DOI: http://dx.doi.org/10.32571/ijct.1024979 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Phenolic profile, antioxidant, DNA protection, acetylcholinesterase, butyrylcholinesterase and urease inhibition activities of *Coriandrum sativum* L. leaf, seed and flower extracts

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Received: 18 November 2021; Revised: 15 January 2022; Accepted: 19 January 2022

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Citation: Ozen, T.; Yenigun, S.; Toka, M. *Int. J. Chem. Technol.* **2022**, 6 (1), 21-32.

ABSTRACT

In this study, chemical content, antioxidant, enzyme inhibition, and DNA protection activities of extracts obtained from different solvents of the *Coriandrum sativum* leaf, flower and seed were determined. Total phenol and flavonoid contents of *C. sativum* leaf, seed and flower hexane extracts were higher than water, methanol, ethanol and ethyl acetate extracts. The highest anthocyanin content was found in the flower part of *C. sativum*. From the antioxidant activity tests, total antioxidant activity of flower aqueous extract, reducing power of seed aqueous extract, H_2O_2 scavenging activity of leaf ethyl acetate extract, $OH^{\cdot}$ radical scavenging activity of leaf hexane extract, free radical scavenging activity of flower methanol extract, metal chelate activity of leaf ethyl acetate extract, superoxide anion scavenging activity of leaf aqueous extract and lipid peroxidation inhibition activity of the leaf ethyl acetate extract had the highest. It was found that the urease inhibition activity of the seed methanol extract and the acetylcholinesterase and butyrylcholinesterase inhibition activities of the seed ethanol extract presented effective inhibition activity as 80.30±0.20%, 112.83±10.75 µg/mL, and 334.28±23.09 µg/mL, respectively. The leaf hexane, flower ethyl acetate, and leaf methanol extracts showed the highest DNA protection activities with values of 71.86%, 70.89%, and 69.38%, respectively. According to the phytochemical content and biochemical activity results, this study is a valuable report proving that the *C. sativum* plant is a natural effective product.

Keywords: *Coriandrum sativum* L. (kinzi), antioxidant activity, enzyme inhibition, DNA protection activity

Coriandrum sativum L. yaprak, tohum ve çiçek ekstraktlarının fenolik profili, antioksidan, DNA koruma, asetilkolinesteraz, butirilkolinesteraz ve üreaz inhibisyon aktiviteleri

ÖZ

Bu çalışmada *Coriandrum sativum* yaprağı, çiçeği ve tohumunun farklı çözücülerinden elde edilen ekstraktların kimyasal içeriği, antioksidan, enzim inhibisyon ve DNA koruma aktiviteleri belirlendi. *C. sativum* yaprak, tohum ve çiçek hekzan ekstraktlarının toplam fenol ve flavonoid içerikleri su, metanol, etanol ve etil asetat ekstraktlarından daha yüksek bulundu. En yüksek antosiyanin içeriği *C. sativum* çiçek kısmında bulunmuştur. Antioksidan aktivite testlerinden, çiçek sulu ekstraktının toplam antioksidan aktivitesi, tohum sulu ekstraktının indirgeme gücü, yaprak etil asetat ekstraktının H_2O_2 süpürme aktivitesi, yaprak hekzan ekstraktının $OH^{\cdot}$ radikal süpürme aktivitesi, çiçek metanol ekstraktının serbest radikal süpürme aktivitesi, metal yaprak etil asetat ekstraktının şelat aktivitesi, yaprak sulu ekstraktının süperoksit anyon temizleme aktivitesi ve yaprak etil asetat ekstraktının lipid peroksidasyon inhibisyon aktivitesi en yüksek değerleri gösterdi. Tohum metanol ekstraktının üreaz inhibisyon aktivitesinin ve tohum etanol ekstraktının asetilkolinesteraz ve butirilkolinesteraz inhibisyon aktivitelerinin sırayla %80.30±0.20, 112.83±10.75 µg/mL ve 334.28±23.09 µg/mL olarak etkin inhibisyon aktivitesi gösterdiği bulunmuştur. Yaprak hekzan, çiçek etil asetat ve yaprak metanol ekstraktları, sırasıyla %71.86, %70.89 ve %69.38 değerleriyle en yüksek DNA koruma aktivitelerini göstermiştir. Fitokimyasal içerik ve biyokimyasal aktivite sonuçlarına göre bu çalışma, *C. sativum* bitkisinin doğal etkili bir ürün olduğunu kanıtlayan değerli bir rapordur.

