



The effect of different solvent selection on the antioxidant capacity and cytotoxicity of *Nigella sativa* extracts

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ABSTRACT: *Nigella sativa* (*N. sativa*) seed is used for treatment as well as food use. In the study, *N. sativa* seed was extracted using methanol (ME), water (SE), vinegar (SiE) and total phenolic/flavonoid content, DPPH radical scavenging activity and effects on human skin cell line (HDF) and human melanoma cell line (451-Lu) were determined. According to the findings, total phenolic substance amount in *N. sativa* extract prepared with methanol (ME) was determined as 72.08 mg GAE/g extract; total flavonoid substance amount as 192.30 mg QE/g extract; DPPH activity as 85.17%. In cytotoxic assays, selective toxicity of the extracts was observed. No significant toxic effect was detected in the HDF cell line, while suppression of cell viability was noted in the 451-LU melanoma cell line. These findings suggest that the extracts may have the potential to selectively target cancer cells without damaging healthy cells and are promising as potential anticancer agents.

KEYWORDS: *Nigella sativa*, Cytotoxicity, Antioxidant, Solvent selection, Extraction

INTRODUCTION

According to data from the Food and Agriculture Organization (FAO), there are approximately 72,000 species of flowering medicinal plants worldwide, and around 500 species in our country are classified as medicinal and aromatic plants [1, 2]. In our country, medicinal and aromatic plants are generally used in folk medicine and have an important economic value [3]. *Nigella sativa*; It is known by different names such as black cumin, grain of abundance and black seed and is located in the *Nigella* genus of the Ranunculaceae family. It is generally distributed in Mediterranean countries, and 12 of the 20 species of the annual herbaceous plant grow in our country [1, 4, 5, 6, 7]. The seeds of the *N. sativa* plant have a special scent and are used as a spice as well as in the pharmaceutical, perfumery and cosmetic industries [8]. The fixed and volatile oils of the *N. sativa* plant are widely used in modern medicine due to their analgesic, antibacterial, antitumor and blood sugar lowering effects [9]. Thymoquinone found in *N. Sativa* provides the plant with different pharmacological properties such as anticancer, antioxidant, antihistamine, anti-inflammatory and antimicrobial [10]. The process of taking one or more compounds in a solid or liquid phase into another liquid phase by taking advantage of their different solubility properties is called extraction [11]. Extraction is

the first step in food analysis to obtain fast, accurate and reliable results [12]. This study was carried out to determine the effectiveness of solvent systems with different polarity levels on *N. sativa*. The effectiveness of *N. sativa* extractions in extracts taken from solvents with different polarity levels on total phenolic content, total flavonoid content, DPPH radical scavenging activity and cytotoxicity were evaluated.

MATERIALS AND METHODS

N. sativa Extraction

10 g of *N. sativa* seeds, which were ground into powder, were extracted using 100 mL of methanol (PI = 5.1), deionized water (PI= 9.0) and food grade vinegar (pH = 3) at 40 °C with a magnetic stirrer for 48 hours. Then, the mixture was filtered using filter paper after waiting to reach room temperature. It was kept in a +4 °C refrigerator for 24 hours and the analyses were carried out the following day [13]. In experimental analyses, calculations were made based on extract weight.

Antioxidant analyses

Total phenolic content determination

The total phenolic contents of *N. sativa* extracts obtained from different solvents were determined according to the

method of Mohammed Hajji et al. [14]. Samples were taken into test tubes in 500 µL volumes, and 500 µL of Folin reagent was added to them. Then, 500 µL of Na₂CO₃ and 3500 µL of distilled water were added, and each test tube was vortexed. The test tubes were kept in a dark laboratory environment for 90 minutes. At the end of the waiting period, absorbance measurements were made at 760 nm using a spectrophotometer (Shimadzu UV-1800). The results were calculated as gallic acid equivalence [14]. The experiments were designed and carried out in three replications.

Total flavonoid content determination

Total flavonoid contents of *N. sativa* extracts obtained from different solvents were taken into 250 µL test tubes according to the method of Zhishen et al. [15], 150 µL NaNO₂ was added to them and after waiting for 6 min, 75 µL AlCl₃ was added. 1000 µL NaOH and 2500 µL dH₂O were added to the test tubes that were kept in the laboratory environment for 5 min. After the additions, the samples were vortexed. The absorbance measurements were made at 510 nm using a spectrophotometer (Shimadzu UV-1800) and calculated as quercetin equivalence [15]. The experiments were designed and carried out in three replications.

