembling those of a drug. Several ADME properties were considered significant, including the molecular weight (MW), H-bond donor, H-bond acceptor, and logarithm of n-octanol/water partition coefficient (log P (O/W)).

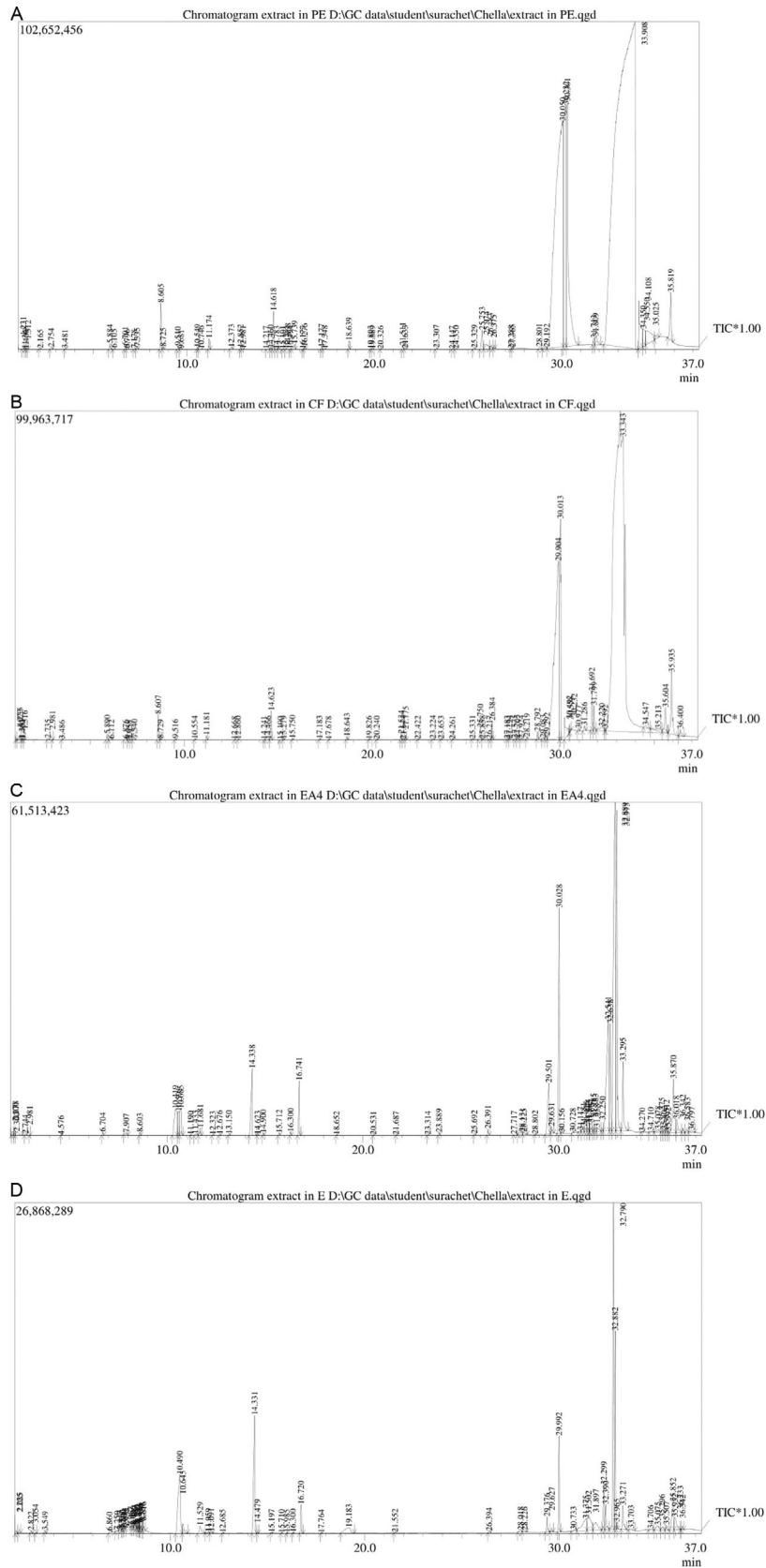
### Statistical analysis

Data were analyzed using [GraphPad Prism](#) (RRID:SCR\_002798) version 5 software to assess the significance of individual variations between the control and treatment groups by one-way analysis of variance (ANOVA) and Duncan's multiple range test. Approximately P<0.05 was considered significant in Duncan's test.

## Results

### GC-MS analysis

GC-MS analysis identified a total of 268 phytochemicals in *N. sativa* seed extracts ([Figure 1](#)).<sup>42</sup> Petroleum ether, chloroform, ethyl acetate and ethanolic extracts showed 65, 70, 67 and 66 peaks, respectively, which are indicating the presence of phytochemicals (Tables 1-4 in *Underlying data*).<sup>42</sup> Among these, the highest peak levels were observed such as, 66.81% linoleic acid (PubChem CID: 5280450) at 33.908 min retention time (petroleum ether extract), 42% cis-vaccenic acid (PubChem CID: 5282761) at 33.343 min retention time (chloroform extract), 29.24% ethyl palmitate (PubChem CID: 12366) at 30.050 min retention time (petroleum ether extract), 20.09% oleic acid (PubChem CID: 445639) at 34.550 min retention time (petroleum ether extract), 16.72% palmitic acid (PubChem CID: 985) at 29.904 min retention time (chloroform extract), 16.57% tetradecanoic acid (PubChem CID: 11005) at 25.974 min retention time (petroleum ether extract), 16.37% 3-(3-Chlorophenyl)imidazolidine-2,4-dione (PubChem CID: 285803) at 31.375 min retention time (ethanolic extract), 15.83% methyl linoleate (PubChem CID: 5284421) at 32.22 min retention time (chloroform extract), 15.34% adaphostin (PubChem CID: 387042) at 30.733 min retention time (ethanolic extract), 14.07% glyceryl diacetate 2-oleate (PubChem CID: 5363238) at 31.897 min retention time (ethanolic extract), 12.62% 2-linoleoylglycerol (PubChem CID: 5365676) at 31.502 min retention time (ethanolic extract), 11.34% monopalmitin (PubChem CID: 14900) at 28.131 min retention time (ethyl acetate extract), 11.14% (z)-tetradec-7-enal (PubChem