Anahtar Kelimeler: *Coriandrum sativum* L. (kinzi), antioksidan aktivite, enzim inhibisyonu, DNA koruma aktivitesi.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

1. INTRODUCTION

One of the main changes that occur during preparing and consuming food is oxidation. Lipid oxidation, which initiates other changes in the nutritional system, affects the quality, nutrition, color, smell, structure, and safety of nutrients. The reactive oxygen species (ROS) and antioxidant protective systems can interact with the chemical changes in biological relevant macromolecules. This imbalance provides appropriate pathobiochemical mechanisms which start and develop many diseases. One process that can apply to eliminate the adverse effects of these ROS is using antioxidant substances.[1]

Antioxidants are low concentrations of organic molecules preventing free radical oxidation of different compounds. In the last century, synthetic antioxidants were used for preservation purposes in the food industry since they were especially effective for a more extended period. However, recent reports of the findings of the carcinogenic effects of synthetic antioxidants, legal restrictions on the use of synthetic antioxidants have begun to introduce in many countries.[2] Recently, the demand for natural antioxidants of herbal has been raised for the food industry and pharmaceutical medicine. As a natural result, attention to natural antioxidants continues to grow, especially those of plant origin.[3] Some plant phenolics have recently been recognized as antioxidants and are produced commercially. In this respect, it is crucial to know the biological availability and required levels of these antioxidants that provide a protective effect on the diet. Natural antioxidants have therapeutic potential as medicinal plants, singlet oxygen suppressors, reducing agents, and free radical scavengers. These plant antioxidant activities are due to bioactive compounds such as isocatechins, flavones, lignans, flavonoids, coumarins, isoflavones, catechins, and anthocyanins.

Nowadays, pharmacological research on natural antioxidants with low or no side effects increases to use in preventive medicine. These spices are known to have a health effect (diuretic, expectorant, laxative, antibacterial, antipyretic) and have been used effectively in local treatments in many countries. Moreover, those natural products' (plants) in vivo physiological effects have been determined by applying many models of experimental animals such as beneficial effects on lipid metabolism, antidiabetic activities, ability to stimulate anti-inflammatory, antipathogenic, antioxidant, and digestion studies.[1]

Coriandrum sativum, which has nutritional and medicinal properties, is widely used and distributed spices due to its monoterpenoid-linalool and essential fatty acids, especially in its seeds. *C. sativum* is used to prepare many home remedies used for flu, seasonal fever, nausea, vomiting, and stomach ailment treatments; it is also used for indigestion, intestinal worms, rheumatism, and joint pain. Many of *C. sativum* curative properties are attributed to its special phytonutrient status, and for this reason, it is referred to as a source of bioactive compounds.[4] *C. sativum* is among the essential plants that produce essential oil globally, with 750 tons. In addition to the production of essential oil, *C. sativum* is used as a spice with fresh and dried herbs, called Chinese parsley or "Cilantro".[5] The homeland of *C. sativum* is Anatolia and the Caucasus. Additionally, it is found naturally in Asia and Europe. *Coriander* cultivation, which belongs to the *Umbelliferae* (umbrella flower) family, is cultivated in Hungary, Russia, Poland, England, Bulgaria, Netherlands, Egypt, and Morocco. In Turkey, it is grown in the Lakes Region, Ankara, Eskişehir, and Konya.[6,7] Although the green parts of *C. sativum* are used as "Chinese parsley" in some countries, the principal used parts of the plant are the seeds (fruits). *C. sativum* seeds use as whole or powdered by mixing them into candies, sauces, milk, and meat products to impart flavor and smell. Linalool is a significant raw material in perfume and cosmetic production. It is also used as a protective material in pharmaceutical and food products for bactericidal and fungicidal.[8] Further, the green part of coriander is used as a spice, either fresh, dried, or in brine.[9] Also, it is known that *C. sativum* is used in various drug preparations to remove foul odors.[10] Because it shows drug properties, *C. sativum* also has delicious and gas-digesting properties.[4] Thus, determining the antioxidant activity of the *C. sativum* plant, which has many properties, will contribute to the literature.

In the literature, *C. sativum* has an anxiolytic effect in mice[11] and antibacterial activity to *S. choleraesuis*, *B. megaterium* and *E. coli*.[12,13] It has been reported to reduce triglyceride and cholesterol levels in rats,[14] be effective in treating inflammatory bowel diseases, and have *in vivo* antidiabetic properties.[15] Five components (Q-carotene, Q-cryptoxanthin epoxide, violaxanthin, neoxanthin, and lutein-5,6-epoxide) were obtained from *C. sativum* ether extract and compared their antioxidant activities with synthetic antioxidant, BHT.[16] Although these components did not show antioxidant activity as much as BHT. Q-carotene showed the most remarkable effect among these fractions. Besides, *C. sativum* ether crude extract showed more antioxidant activity, which attributed to a synergistic effect between carotenoid fractions.