DPPH radical scavenging activity

The DPPH radical scavenging activity of *N. sativa* extracts obtained from different solvents was determined according to the method of Hatano et al. [16]. 0.3 mL of samples were taken, 2.7 mL of freshly prepared DPPH solution was added to the test tubes and the samples were vortexed. They were kept in the dark laboratory environment for 60 min. Pure water was used instead of the sample as a control. Absorbance measurements at 517 nm with a spectrophotometer (Shimadzu UV-1800) were calculated by comparing with BHA [16]. The experiments were designed and carried out in three replications.

$$\text{DPPH scavenging (\%)} = \left[\frac{(A_C - A_E)}{A_C} \times 100 \right]$$

* A_C: Absorbance of control sample

* A_E: Absorbance of the example sample

Cytotoxicity analyses

10% (50 mL) FBS and 1% (5 mL) penicillin-streptomycin were added to 500 mL DMEM medium. It was stored at +4°C. The 451-Lu and HDF cell lines, stored at -80 °C, were used. Cells thawed for karyotyping were placed in 15 ml falcon tubes, and after adding 4 ml of DMEM medium, they were centrifuged at 4500 rpm for 4 minutes, and the supernatant was discarded. 4 mL of medium was added to the cells again, and pipetting was performed. After this procedure, the cells were transferred to 25 cm² flasks,

cultured in a 37°C and 5% CO₂ incubator, and seeded into wells of a 96-well plate at a density of 10,000 cells per well. The concentrations of the samples were adjusted to 2 mg/mL. Different concentrations were applied using serial dilutions (1000, 500, 250, 125, 62.5, 31.25 µg/mL). It was incubated at 37°C and 5% CO₂ for 24 hours. 50 µL of XTT (*sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate*) was added to each well, and after a 4-hour incubation, measurements were taken at 450-630 nm (Spectramax Plus 384 Elisa microplate) [17].

Statistical analysis

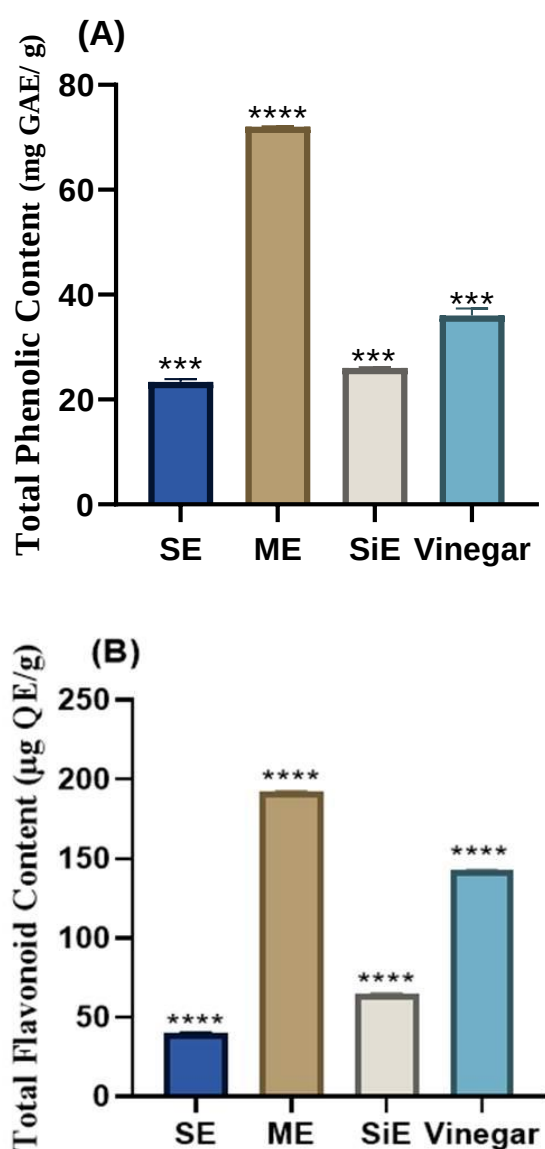
Experiments were performed in duplicate and are shown as mean ± standard error mean (SEM). One-way analysis of variance (ANOVA) was used to evaluate the parameters of different groups using GraphPad Prism (version 8.3.0 GraphPad Software Inc., San Diego, CA, USA). Tukey analyses were performed to compare the parameters of different groups and p < 0.05 was considered statistically significant. The levels of statistical significance in the analysis results are indicated as follows: ns (not significant): p > 0.05; *: p < 0.05; **: p < 0.01; ***: p < 0.001; **: p < 0.0001.