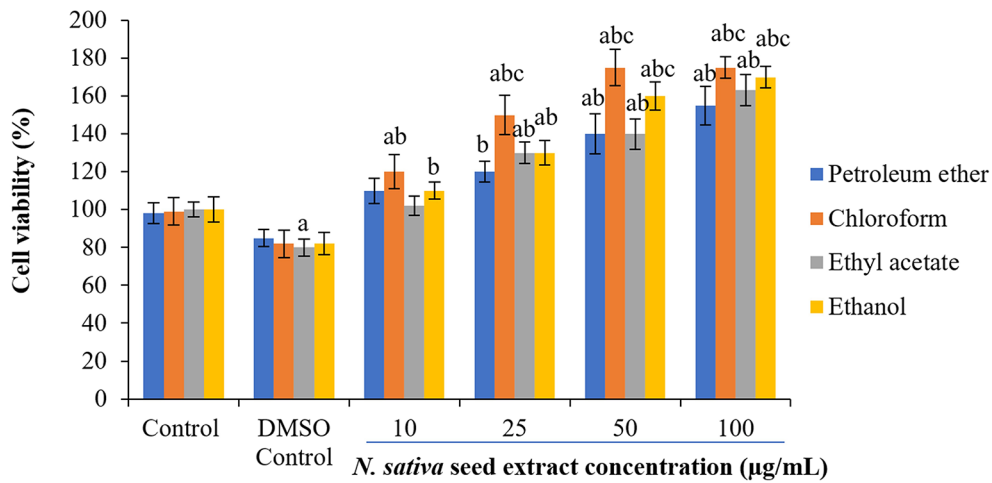


**Figure 1.** Analysis of different solvent extracts of *N. sativa* seeds using GC-MS.

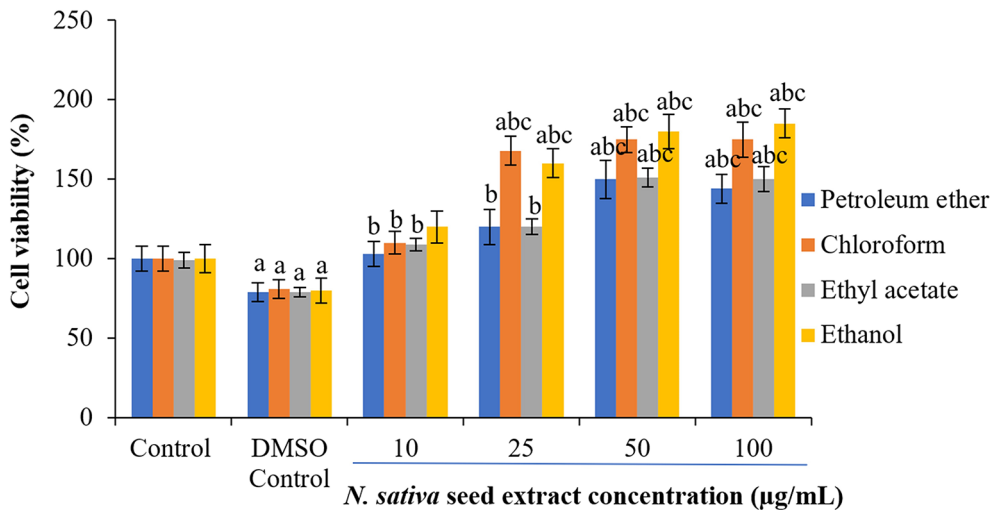
CID: 5364468) at 2.3 min retention time (ethyl acetate extract), 10.54% glycerol, 2-octadecanoate, diacetate (PubChem CID: 539925) at 33.703 min retention time (ethanolic extract), 10.52% glyceryl diacetate 1-linolenate (PubChem CID: 6434505) at 35.075 min retention time (ethanolic extract), 10.45% olealdehyde (PubChem CID: 5364492) at 36.797 min retention time (ethyl acetate extract), 10.25% 2,3-dihydroxypropyl acetate (PubChem CID: 33510) at 10.419 min retention time (ethyl acetate extract), 10.23% 16-trimethylsilyloxy-9-octadecenoic acid, methyl ester (PubChem CID: 6421149) at 34.547 min retention time (chloroform extract), 10.08% propyl ester (PubChem CID: 221069) at 34.71 min retention time (ethyl acetate extract).

**Effect of *N. sativa* seeds on cell viability of NHDFs and HUVECs**

To check the cytotoxicity of *N. sativa* seed extracts, two different normal cell lines (NHDF and HUVECs) were used at the different concentrations of crude extracts. Cell viability percentages were plotted against extracts treatment concentrations to obtain treatment-response curves. A concentration-dependent increase in cell viability was observed with *N. sativa* seed extracts (Figures 2 and 3). In response to 25-50 µg/mL of ethanolic and chloroform extract on both cell lines, the viability of cells were increased by 60-68% following 48 h treatment (Figures 2 and 3).



**Figure 2. Effect of extracts on HUVEC viability.** Cells were cultured in DMEM supplemented with 10% FBS were incubated with indicated concentrations of extracts (0–100 µg) for 48 h. Each bar represents the mean ± SEM of six independent observations. Significance was considered as the levels of p < 0.05 level using Duncan’s multiple range test. a - compared to control; b - compared to DMSO control; c - compared with 10 µg treated cells; d - compared with 25 µg treated cells.