C. sativum seeds and leaves extracted with different polarities were evaluated for their antioxidant activities and the inhibition of Fe^{+2}-induced phospholipid peroxidation and observed a correlation between their antioxidant activity and total phenol content.[17] In addition, it was determined that the ethyl acetate extract, which has a medium polarity, showed more effect than the other extracts. In the study by de Almeida Melo and co-worker,[18] the aqueous extract of *C. sativum* was obtained by successive extraction. Four different fractions were also acquired using silica gel column chromatography. Caffeic acid was determined as 4.34

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and 2.64 µg/mL in the first and third fractions, respectively, and protocatechuic acid and glycate in the second and fourth fractions, 6.43 and 3.27 µg/mL, respectively. Additionally, using the Q-carotene/linoleic acid model, they suggested that the antioxidant activities of these fractions were the same, and thus the antioxidant activity of the *C. sativum* aqueous extract was due to its content of phenolic acids. *C. sativum* whole seeds or powdered ones are mixed into candies, sauces, milk and meat products, and alcoholic and non-alcoholic beverages to impart flavor and smell. Linalool, the main component of essential oil, is a significant raw material in perfume and cosmetic products. It is also used as a preservative in pharmaceutical products and food due to its bactericidal and fungicidal. In addition, the green parts of *C. sativum* using as a spice, either fresh, dried, or in brine. It stated that *C. sativum* is used in various drug preparations to remove foul odors. *C. sativum* also has delicious and gas-digesting properties because it shows drug properties.[19]

Although there are studies on antioxidant activity for *C. sativum* leaf and seed extracts in the literature, no study has been found on flower extracts. In addition, there are no studies on the inhibition activities of urease and esterases (acetylcholinesterase, butyrylcholinesterase); also, no one involves DNA protection activities of *C. sativum* seed, leaf, and flower extracts. In this study, considering the integrity of the aerial part of the *C. sativum* plant of the extracts of three different parts obtained with five solvents were investigated phytochemically and biochemically by tests for the chemical contents, antioxidant, enzyme inhibition, and DNA protection activities.

In this work, bioactive compounds (total phenolic, flavonoid, and anthocyanin), antioxidant activity tests (total antioxidant, metal chelating, lipid peroxidation, superoxide radical, free radical, hydroxyl radical, H_2O_2 scavenging activity, and reducing power capacity,) and enzyme inhibition activity tests (acetylcholinesterase, butyrylcholinesterase, and urease) were applied in the extracts of three different parts of the plant, obtained from different polarity solvents (ethanol, methanol, aqueous, hexane, and ethyl acetate). Thus, a significant potential of the *C. sativum* plant in Turkey in terms of the production of medicinal and spice plants was investigated, and its advantages and valuable results were obtained.

2. MATERIALS AND METHODS

2.1. Chemicals

Methanol, ethanol, ethyl acetate, hexane, acetone, gallic acid, catechin, acetylcholinesterase (AChE), urease, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), butyrylcholinesterase (BChE), gel loading dye (blue), ethidium bromide, galantamine, trolox, tert-butyl hydroquinone (TBHQ), α-tocopherol, ammonium molybdate, sodium phosphate, $K_3Fe(CN)_6$, $FeCl_3$, KCl, HCl, Na_2CO_3, $NaNO_2$, $AlCl_3$, NaOH, nitro blue tetrazolium (NBT), trichloroacetic acid (TCA), $FeSO_4$, phenazine meta sulfate (PMS), thiobarbituric acid (TBA), nicotinamide adenine dinucleotide (NADH), $FeCl_2$, ferrozine, salicylic acid, $FeSO_4$, $FeCl_2$, potassium iodide (KI), $Na_2S_2O_3$, DTNB, phenol, sodium nitroprusside, NaOCl from Sigma-Aldrich; Folin-Ciocalteu, ascorbic acid, thiourea from Merck; 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) from Fluka; pBR322 plasmid DNA from ThermoFisher and ethylenediaminetetraacetic acid (EDTA) from Carlo Erba were purchased.

2.2. Sample

The *C. sativum* growing in the natural environment was purchased from the local market in Samsun, Atakum, İncesu Village in 2019. The plant was identified by Prof. Dr. Erkan Yalçın, Ondokuz Mayıs University, Faculty of Arts and Sciences, Department of Biology, and its voucher numbers were OMUB 8679 and OMUB 1861. The leaves, seeds, and flower parts of the plant were dried in a cool and air-flowing condition and ground into powder in the grinding mill.

2.3. Extraction

The extraction processes were applied to the seed (10 g), flower (10 g), and leaf (10 g) parts of the *C. sativum* plant with different polarities (methanol, ethanol, hexane, and ethyl acetate) using a soxhlet device and filtered on Whatman no:1 paper. The solvents were evaporated rotary at 40 °C and received dry crude extracts. Aqueous extracts were prepared with hot aqueous using a magnetic stirrer and filtered on Whatman no:1 paper. The clear extract was lyophilized at -50 °C, and under low pressure, the crude extract was obtained in powder form. The crude extracts were stored at -20 °C for chemical content analysis and activity determination.