RESULTS AND DISCUSSION

Antioxidant analyses

There is a great interest in the production and use of natural antioxidants and antimicrobial substances of plant origin as an alternative to synthetic antioxidants [18]. Due to the antioxidant properties of *N. sativa* seeds and extracts obtained from its seeds, it is used extensively in different areas such as pharmacy, cosmetics and food industry, and *N. sativa* oil is among the herbal essential oils used to prevent the deterioration of foods during storage [19]. Phenolic substances constitute an important group of natural antioxidants [20]. The bactericidal, anti-cancer, anti-inflammatory, anti-diabetic and anti-radical properties of *N. sativa* essential oil were reported by Sultan [21]; Lutterodt et al. [22]; Bourgou et al. [23]. In the study conducted by Ilhan et al. [24], it was determined that *N. sativa* oil exhibited antioxidant properties in mice [24]. In their study, Kar et al. [25], found that the total phenolic content of *N. sativa* seeds obtained from two different regions was 1451 mg/GAE g and 1588 mg/GAE g according to the Folin-Ciocalteu method [25]. Total phenolic content of methanolic extracts of *N. sativa* seeds taken from India region was determined as 4.1 mg GAE/g extract [26]. In the study conducted by Buran et al. [27], in which the antioxidant, phenolic and flavonoid

substance amounts of grape seed, *N. sativa* and flaxseed powders were determined, it was determined that *N. sativa* seeds had the highest phenolic substance amount (39.18 ± 0.87 mg/g GAE) in methanol extracts [27]. In this study, in the analyzes performed using three different solvents, it was determined that methanol extracts had the highest phenolic and flavonoid content. Total phenolic content was found as SE 23.77 mg GAE/g extract, SiE 26.12 mg GAE/g extract, ME 72.08 mg GAE/g extract, vinegar 36.99 mg GAE/g (Figure 1(A)); total flavonoid content was found as SE 40.3 mg QE/g extract, SiE 64.63 mg QE/g extract, ME 192.30 mg QE/g extract, vinegar 142.46 mg QE/g (Figure 1(B)).



****: $p < 0.0001$

Figure 1(A): Total Phenolic Content, **(B):** Total Flavonoid Content

In the study conducted by Kar et al. [25], in which the antioxidant activity of *N. sativa* seeds obtained from Samsun and Egypt was examined, it was determined that *N. sativa* extracts had better activity than synthetic antioxidants such as BHA and BHT, which are widely used in the industry [25]. As the absorbance values read at 517 nm in the DPPH method decrease, the free radical scavenging activity increases [28]. The DPPH radical scavenging activity of our samples was calculated as percent inhibition and SE was found to be 66.23%, SiE was 64.77%, ME was 85.17%, vinegar was 76.87% and BHA, which is one of the synthetic antioxidants, was found to be 88.37% (Figure 2). The numerical differences between the results are thought to be due to differences in plant cultivation time, climate and environmental conditions, and changes in soil structure, as stated by Ashraf et al. [29].

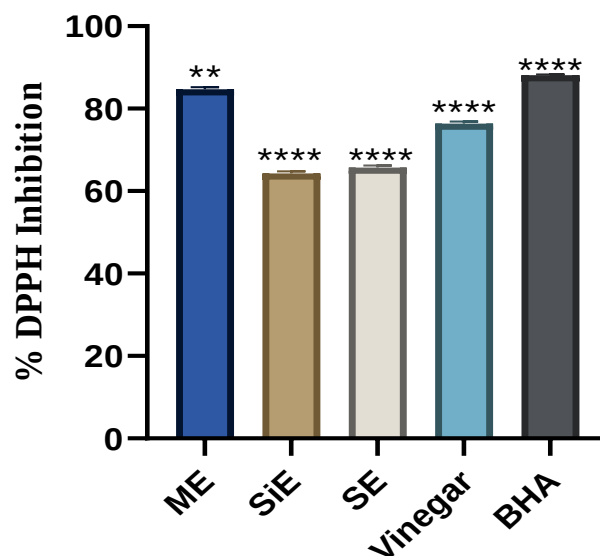


Figure 2. DPPH scavenging activity of different extracts

Cytotoxicity assays

In the graphs, the cytotoxic effects of a tested substance on HDF (Human Dermal Fibroblast) and 451-Lu (melanoma cell line) are shown with cell viability percentages at different concentrations. In both graphs, the cell viability of the control group is accepted as 100%.