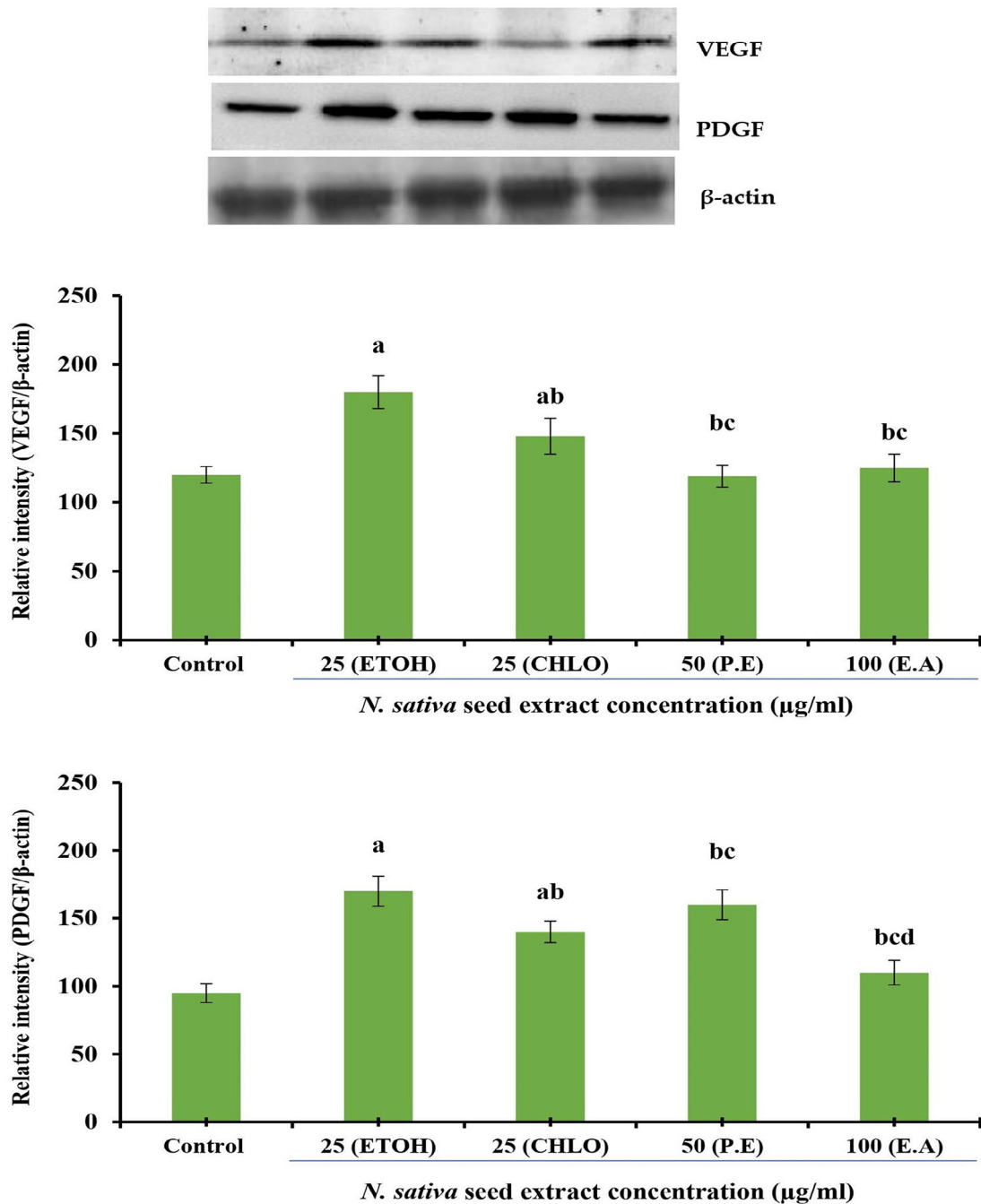


**Figure 3. Effect of extracts on cell viability in NHDF cell lines.** Cells cultured in DMEM supplemented with 10% FBS were incubated with indicated concentrations of extracts (0–100 µg) of 48 h. Each bar represents the mean ± SEM of six independent observations. Significance was considered at p < 0.05 level using Duncan’s multiple range test. a - compared to control; b - compared to DMSO control; c - compared with 10 µg treated cells; d - compared with 25 µg treated cells.

### Protein expression analysis

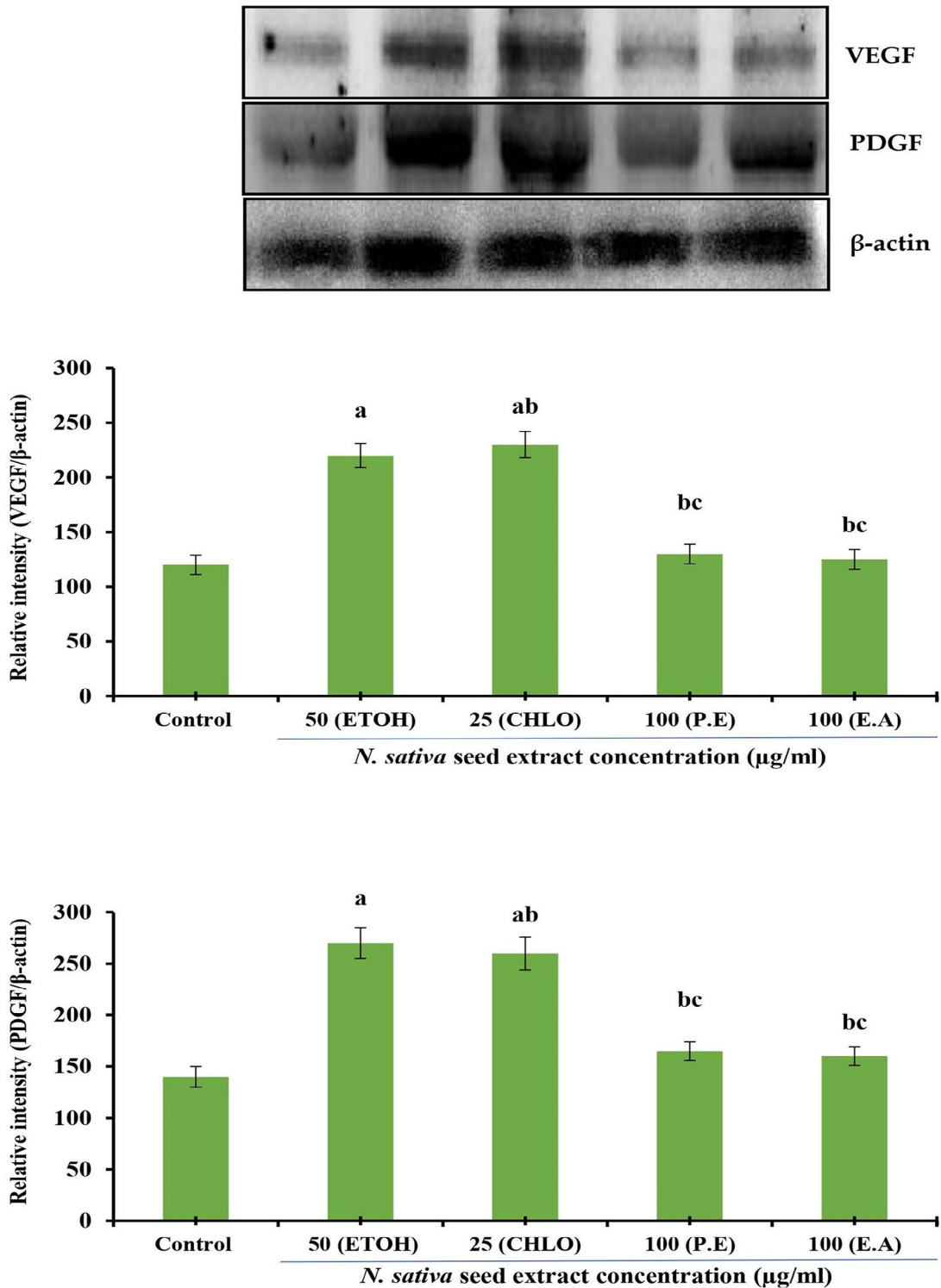
#### Effect of crude seed extracts on VEGF and PDGF protein expression in NHDF cell lines

The effect of different solvent extracts of *N. sativa* seeds on VEGF and PDGF protein expression in NHDF cell lines were investigated. Incubation for 24 h with extracts of indicated concentrations was conducted in DMEM supplemented with



**Figure 4.** Effect of different *N. sativa* seed extracts on VEGF and PDGF protein expression in NHDF cells. Protein expression was analyzed by western blotting using specific antibodies. Each bar represents the mean  $\pm$  SEM of six independent observations. Significance was considered as the levels of  $p < 0.05$  level using Duncan's multiple range test. a, compared with control; b, compared with ethanolic extract (25  $\mu$ g) treated cells; c, compared with chloroform extract (25  $\mu$ g) treated cells; *N. sativa*, *Nigella sativa* L.; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; NHDF, normal human dermal fibroblast; P. E, Petroleum Ether extract; CHLO, Chloroform extract; E. A, Ethyl Acetate extract; ETOH, Ethanolic extract.