2.4. Chemical Component Analysis

2.4.1. Total phenol determination

The total phenol content of the extracts was determined by expressing gallic acid equivalent (GAE)/g.[20] 1 mL of extract, and 1 mL of Folin & Ciocalteu's reagent solutions were mixed in the beaker. Then, 3 mL of 2% Na_2CO_3 solution was added to the reaction mixture. After the mixture was kept in the dark for 2 hours at room temperature, its absorption was read at 760 nm. A calibration curve was drawn using gallic acid as a standard (0.025-6.25 mg/mL; y= 0.0639x - 0.0054, R^2= 0.99) and determined the total phenolic contents of the extracts.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

2.4.2. Total flavonoid determination

The total flavonoid quantity of the extracts was calculated by stated as catechin equivalent (CE)/g.[21] 250 µL of extract solution and 75 µL of 5% $NaNO_2$ were mixed homogeneously. After 5 minutes, 150 µL of 10% $AlCl_3$ and 500 µL of 1 M NaOH solutions were inserted into the mixture. Finally, after adding 275 µL of aqueous to the reaction mixture, the absorption of the mixture was read at 510 nm. A calibration curve was drawn using catechin as a standard (0.06-1000 µg/mL; y= 0.2268x + 0.0216, R^2= 0.99) and calculated the total flavonoid contents of the extracts.

2.4.3. Total anthocyanin determination

The total anthocyanin content of the extracts was applied by modifying the previously applied methods.[22] 1 g of dry ground plants was mixed homogeneously with 1% HCl solution and centrifuged. The supernatant of the 0.2 mL extract with 1.8 mL of the buffer was mixed. In the analysis, KCl buffer solution for the first pH=1 buffer solution and sodium acetate buffers for the second pH=4.5 buffer solution was used. The extracts were diluted using pH 1.0 and pH 4.5 buffers. The absorbance of the mixtures was then measured at 520 nm. After 15 minutes, absorbance values of the mixture at 700 and 520 nm were recorded. The total anthocyanin concentration of the plant was expressed as mg cyanidin 3-glucoside equivalent/mL.

2.5. Antioxidant activity

The antioxidant activity of *C. sativum* extracts was determined by the following spectroscopic methods. BHA, TBHQ, BHT, α-tocopherol, ascorbic acid, and trolox were used as standard antioxidants. All activity tests were applied at a dose of 500 µg/mL of the extracts and performed in triplicate. While the reducing power capacities and total antioxidant activities of the extracts were expressed as absorbance values, the results of other activity tests were given as %.

2.5.1. Determination of total antioxidant activity

The activity was determined by the ammonium molybdenum method that is based on the reduction of molybdenum in an acidic medium and the formation of the green color phosphate/Mo(V) compound formed at 695 nm.[23] It was mixed with 1 mL of reagent solution (28 mM sodium phosphate:0.6 M sulfuric acid:4 mM ammonium molybdate) and 0.1 mL of extract.

The reaction medium was incubated in a lidded tube in an aqueous shaker bath for 90 minutes at 95 °C. Then the mixture was cooled to 25 °C, and it was recorded the absorbance values at 695 nm.

2.5.2. Determination of reducing power

The reducing capacities of *C. sativum* extracts were observed with Fe^{3+} to Fe^{2+} reduction assay spectroscopically.[24] Briefly, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ was mixed with 1 mL of extract. The mixture waited in an aqueous bath at 50 °C for 20 minutes. After, 2.5 mL of 10% TCA was added to the mixture medium, and the mixture was centrifuged at 2500 x g. The 2.5 mL of the obtained supernatant was mixed with 0.5 mL of 0.1% $FeCl_3$ and 2.5 mL of ddH_2O. The reducing power capacities of the standards and extracts were measured at 700 nm, and changes in absorbance were followed.

2.5.3. Determination lipid peroxidation inhibition

The lipid peroxidation inhibition capacities of the extracts were obtained by monitoring the level of linoleic acid peroxidation.[25] Extract solution 550 µL of linoleic acid (40 µM), 150 µL of ascorbic acid (10 µM), and 500 µL of phosphate buffer (100 µM, pH 7.4) were mixed homogeneously. Linoleic acid peroxidation is initiated by adding 0.1 mL of $FeSO_4$ (10 µM) to the mixture. The reaction mixture is incubated at 37 °C for 60 minutes in the dark. After incubation, 1.5 mL of 10% TCA solution prepared in 0.5% HCl, 3 mL 1% TBA solution prepared in of 50 mM NaOH was added to the reaction mixture. The TBA/extract (or standard) mixture was incubated at 95 °C. Later the mixture was cooled to 25 °C, 3 mL of *n*-butanol was added. The inhomogeneous mixture was centrifuged, and the pink supernatant was removed. The absorption of the mixture was read at 532 nm. The percent inhibition of linoleic acid peroxidation level was estimated according to the following formula;

Activity (%): $((A_0-A_1)/A_0) \times 100$

A_0: absorbance of the control, A_1: absorbance of the sample

2.5.4. Determination of free radical scavenging activity

This assay was evaluated by a 1,1-diphenyl-2-picryl-hydrazyl (DPPH˙) radical scavenging assay.[26] 3 mL of the extract and solutions of standard antioxidant substances were mixed with 1 mL of 0.1 mM DPPH˙ solution. Changes in absorbance at 517 nm were recorded. The activity of the samples was calculated according to the formula written below.