Figure 3(A) HDF Cells: There is no statistically significant decrease in HDF cells at concentrations ranging from 1000–62.5 μ g/mL. This shows that the tested substance is not cytotoxic on HDF cells up to these concentrations. However, at a concentration of 31.25 μ g/mL, cell viability decreased to 92.1296 and this difference was found to be statistically significant ($p < 0.05$). This suggests that significant toxicity may occur on HDF cells at low

concentrations. Contrary to general expectations, toxicity occurred at the lowest concentration. This result may indicate that the dose-response relationship is not linear.

Figure 3(B) 451-Lu Cells:

Cell viability was significantly decreased ($p<0.05$) compared to the control group at all concentrations (1000–31.25 $\mu\text{g/mL}$). This indicates that the tested substance exhibited a significant cytotoxic effect on the melanoma cell line and reduced cell viability in a dose-independent manner.

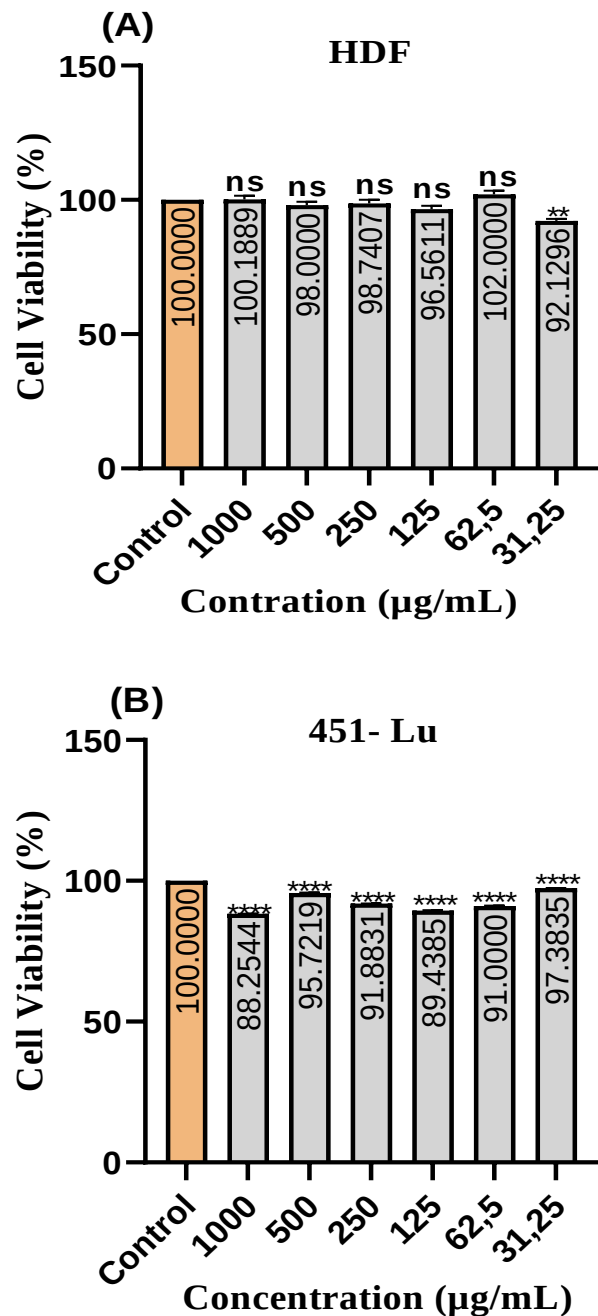


Figure 3. Effect of SE on viability on HDF and 451-Lu

In HDF cells (Figure 4(A)), cell viability was between 95–105% at all concentrations, and it was observed that the extract did not show a cytotoxic effect, and even at some doses it could have a proliferative effect on cell proliferation. Although a similar proliferative effect was observed between 451-LU cells and HDF cells, a slight decrease in cell viability was observed with increasing doses in 451-LU cells (Figure 4(B)), while viability generally remained at high levels and a more proliferative effect was observed in HDF cells. This may suggest that HDF cells were more sensitive to the extract and showed a proliferative response, while 451-LU cells showed a slightly more indecisive response at similar doses.