**Figure 5. Effect of different *N. sativa* seed extracts on VEGF and PDGF protein expression in HUVEC lines.** Protein expression was analyzed by western blotting using specific antibodies. Each bar represents the mean  $\pm$  SEM of six independent observations. Significance was considered as the levels of  $p < 0.05$  level using Duncan's multiple range test. a, compared with control; b, compared with ethanolic extract (25  $\mu$ g) treated cells; c, compared with chloroform extract (25  $\mu$ g) treated cells; *N. sativa*, *Nigella sativa* L.; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; HUVEC, human umbilical vein endothelial cell; P. E, Petroleum Ether extract; CHLO, Chloroform extract; E. A, Ethyl Acetate extract; ETOH, Ethanolic extract.

10% FBS using NHDF cells. Densitometry analysis was used to calculate protein expression, which is expressed in relative intensity. Internal control was performed using  $\beta$ -Actin. Based on six independent observations, each bar represents the mean and standard error of the mean. The significance level was determined by using Duncan's multiple range test at  $p < 0.05$ . VEGF and PDGF expression levels were comparatively increased at 25  $\mu\text{g/mL}$  by both ethanolic and chloroform extracts (Figure 4).

#### Effect of crude seed extracts on VEGF and PDGF protein expression in HUVEC lines

Effect of different solvent extracts on VEGF and PDGF protein expression in HUVEC lines. Incubation of HUVECs with indicated concentrations of extracts for 24 h was performed in DMEM supplemented with 10% FBS. Densitometry analysis was used to quantify protein expression, which is expressed as relative intensity. An internal control was performed using  $\beta$ -actin. Six independent observations are represented by a bar with a mean and standard error of measurement. The Duncan's multiple range test was used to determine significance at  $p < 0.05$ . At a concentration of 25  $\mu\text{g/mL}$ , ethanolic and chloroform extracts of *N. sativa* seed led to comparatively significant increases in the expression levels of VEGF and PDGF proteins (Figure 5).

#### In silico analysis

In the present study, Autodock Vina was used to predict the binding affinity of a total of 268 selected phytochemicals of *N. sativa* with the target proteins of wound healing process such as  $\text{TNF}\alpha$  (PDB ID: 2AZ5), TGFBR1 kinase (PDB ID: 6B8Y), IL-1 $\beta$  (PDB ID: 6Y8M), PKC- $\beta$ II (PDB: 2I0E), VEGF-A (PDB ID: 3QTK) and platelet-derived growth factor receptor alpha (PDGFRA) (PDB ID: 6JOL). The binding energies of 10 ligands that showed the highest binding affinities are indicated in the heatmap (Figure 6). It is clear from Figure 6, three compounds (1, 2, and 3) namely tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-, adaphostin (PubChem CID: 387042), and obeticholic acid (PubChem CID: 447715), respectively, showed highest binding affinity with all the tested target proteins. The best docked protein ligand interactions are shown in Table 1 and Table 2. The 2D and 3D structures of the top docked complexes are shown in Figures 7-9. ADME properties of those compounds were under the acceptable range (Table 3).

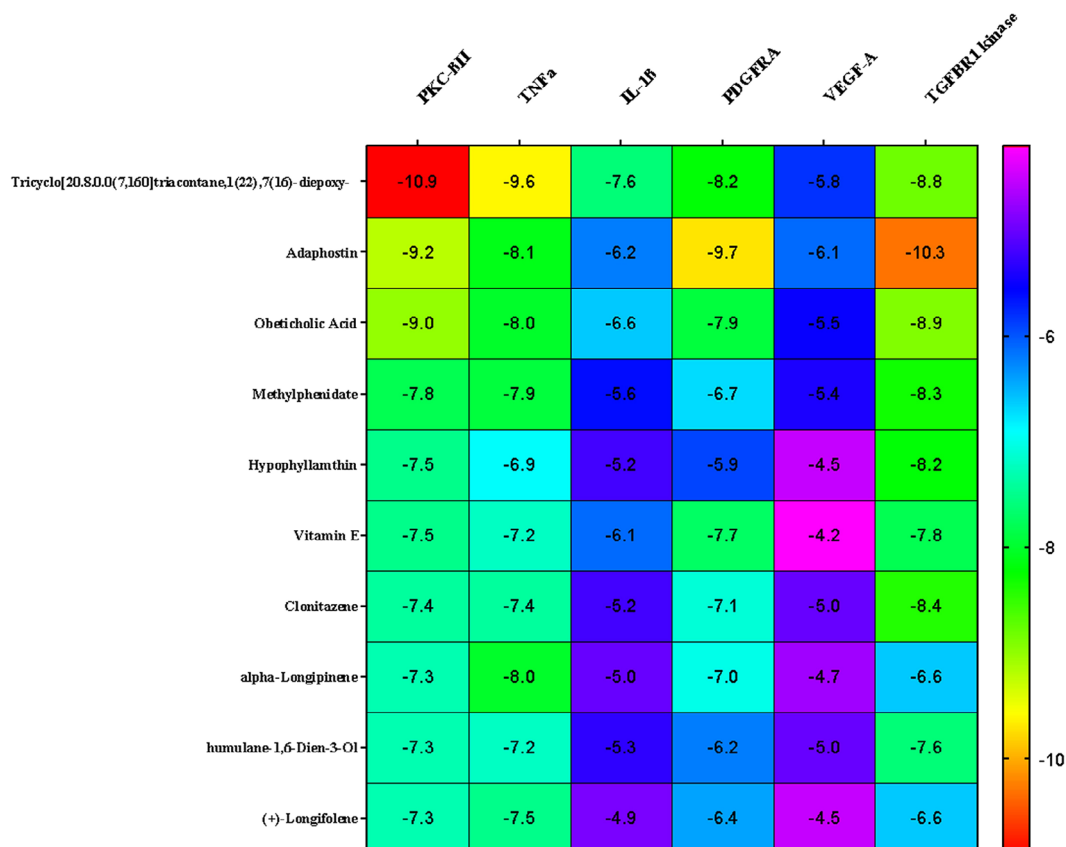


Figure 6. Heat map representation of top protein ligand docking analysis.