Activity (%): $((A_0-A_1)/A_0) \times 100$

A_0: absorbance of the control, A_1: absorbance of the sample

2.5.5. Determination of superoxide anion scavenging activity

This method of the extracts and antioxidant substances was determined according to the Nishikimi Method.[27] In the method, the superoxide radical was produced by the

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NADH-PMS system by reduction of NBT and oxidation of NAD. In the experiment, NBT (156 µM, 1.0 mL), 1 mL of different concentrations of extract and solutions of antioxidant substances, and NADH (468 µM, 1.0 mL) was stirred thoroughly. The reaction was initiated by adding PMS (100 µM, 0.4 mL) to the reaction mixture, incubated, and left at ambient temperature for 5 minutes. The absorbance of the mixture was read at 560 nm, and the superoxide anion scavenging activity of the samples was calculated according to the formula written below;

Activity (%): $((A_0-A_1)/A_0) \times 100$

A_0: absorbance of the control, A_1: absorbance of the sample

2.5.6. Determination of metal chelating activity

The complexation activity of the standard and extract antioxidant substances with Fe^{2+} was evaluated by measuring the absorbance of the Fe^{2+}-ferrozine complex at 562 nm.[28] 0.4 mL of extract and 0.05 mL of 2 mM $FeCl_2$ were mixed by vortex thoroughly. The reaction is initiated by adding 0.2 mL of 5 mM ferrozine. The final volume is complete to 4 mL with ethanol. The mixture was thoroughly mixed by vortex and left at 25 °C for 10 minutes. The absorbance of the mixture is read at 562 nm, and the % inhibition of Fe^{2+}-ferrozine complex formation was estimated according to the equation given below;
Activity (%): $((A_0-A_1)/A_0) \times 100$
A_0: absorbance of the control, A_1: absorbance of the sample

2.5.7. Determination hydroxyl radical ($OH^{\cdot}$) scavenging activity

The activity of the extracts and standard antioxidant substances was determined by modifying the method developed by Smirnoff, Cumbes.[29] $OH^{\cdot}$ radical was formed with a mixture of $FeSO_4$ and H_2O_2 and determined by measuring the spectrophotometric absorption of the compound formed by the radical with salicylic acid. The absorbance of the 3.0 mL reaction mixture consisting of 1.0 mL $FeSO_4$ (1.5 mM), 0.3 mL salicylic acid (20 mM), 0.7 mL H_2O_2 (6 mM), and 1.0 mL plant extract was measured at 562 nm, and percent $OH^{\cdot}$ scavenging activity was calculated according to the formula below.
Activity (%): $((A_0-A_1)/A_0) \times 100$
A_0: absorbance of the control, A_1: absorbance of the sample

2.5.8. Determination H_2O_2 scavenging activity

The activity was evaluated by a titration assay modified by Zhao and co-workers[30]. 1.0 mL, 0.1 mM H_2O_2, and 1.0 mL extract (or standard antioxidant substance) were added and mixed well, then 0.1 mL of 3% ammonium molybdate, 10 mL of sulfuric acid (2M), and 7.0 mL of potassium iodide (1.8 M) were added, sequentially. The mixture was titrated with $Na_2S_2O_3$ (5 mM). According to the obtained volume values, the percent activity was calculated according to the formula below.
Activity (%): $((V_0-V_1)/V_0) \times 100$
V_0 represents the $Na_2S_2O_3$ volume (mL) spent in the presence of H_2O_2 (without extract or standard substance; ddH_2O is used),
V_1 denotes the $Na_2S_2O_3$ volume (mL) in the mixture containing the extract or standard substance.

2.6. Enzyme inhibition activities

Enzyme inhibition activities of *C. sativum* extracts were observed by the following spectroscopic methods. Galantamine was used as a standard in acetylcholinesterase and butyrylcholinesterase inhibition activities, and thiourea was used as a standard in urease inhibition activities. Enzyme inhibition activity tests were applied to the extracts and standard substances under the same conditions and performed in triplicate.