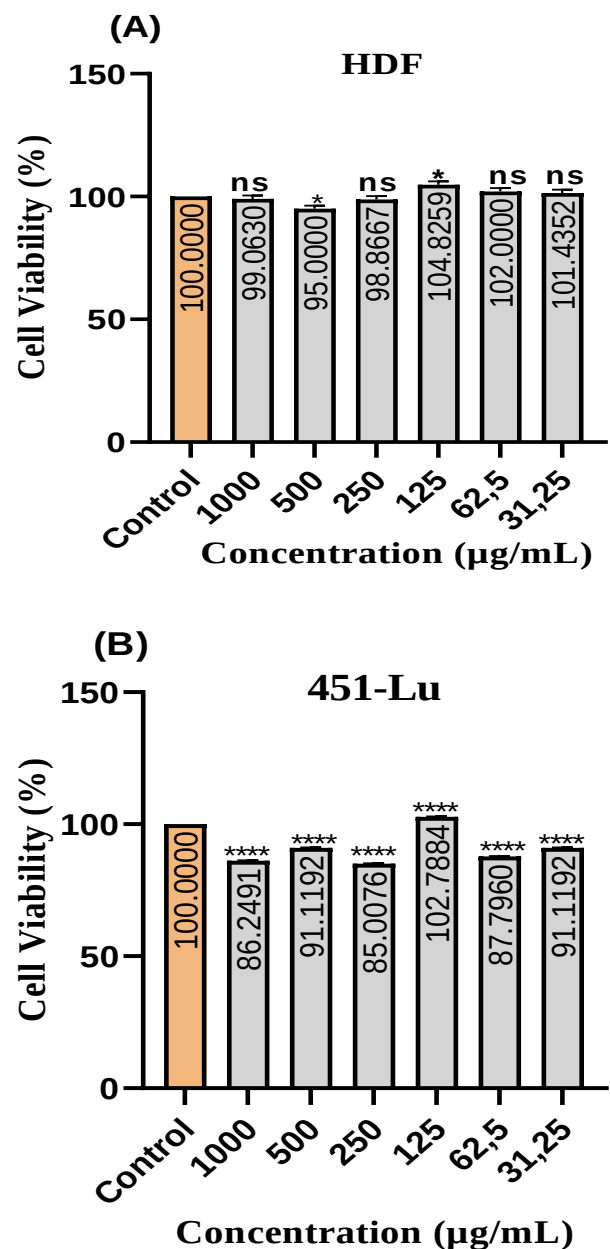


Figure 4. Effect of ME on viability on HDF and 451-Lu.

In HDF cells (Figure 5(A)), SiE extract generally did not show cytotoxic effect and cell viability was determined to be in the range of 95-106% at most doses. Vinegar application generally maintained the viability of HDF cells and it was observed that it may have a proliferative effect at some doses. When the effect of SiE extract on 451-LU cells was examined, a slight decrease in cell viability was observed at some concentrations (especially 1000 and 250 $\mu\text{g/mL}$), suggesting that the extract may have limited cytotoxic effect. These findings suggest that SiE extract may have limited anticancer potential on 451-LU cells (Figure 5(B)); however, dose-dependent responses should be carefully evaluated, especially in healthy cells. However, the fact that the viability was above 100% at the dose of 500 $\mu\text{g/mL}$ and increased to 106% at the dose of 31.25 $\mu\text{g/mL}$ suggests that the anticancer effect of the extract does not show a significant dose-response relationship.