**Table 1. Molecular docking analysis of screened compounds with targeted proteins.**

Compound name (PubChem CID)	AutoDock Score					
	PKC-βII (2I0E)	TNFα (2AZ5)	IL-1β (6Y8M)	PDGFRA (6JOL)	VEGF-A (3QTK)	TGFBR1 (6B8Y)
Tricyclo[20.8.0.0(7,16)]triacontane, 1 (22),7(16)-diepoxy- (543764)	-10.9	-9.6	-7.6	-8.2	-5.8	-8.8
Adaphostin (387042)	-9.2	-8.1	-6.2	-9.7	-6.1	-10.3
Obeticholic acid (447715)	-9.0	-8.0	-6.6	-7.9	-5.5	-8.9
Methylphenidate, HFB (91747145)	-7.8	-7.9	-5.6	-6.7	-5.4	-8.3
Hypophyllanthin (182140)	-7.5	-6.9	-5.2	-5.9	-4.5	-8.2
Vitamin E (14985)	-7.5	-7.2	-6.1	-7.7	-4.2	-7.8
Clonitazene (62528)	-7.4	-7.4	-5.2	-7.1	-5.0	-8.4
α-Longipinene (520957)	-7.3	-8.0	-5.0	-7.0	-4.7	-6.6
Humulane-1,6-dien-3-ol (5353015)	-7.3	-7.2	-5.3	-6.2	-5.0	-7.6
(+)-Longifolene (1796220)	-7.3	-7.5	-4.9	-6.4	-4.5	-6.6

**Table 2. Top protein-ligand docking complex interactions in the active sites.**

Interaction residues	Distance	Category	Types
<b>PKC-βII complexed with tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-</b>			
LEU10	5.01131	Hydrophobic	Alkyl
VAL18	5.47023	Hydrophobic	Alkyl
VAL18	4.98992	Hydrophobic	Alkyl
LYS33	5.44287	Hydrophobic	Alkyl
MET82	4.97728	Hydrophobic	Alkyl
ALA145	4.46926	Hydrophobic	Alkyl
<b>TNFα complexed with tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-</b>			
TYR59	4.46433	Hydrophobic	Pi-Alkyl
TYR59	5.31031	Hydrophobic	Pi-Alkyl
<b>IL-1β complexed with tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-</b>			
LEU108	4.8333	Hydrophobic	Alkyl
PHE44	4.68795	Hydrophobic	Pi-Alkyl
<b>PDGFRA complexed with tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-</b>			
ILE65	5.13132	Hydrophobic	Alkyl
<b>VEGF-A complexed with tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-</b>			
LYS77	4.91527	Hydrophobic	Alkyl
<b>TGFBR1 kinase complexed with tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-</b>			
VAL219	4.46222	Hydrophobic	Alkyl
ALA230	5.2525	Hydrophobic	Alkyl
LEU260	5.30162	Hydrophobic	Alkyl
LEU340	5.03535	Hydrophobic	Alkyl
ALA350	4.70449	Hydrophobic	Alkyl
<b>PKC-βII complexed with adaphostin</b>			
THR66	2.8462	Hydrogen Bond	Conventional Hydrogen Bond
VAL85	2.83916	Hydrogen Bond	Conventional Hydrogen Bond
ASP89	2.08593	Hydrogen Bond	Conventional Hydrogen Bond
MET135	4.31632	Other	Pi-Sulfur
MET135	3.86336	Other	Pi-Sulfur
VAL18	4.67941	Hydrophobic	Alkyl
LYS33	5.4335	Hydrophobic	Alkyl
LYS33	4.02333	Hydrophobic	Alkyl
MET82	5.17432	Hydrophobic	Alkyl

Table 2. Continued

Interaction residues	Distance	Category	Types
ALA145	4.50014	Hydrophobic	Alkyl
MET82	4.19605	Hydrophobic	Alkyl
VAL18	4.38234	Hydrophobic	Alkyl
LYS33	3.85054	Hydrophobic	Alkyl
PHE15	5.32592	Hydrophobic	Pi-Alkyl
ALA31	3.89466	Hydrophobic	Pi-Alkyl
VAL85	5.13226	Hydrophobic	Pi-Alkyl
<b>TNF<math>\alpha</math> complexed with adaphostin</b>			
TYR151	4.02119	Hydrogen Bond	Pi-Donor Hydrogen Bond
TYR59	3.95293	Hydrophobic	Pi-Sigma
TYR151	5.8628	Hydrophobic	Pi-Pi T-shaped
TYR119	5.29859	Hydrophobic	Pi-Alkyl
TYR119	4.93237	Hydrophobic	Pi-Alkyl
TYR151	5.48208	Hydrophobic	Pi-Alkyl
<b>IL-1<math>\beta</math> complexed with adaphostin</b>			
THR145	3.43485	Hydrogen Bond	Pi-Donor Hydrogen Bond
THR145	3.76017	Hydrophobic	Pi-Sigma
LEU108	5.31577	Hydrophobic	Alkyl
LEU108	4.56027	Hydrophobic	Alkyl
LEU108	3.89181	Hydrophobic	Alkyl
<b>PDGFRA complexed with adaphostin</b>			
LYS45	2.9452	Hydrogen Bond	Conventional Hydrogen Bond
ILE90	2.07672	Hydrogen Bond	Conventional Hydrogen Bond
LYS45	2.72555	Hydrophobic	Pi-Sigma
LEU171	2.83413	Hydrophobic	Pi-Sigma
MET66	5.90633	Other	Pi-Sulfur
LEU17	5.20756	Hydrophobic	Alkyl
LEU17	4.23893	Hydrophobic	Pi-Alkyl
VAL25	5.17683	Hydrophobic	Pi-Alkyl
ALA43	4.72332	Hydrophobic	Pi-Alkyl
VAL25	4.60138	Hydrophobic	Pi-Alkyl
ALA43	5.20594	Hydrophobic	Pi-Alkyl
LYS45	4.49156	Hydrophobic	Pi-Alkyl
<b>VEGF-A complexed with adaphostin</b>			
HIS79	3.30248	Hydrogen Bond	Conventional Hydrogen Bond
TYR38	2.0803	Hydrogen Bond	Conventional Hydrogen Bond
LYS77	5.09656	Hydrophobic	Alkyl
LYS77	4.90891	Hydrophobic	Alkyl
HIS79	4.92344	Hydrophobic	Pi-Alkyl
PRO78	5.47674	Hydrophobic	Pi-Alkyl
ILE39	3.81041	Hydrophobic	Pi-Alkyl
<b>TGFR1 kinase complexed with adaphostin</b>			
SER280	2.75731	Hydrogen Bond	Conventional Hydrogen Bond
GLY286	3.52311	Hydrogen Bond	Carbon Hydrogen Bond
SER287	3.75326	Hydrogen Bond	Carbon Hydrogen Bond
LYS232	4.0013	Electrostatic	Pi-Cation
VAL219	3.85337	Hydrophobic	Pi-Sigma
LEU260	3.84277	Hydrophobic	Pi-Sigma
LEU340	3.95459	Hydrophobic	Pi-Sigma
LYS337	4.03168	Hydrophobic	Alkyl
ILE211	5.34457	Hydrophobic	Pi-Alkyl
ALA230	5.22616	Hydrophobic	Pi-Alkyl
LYS232	4.61046	Hydrophobic	Pi-Alkyl
<b>PKC-<math>\beta</math>II complexed with obeticholic acid</b>			
ASP146	2.90673	Hydrogen Bond	Conventional Hydrogen Bond
VAL18	5.21721	Hydrophobic	Alkyl
VAL18	3.82528	Hydrophobic	Alkyl
ALA145	3.10588	Hydrophobic	Alkyl
LYS33	4.49913	Hydrophobic	Alkyl
LEU10	4.55211	Hydrophobic	Alkyl
PHE15	4.14543	Hydrophobic	Pi-Alkyl
PHE15	4.92236	Hydrophobic	Pi-Alkyl