2.6.1. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activity

AChE and BChE inhibition levels of the extracts were measured spectrophotometrically.[31] 750 µL of 100 mM Na-K buffer (pH 8.0), 50 µL of different concentrations (0.025-5.0 µg/mL) of extract solutions (or galantamine), 100 µL of enzyme solution (0.03 U/mL, 100 mM pH 8.0, Na-K buffer) into the tubes was mixed, respectively and incubated for 15 minutes at 25 °C. 50 µL of 3.3 mM DTNB and 50 µL of 1 mM acetylcholine iodide (or butyrylcholine chloride) were added to the mixture. The absorption values of each mixture were recorded at 412 nm. The IC_{50} values of the extract samples and galantamine were determined and expressed as µg/mL. IC_{50} values (µg/mL) were also presented with effective concentration inhibition of AChE (or BChE) for inhibition activities.

2.6.2. Urease inhibition activity

The urease inhibition activities of the extracts were determined by spectrophotometrically.[32] 125 µL of 0.47 U urease (100 mM pH 6.8 PBS), 25 µL of extract (or thiourea, 50-500 µg/mL), 275 µL of 0.2 mM urea were placed in test tubes, respectively. The mixture was kept waiting for 15 minutes at 30 °C in a shaking aqueous bath. 225 µL of phenol reagent (0.005%, w/v sodium nitroprusside + 1%, w/v phenol) and 350 µL of alkaline reagent (1%, w/v NaOH + 0.075%, v/v NaOCl) were added, respectively. The mixture was incubated for 50 minutes at 30 °C in a shaking aqueous bath. The absorptions of each mixture were measured at 630 nm, and percent urease inhibition activity was calculated according to the formula below. The activities of the extracts and thiourea at 100 µg/mL were expressed as %.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

Inhibition activity (%): $((A_0-A_1)/A_0) \times 100$
A_0 is the absorbance of the control, A_1 is the absorbance of the extract or standard substance

2.7.DNA Protection Activity

The DNA protection activity of *C. sativum* extracts was estimated using the agarose gel electrophoresis method. The capacities of the extracts to protect plasmid DNA (pBR322, ThermoFisher) from the oxidizing effects of H_2O_2 and UV treatment were evaluated by their DNA breaking forms.[33,34] In summary, assay 5 µL of extract (1000 µg/mL), 3 µL of plasmid DNA (1:3, v/v), and 1 µL of H_2O_2 (30%) were mixed. The mixture of 6 µL H_2O and 3 µL plasmid DNA (1:3, v/v) as negative control (C1) and 6 µL H_2O, 3 µL plasmid DNA (1:3, v/v) as other positive control (C2), and 1 µL of H_2O_2 (30%) were formed. In addition, quercetin (1000 µg/mL) was used as a standard. The reaction was initiated by the application of UV irradiation for 5 minutes. After irradiation, 2 µL of loading dye was mixed into the mixture and loaded on a 1% agarose gel to which 2 µL of ethidium bromide was added. Electrophoresis was performed at 90 volts for 60 minutes and then photographed with a UV transilluminator (320 nm, 8000 µW/cm). In addition, the percent protection level of the super-coiled DNA form (Form I) and broken DNA form (Form II) of the extracts and quercetin were calculated using the ImageJ Program.

2.8. Statistical analysis

Each parameter of *in vitro* biological activity studies was expressed as triplicate analysis results ± standard deviation values. All data were analyzed in the IBM Statistical Package for Social Studies (SPSS) 20.0 program. ANOVA was used because the mean of more than two independent groups between the analysis means and the data was with normal distribution variance and homogeneous. Tukey HSD[a,b] was used for multiple comparisons based on the data obtained. The statistical significance of the values was compared with the activity analysis result group, and the level of significance was expressed with $p<0.05$ values and considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Extracion yield, total phenol, flavonoid, and anthocyanin contents

Phenolic compounds exhibit high antioxidant activity. They have an important role in the protection of plants against UV radiation, beast of prey, and pathogens.[35] Phenolics are all-important plant components because of their capacity to scavenge radicals such as active oxygen types like singlet oxygen and hydroxyl and free radicals.[3,36] Flavonoids are phenolic compounds that impressive against microbial infections and are synthesized by plants. And also, they are abundantly present in nearly whole plants and have many effects like antioxidant, antiviral and antimutagenic.[37] In recent, bioactive compounds from natural sources obtaining interest increase. Therefore, vast areas of bioactivity methods and assays have been developed.[38,39]