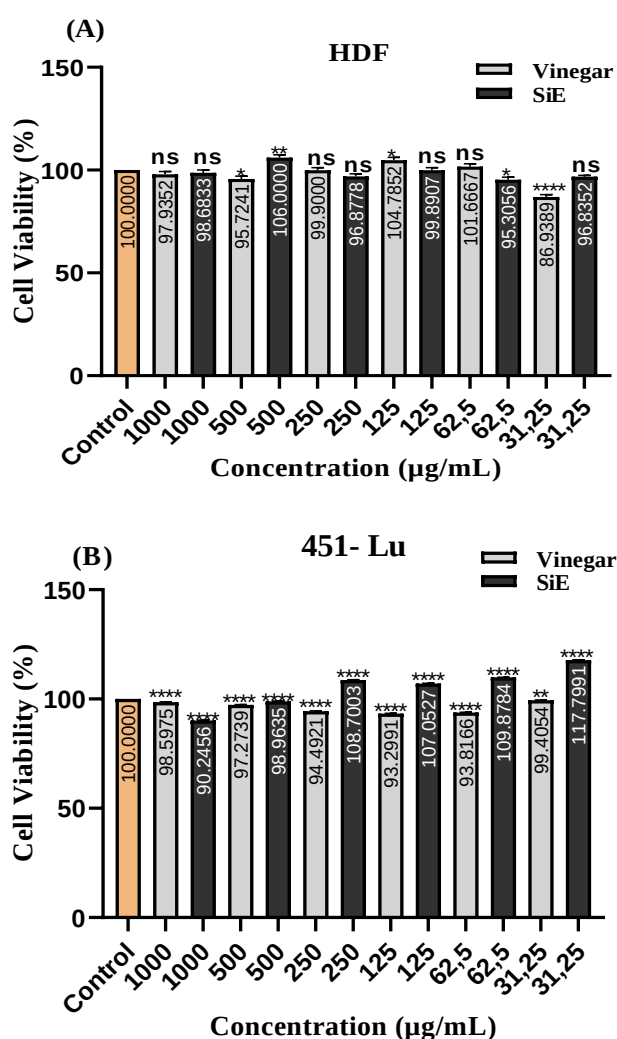


Figure 5. Effect of SiE and Vinegar on viability on HDF and 451-Lu.

Various methods are available to assess the cytotoxicity of extracts from plants. Concentrations that may result in lower and higher toxicity can be determined by standard test methods used for cytotoxicity assessments [30, 31]. Natural compounds can cause cellular morphological changes through cytotoxic action mechanisms, prevent attachment, inhibit cell cycle and DNA replication, and significantly reduce cell viability [32]. Thymoquinone, found in *N. sativa* seeds, controls the cell cycle and can affect the expression of gene proteins that regulate cell proliferation. Previous studies have reported that thymoquinone, which affects different points in the cell cycle, has a lethal effect on cancer cells. Studies show that *N. sativa* seed extracts obtained using methanol as a solvent do not have a cytotoxic effect on healthy cells, but reduce the viability of cancer cells [30, 31, 32, 33]. During the carcinogenesis process, cells tend to grow and proliferate uncontrollably. This can lead to an increase in tumor volume and delay the response to drugs used in cancer treatment. Studies have shown that thymoquinone can modulate the expression or activity of genes that regulate the cell cycle and the proteins belonging to these genes. It has been reported that thymoquinone can induce cell death by showing cytotoxic effects on cancer cells, especially at different stages of the cell cycle (G0/G1-G1/S-G2/M) [10, 34, 35, 36]. In addition, the effect of thymoquinone on apoptosis mechanisms is also remarkable. It is reported that it can trigger apoptosis by both p53-dependent and p53-independent pathways by interacting with the p53 protein, which plays an important role in the regulation of the cell cycle and the induction of apoptosis [10, 36, 37]. While thymoquinone increases the expression of proapoptotic factors in the suppression of cancer, it reduces the levels of antiapoptotic proteins and thus activates apoptosis mechanisms. It has also been stated that it plays an important role in the cell death process by regulating caspase pathways [10,36,38,39].

CONCLUSION

The findings of this study revealed that *N. sativa* seed extracts possess both anticancer and antioxidant properties. Cytotoxicity assays demonstrated that the extracts exhibited selective toxicity; no significant toxicity was observed in the HDF cell line, whereas a notable reduction in cell viability was detected in the 451-LU melanoma cell line. This suggests that the extracts may selectively target cancer cells without harming healthy cells, indicating their potential as anticancer agents. Furthermore, the antioxidant capacity data showed that the bioactive compounds present in the extracts were effective in scavenging free radicals and reducing

oxidative stress. In particular, compounds such as thymoquinone, which naturally occurs in *N. sativa* seeds, have been reported in the literature to regulate the cell cycle and induce apoptosis in cancer cells. Accordingly, the biological activities of the *N. sativa* extracts obtained using different solvents may vary depending on their chemical composition, but overall, they demonstrate significant therapeutic potential.

DECLARATIONS

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Authorship Contributions

Concept and Writing: E.B., Z.D. ; Data Collection and Interpretation: E.B., Z.D.; Proofreading: B.K.

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Consent for publication

Not applicable.

Competing interests

The authors declared that there is no conflict of interest.

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