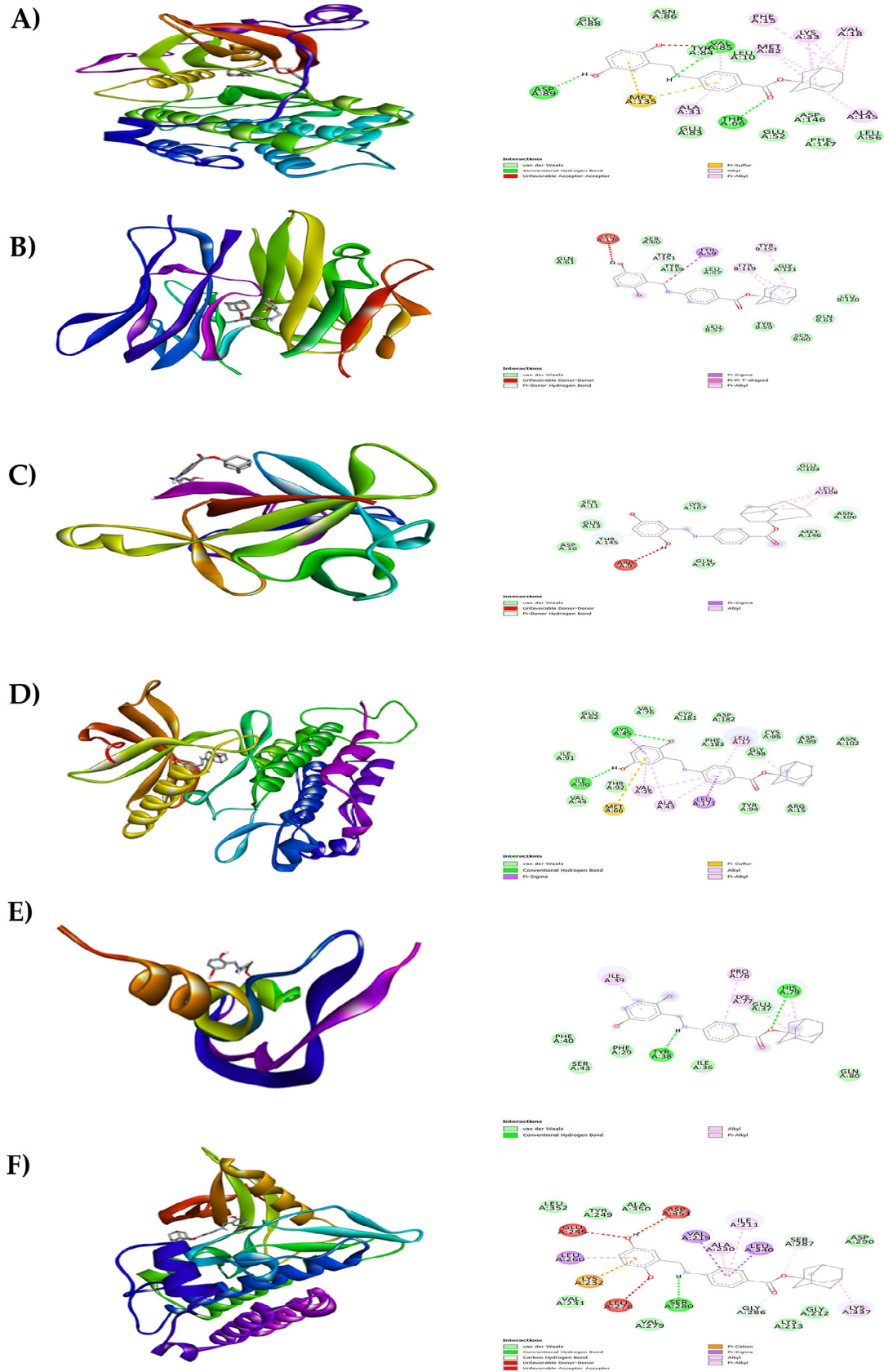
**Table 2.** *Continued*

Interaction residues	Distance	Category	Types
<b>TNF<math>\alpha</math> complexed with obeticholic acid</b>			
TYR59	3.01852	Hydrogen Bond	Conventional Hydrogen Bond
HIS15	5.22368	Hydrophobic	Pi-Alkyl
TYR59	3.82176	Hydrophobic	Pi-Alkyl
TYR59	4.23758	Hydrophobic	Pi-Alkyl
TYR151	4.27561	Hydrophobic	Pi-Alkyl
TYR59	4.18769	Hydrophobic	Pi-Alkyl
<b>IL-1<math>\beta</math> with obeticholic acid</b>			
LYS101	2.78583	Hydrogen Bond	Conventional Hydrogen Bond
GLU103	3.26326	Hydrogen Bond	Conventional Hydrogen Bond
PHE44	3.61118	Hydrophobic	Pi-Sigma
ILE54	5.26877	Hydrophobic	Alkyl
ILE54	5.16643	Hydrophobic	Alkyl
LEU4	5.43239	Hydrophobic	Alkyl
PHE44	4.93858	Hydrophobic	Pi-Alkyl
<b>PDGFRA with obeticholic acid</b>			
VAL161	2.55777	Hydrogen Bond	Conventional Hydrogen Bond
MET66	2.60333	Hydrogen Bond	Carbon Hydrogen Bond
ILE65	4.98082	Hydrophobic	Alkyl
CYS160	5.23492	Hydrophobic	Alkyl
ILE65	5.20837	Hydrophobic	Alkyl
ILE65	4.90546	Hydrophobic	Alkyl
MET66	4.8268	Hydrophobic	Alkyl
VAL76	4.91524	Hydrophobic	Alkyl
<b>VEGF-A with obeticholic acid</b>			
A:TYR38	2.2914	Hydrogen Bond	Conventional Hydrogen Bond
GLU37	2.05672	Hydrogen Bond	Conventional Hydrogen Bond
PRO78	5.08241	Hydrophobic	Alkyl
LYS77	4.12618	Hydrophobic	Alkyl
<b>TGFB1 kinase with obeticholic acid</b>			
LYS213	3.18529	Hydrogen Bond	Conventional Hydrogen Bond
SER280	2.94006	Hydrogen Bond	Conventional Hydrogen Bond
VAL219	5.27115	Hydrophobic	Alkyl
VAL219	3.93297	Hydrophobic	Alkyl
LYS337	5.0964	Hydrophobic	Alkyl
ALA350	4.27292	Hydrophobic	Alkyl
ILE211	5.2547	Hydrophobic	Alkyl
LEU340	5.15809	Hydrophobic	Alkyl
LEU340	4.34364	Hydrophobic	Alkyl
LYS337	3.41833	Hydrophobic	Alkyl
VAL219	5.19865	Hydrophobic	Alkyl