The yields of the extracts were obtained from seed, leaf, and flower extracts of *C. sativum*. Total flavonoid and phenolic contents were applied to methanol, aqueous, ethanol, hexane, ethyl acetate, and extracts of *C. sativum* flower, seed, and leaf parts. The results of the total phenolic, anthocyanin, and flavonoid constituents of *C. sativum* leaf, seed, and flower extracts and dried samples were summarized in Table 1. The amounts of total phenol contents were determined as the GAE using a calibration equation and calculated from the GAE graph ($y= 0.0639x - 0.0054$, $R^2= 0.99$). The highest total phenolic contents identified in leaf hexane extract, seed hexane extract, and flower hexane extract as 250.40±17.84, 423.65±21.15, and 238.82±10.46 mg GAE/g dry plant, respectively, while leaf methanol extract, aqueous seed extract, and flower ethyl acetate extract exhibited the lowest phenolic compounds as 5.46±0.07, 30.07±0.16 and 25.80±10.46 mg GAE/g dry plant, respectively. The previous study done by Wangensteen and co-workers[17] found that the total phenolic contents of *C. sativum* seed ethanol, dichloromethane, ethyl acetate, and *n*-butanol extracts as 0.15±0.01, 0.09±0.01, 1.89±0.08, and 1.16±0.01 g GAE/100 g plant, respectively. In our study, the total phenolic contents of *C. sativum* seed ethanol and ethyl acetate extracts were determined to be higher. Demir and Korukluoglu[40] determined the total phenol contents of *C. sativum* seed methanol and ethanol extracts as 4.20±0.30 and 2.10±0.40 mg GAE/g. However, in our study, the total phenol contents of *C. sativum* seed methanol and ethanol extracts were determined to be higher. In a different study, Muñiz-Márquez and co-workers[41] calculated the total phenol content of all parts of *C. sativum* ethanol extract as 1.38±0.06 mg GAE/g plant. In contrast, in our study, the total phenol contents of *C. sativum* seed, leaf, and flower ethanol extracts were calculated to be higher. On the other hand, Yildiz[42] observed that the phenolic content of all parts of *C. sativum* ethanol extract was 14.97±0.05 mg GAE/g. In our study, the total phenol contents of *C. sativum* seed, leaf, and flower ethanol extracts were determined to be higher. Harsha and Anilakumar[43] found that the total phenol contents of *C. sativum* leaf ethanol extract was 133.74 µg GAE/mg extract. However, in our study, the total phenol content of *C. sativum* leaf ethanol extract was determined to be higher. The work of Msaada and co-workers[44] reported that the total phenol contents of *C. sativum* fruits methanol extracts obtained from Tunisia, Syria, and Egypt were as 1.00±0.06, 1.09±0.02, and 0.94±0.05 mg GAE/g, respectively. In our study, the total phenol contents of *C. sativum* fruit methanol extract was determined to be higher. In a different study, Gallo

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

and Co-workers[45] observed that the total phenol contents of aqueous and ethanol extract obtained using *C. sativum* seed ultrasound and microwave were determined as 41.81±2.77 and 82.09±8.43 mg GAE/100 g plant, respectively. In our study, the total phenol contents of *C. sativum* seed ethanol and aqueous extracts were determined to be higher. Sreelatha and Inbavalli[46] reported the phenol contents of *C. sativum* seed and leaf ethanol extracts as 15.14±1.62 and 25.23±2.17 g GAE/100 g plant, respectively, while, in our study, the total phenol contents of *C. sativum* seed and leaf ethanol extracts were determined to be higher. The total flavonoid contents were performed as the catechin equivalent (CE) using a graph equation drawn from a standard quercetin graph (y= 0.2268x + 0.0216, R^2= 0.99). The highest total flavonoid contents identified in leaf aqueous extract, seed, and flower hexane extract as 32.46±1.10, 132.35±14.08, and 68.66±9.09 mg CE/g dry plant, respectively, while leaf methanol extract, aqueous seed extract, and flower ethyl acetate extract exhibited the lowest phenolic compounds as 3.12±0.02, 17.61±0.20 and 11.43±0.13 mg CE/g dry plant, respectively. Harsha and Anilakumar[43] found the total flavonoid contents of *C. sativum* leaf ethanol extract as 44.5 □g CE/mg extract. However, in our study, the total flavonoid contents of *C. sativum* leaf ethanol extract were determined to be higher.

Table 1. Extraction yield, total phenolic, flavonoid, and anthocyanin contents of *C. sativum* leaf, seed, and flower extracts