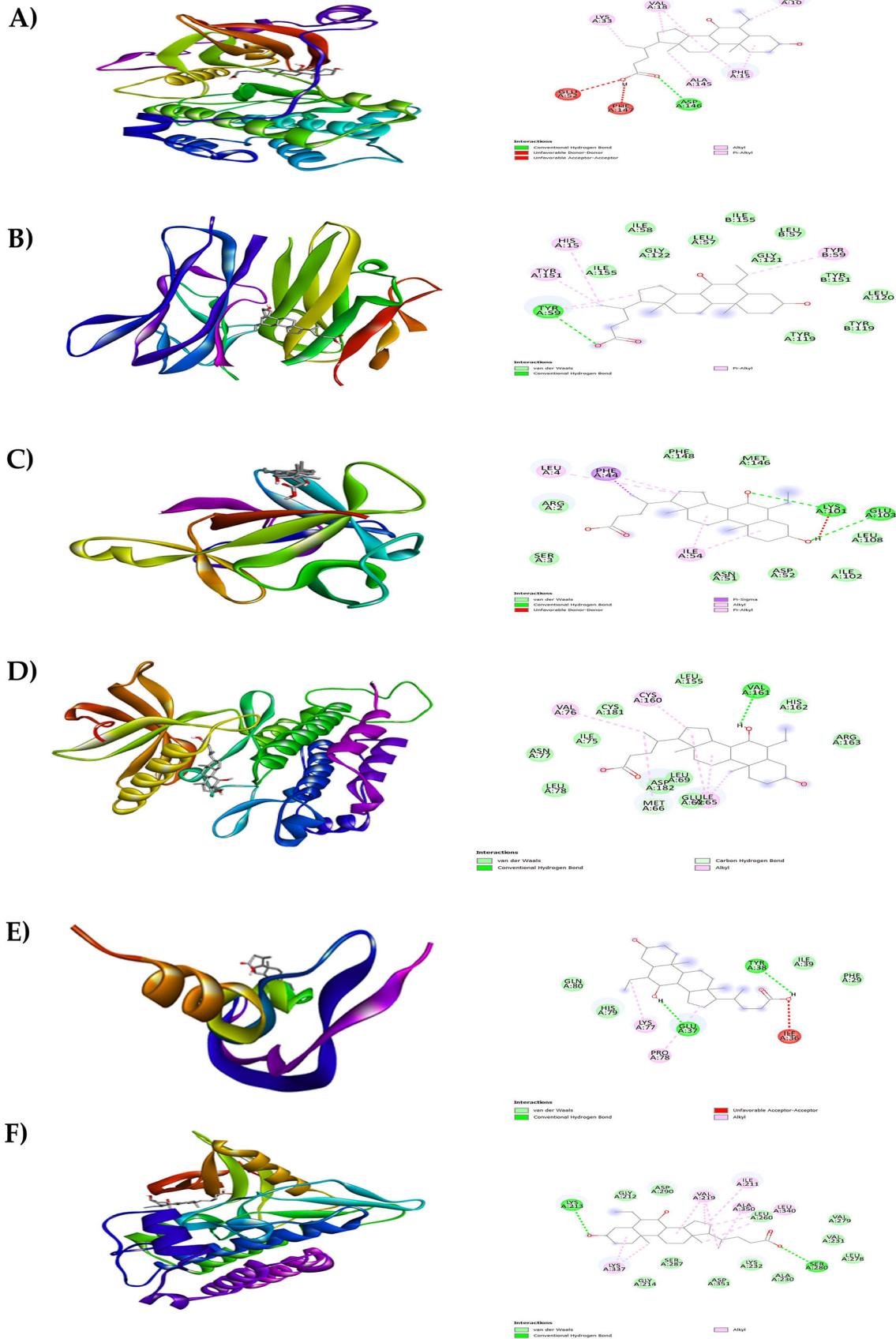
## Discussion

A wound is defined as a disruption of anatomical integrity of any biological tissue by physical, mechanical, chemical, or microbial factors. The wound healing process starts following the wound formation and repairs the injured or damaged tissues.<sup>28</sup> Development of natural wound healing agents is of current interest to mitigate the side effects of wound care products.<sup>29</sup> Nature has gifted us with a diverse range of medicinal plants to treat various ailments including wound healing.<sup>30</sup> It has been reported that *N. sativa* has a wide range of pharmaceutical properties.<sup>31</sup> The complete extracts and their phytochemicals from *N. sativa* seeds have not been investigated for their wound healing properties. Therefore, this study has been conducted to determine wound healing activity of various solvent extracts of *N. sativa* seeds by *in vitro* and *in silico* analyses. Cells, cellular components, and chemical mediators interact to heal wounds in a complex way.<sup>32</sup> The process of wound healing is broadly divided into four phases, namely coagulation and hemostasis, inflammation, proliferation, and scar tissue formation (maturation). The process of angiogenesis involves the formation of new blood vessels, and it is one of the most important steps in wound healing.<sup>33</sup> In the wound area, angiogenic signals from the macrophage-derived factors stimulate the proliferation, migration and differentiation of endothelial cells, and subsequent increase in blood vessel formation.<sup>34</sup> During the wound healing process, the new capillaries develop into the fibrin clots, which subsequently form a microvascular network that is a critical for the formation tissue formation. HUVECs are primary endothelial cells from umbilical cord and are widely used for *in vitro* investigation of angiogenesis.<sup>35</sup> In order to





**Figure 8. Results of molecular docking analysis.** Molecular docking analysis of adaphostin complexed with targeted proteins of A) PKC- $\beta$ II, B) TNF $\alpha$ , C) IL-1 $\beta$ , D) PDGFRA, E) VEGF-A and F) TGFBR1.



**Figure 9. Results of molecular docking analysis.** Molecular docking analysis of obeticholic acid complexed with targeted proteins of A) PKC-βII, B) TNFα, C) IL-1β, D) PDGFRA, E) VEGF-A and F) TGFBR1.



**Table 3. ADME properties of screened phytochemicals.**

Compound name (PubChem CID)	Mol. Wt	H. Bond Donor	H. Bond Acceptor	Log P	Rotatable Bond
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- (543764)	444.7	0	2	9.5	0
Adaphostin (387042)	393.5	3	5	5.2	6
Obeticholic Acid (447715)	420.6	3	4	5.7	5

determine whether different crude extracts of *N. sativa* are cytotoxic, this study first carried out MTS tests on HUVECs. As shown in the [Figure 2](#), all tested crude extracts did not exert any cytotoxic activity on the HUVECs. Notably, ethanol and chloroform extracts significantly enhanced the viability of HUVECs. Angiogenesis involves a complex series of molecular events mediated by several factors. In the wound healing process, there are a number of growth factors that play key roles, including PDGF, TGF- $\beta$ 1, EGF, VEGF and bFGF.<sup>36</sup> Proangiogenic factors, such as VEGF, promote the survival, migration, differentiation, self-assembly, and self-repair of endothelial cells. As soon as VEGF binds to the VEGF receptor, multiple downstream protein kinase pathways are activated and new blood vessels are formed.<sup>37</sup> The wound healing process is also affected by PDGF, another important growth factor. Additionally, PDGF stimulates the formation of new blood vessels by acting as a pro-angiogenic factor.<sup>38</sup> Thus, the onset of angiogenesis is positively regulated by both PDGF and VEGF. Therefore, this study has analyzed the expression levels of VEGF and PDGF in both tested cell lines. As seen in [Figures 4 and 5](#), both ethanol and chloroform extracts increased the expression levels of VEGF and PDGF in both NHDFs as well as HUVECs. This indicates that the *N. sativa* seed extracts might promote the cell survival and self-repair of cells, and subsequent wound healing efficacy.

Wounds are characterized by excessive inflammation due to increased local and systemic levels of TNF $\alpha$ .<sup>39</sup> Evidence suggests that inhibition of TNF $\alpha$  is critical for the treatment of wounds. It plays an important role in wound healing by re-epithelializing, inducing inflammation, stimulating angiogenesis, and forming new skin tissue.<sup>40</sup> The docking studies were used to predict the possible therapeutic effects of phytochemicals of *N. sativa* against wound healing related molecular targets including TNF $\alpha$ , TGFBR1 kinase, IL-1 $\beta$ , PKC- $\beta$ II, VEGF and PDGF. Based on the docking studies, it was predicted that bioactive compounds *N. sativa* showed strong binding affinity to select the wound healing related targets. Together, the current study results suggest that *N. sativa* seeds might exert wound healing effects mainly through the modulation of proangiogenic factors.

## Conclusions

Management of chronic wounds and the development of natural wound healing products is of critical importance in the area of clinical research. Medicinal plants have long served as a potential source of wound healing medications since ancient times, with their use going as far back as 3,000 BC).<sup>41</sup> *N. sativa* is one such medicinal herb that has been shown to possess a wide range of pharmacological properties. In this aspect, this study investigated the wound healing properties of different solvent extracts of *N. sativa* seeds. Both ethanolic and chloroform extracts significantly improved the viability in NHDF and HUVEC cell lines. Besides, both ethanolic and chloroform extracts increased the expression levels of VEGF and PDGF proteins indicating *N. sativa* can have significant impact on the rate of wound healing by promoting the angiogenesis and cell proliferation. The computational analysis of identified phytochemicals from the GC-MS spectrum showed potent binding affinity towards the wound healing-associated target proteins such as PKC- $\beta$ II, TNF $\alpha$ , IL-1 $\beta$ , PDGFRA, VEGF-A, and TGFBR1 kinase. Based on the current findings, *N. sativa* seed extracts can exert potent wound healing activity *via* activating the VEGF and PDGF signaling pathways. However, further *in vitro* and *in vivo* studies are still required to confirm the current findings.

## Data availability

### Underlying data

Zenodo: *Nigella sativa* L. seed extracts promotes wound healing progress by activating VEGF and PDGF signalling pathways: An *in vitro* and *in silico* study, <https://doi.org/10.5281/zenodo.7712528>.<sup>42</sup>

This project contains the following data:

- Cell viability assay.zip
- Docking results.zip

- GC-MS identified Compounds from *N. sativa* seed extracts.zip
- Protein structures.zip
- Westernblot raw data.pptx
- List of tables.docx

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

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In this study, the authors examined the wound healing properties of *Nigella sativa* seed extracts using normal cell lines such as normal human dermal fibroblasts (NHDFs) and normal human umbilical vein endothelial cells (HUVECs). An analysis of western blots was performed to determine VEGF and PDGF expression. Moreover, computational analysis was conducted to screen the potential bioactive compounds for wound healing. The overall quality of the research work and its clarity are excellent. The figures are clear and of good quality; in fact, they are intended to improve the reader's experience with the paper. The work is sufficiently scientifically interesting and technically sound to merit indexing.

Although there are many minor corrections that need to be made, the authors should take time to revise the manuscript carefully.

1. Provide a detailed description of the incubation period, treatment period of the cells, and concentration of the extracts used in the cell viability analysis.
2. The international unit system should be checked and corrected throughout the manuscript.
3. The author stated that each bar represented the mean + SEM of six independent observations, but the figure only shows five independent bars. The author should correct the figures.
4. There is a need for the author to clarify whether the treatment period was 24 or 48 hours.
5. 'Effect of crude seed extracts on VEGF and PDGF protein expression in HUVEC lines' it should be 'Effect of crude seed extracts on VEGF and PDGF protein expression in HUVEC cell line'.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Environmental Science

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 18 May 2023

<https://doi.org/10.5256/f1000research.145007.r171685>

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**Monica Mironescu**

Faculty of Agricultural Sciences Food Industry and Environmental Protection, Lucian Blaga University of Sibiu, Sibiu, Romania

*Nigella sativa* is native to Southeast Asia and its seed extract has been used for many centuries to treat diseases and more recently, the main active compound - thymoquinone has been tested for its effectiveness against diseases such as cancer. There are numerous researches and reviews that evaluate the medicinal use of *Nigella sativa* in various conditions. A series of preclinical and clinical studies describe multiple effects of *Nigella sativa* in animal or cellular models such as bronchodilator, antihistaminic, anti-inflammatory, anti-leukotriene and immunomodulatory effects.

This research is investigating the action of *Nigella sativa* seed extracts on wound healing and its progress by activating VEGF and PDGF signaling pathways. In vitro and in silico studies are realized. The research is very complex and well done.

Still, there are two aspects to be improved by the authors:

1. There is not enough discussion of the results obtained by the authors in comparison with other researches.
2. Some spelling mistakes are given below:
  - To connect the time of the verb with the subject in the proposition: Infection by pathogenic bacteria delay the healing process and increase health risks to the general public;
  - 'Aliments' instead of 'ailments';
  - 'Tap water' instead of 'tab water';
  - To finish the proposition: A humidified atmosphere containing 5% CO<sub>2</sub> was used for HUVEC and NHDF cell lines during the experiment to maintain 37°C for the duration ...;
  - Comma in the phrase "...as the control)" has to be removed.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** microbiology, antimicrobials

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 16 May 2023

<https://doi.org/10.5256/f1000research.145007.r171686>

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**Balasubramani Ravindran**

Department of Environmental Energy & Engineering, Kyonggi University, Suwon, South Korea

Palanisamy *et al.*, have demonstrated the role of *Nigella sativa* L. (*N. sativa*) seed extracts for wound healing potential using two different cell lines and with computational work in order to show the mechanisms via regulates VEGF and PDGF signalling. This research work has scientific merit and sounds enough to be considered in the F1000Research journal.

However, the authors should justify the following:

1. Whether the authors have done phytochemical screening analysis in all the extracts?
2. What was the dilution of primary antibody? Please mention.
3. The plant name should be in italic style, please check it throughout the manuscript.
4. The author may continue these findings in *in vivo* experimental animal model.
5. How can the authors conclude *N. sativa* seed extracts possess wound healing properties with basic experiments? Please justify.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Plants

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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