Samples	Code	Yield, %	Total phenolic, mg GAE/g	Total flavonoid, mg CE/g	Total anthocyanin, µg cy-3-glu/g
***C. sativum* leaf (1 g)**	**CSL**	-	-	-	136.56±0.16
***C. sativum* leaf aqueous extract**	**CSLA**	23.90	87.77±0.43	32.46±1.10	-
***C. sativum* leaf methanol extract**	**CSLME**	1.80	5.46±0.07	3.12±0.02	-
***C. sativum* leaf ethanol extract**	**CSLET**	12.00	57.09±0.75	26.60±0.90	-
***C. sativum* leaf ethyl acetate extract**	**CSLEA**	3.40	26.35±0.84	15.25±0.38	-
***C. sativum* leaf hexane extract**	**CLHE**	10.70	250.40±17.84	22.78±0.81	-
***C. sativum* seed (1 g)**	**CSS**	-	-	-	14.07±0.21
***C. sativum* seed aqueous extract**	**CSSA**	8.76	30.07±0.16	17.61±0.20	-
***C. sativum* seed methanol extract**	**CSSME**	18.50	62.47±1.03	35.34±0.79	-
***C. sativum* seed ethanol extract**	**CSSET**	38.00	141.53±2.38	68.25±3.04	-
***C. sativum* seed ethyl acetate extract**	**CSSEA**	30.60	99.26±1.47	49.89±0.16	-
***C. sativum* seed hexane extract**	**CSSHE**	26.30	423.65±21.15	132.35±14.08	-
***C. sativum* flower (1 g)**	**CSF**	-	-	-	184.26±0.74
***C. sativum* flower aqueous extract**	**CSFA**	9.30	39.20±0.17	20.28±0.21	-
***C. sativum* flower methanol extract**	**CSFME**	19.50	93.18±0.93	34.79±0.87	-
***C. sativum* flower ethanol extract**	**CSFET**	11.80	42.72±0.93	21.64±1.78	-
***C. sativum* flower ethyl acetate extract**	**CSFEA**	5.30	25.80±0.10	11.43±0.13	-
***C. sativum* flower hexane extract**	**CSFHE**	5.40	238.82±10.46	68.66±9.09	-

In the work of Msaada and co-workers[44] reported that the total flavonoid contents of *C. sativum* fruits obtained from Tunisia, Syria, and Egypt were as 2.03±0.04, 2.51±0.08, and 2.07±0.05 mg CE/g, respectively. In this study, the total flavonoid content of *C. sativum* fruit methanol extract was determined to be higher. Sreelatha and Inbavalli[46] reported the total flavonoid contents were as 18.41±2.85 and 19.15±2.33 g QE/100 g plant, respectively, while, in our study, the total flavonoid contents of *C. sativum* seed and leaf ethanol extracts were determined to be higher. The total anthocyanin concentration of the plant was expressed as mg cyanidin 3-glucoside equivalent/mL. The anthocyanin compound amounts of *C. sativum* leaf, seed, and flower (1 g plant) were 136.56±0.16, 14.07±0.21, and 184.26±0.74 µg cyanidin-3-glucoside equivalent/g, respectively.

3.2. Antioxidant activity

Free radicals to include their orbitals unpaired electrons. These molecules or atoms generated consistently in the body contain ROS. Reactive radicals that carry unpaired electrons can quickly bond with biomolecules.[47] Free radicals are the reason for most of the diseases.[48] Many serious diseases such as cancer and cardiovascular have been deliberated to be the conclusion that this radical does harm lipids, nucleic acid, and proteins. The reduced ability of a compound can concern with antioxidant capacity. Those are the ability to transfer electrons into reactive radicals, thereby its reducing unreactive species and into more stable.[47] Some plants have the capacity to both balance and avoid series radical reactions owing to definite specific reducing substances in their structure.[49] Eight different antioxidant activity tests were applied to test the methanol, aqueous, ethyl acetate, hexane, and ethanol extracts of *C. sativum* leaf, seed, and flower parts at 500 µg/mL, and their results were exhibited in Table 2. The extracts of *C. sativum* seed, flower, and leaf exhibited relatively effective antioxidant activities. The results of activities were significant differences between control and extracts, statistically ($p < 0.05$). The total antioxidant activities of *C. sativum* of leaf hexane, seed aqueous, and flower aqueous extracts were observed the highest as 1.043, 0.557, and 0.272, respectively.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

Sreelatha and Inbavalli[46] determined the total antioxidant activities as 55.36±0.28% and 64.56±0.51% for the *C. sativum* seed and leaf ethanol extracts. However, in our study, total antioxidant activities for the *C. sativum* seed and leaf ethanol extracts were calculated to be lower. This is thought to be due to the phenolic and flavonoid content it contains. The reducing power capacity of *C. sativum* of leaf methanol, seed aqueous, and flower aqueous extracts was determined to be the highest as 0.564, 0.235, and 0.370. Harsha and Anilakumar,[43] IC_{50} values of reducing power capacities of *C. sativum* leaf ethanol extract was found as 251.80 µg/mL. In another study, Msaada and co-workers,[44] observed the EC_{50} values of reducing power activities as 122.01±13.25, 54.20±6.22, and 56.11±7.45 µg/mL of methanol extracts of *C. sativum* fruits obtained from Tunisia, Syria, and Egypt. Martins and co-workers[50] found the EC_{50} values of reducing power capacities of *C. sativum* seed hydro-methanolic extract as 2069.00±55.00 µg/mL. In our study, the reducing power activity results were expressed as absorbance. Therefore, it could not be compared with the data in the literature due to unit differences. The H_2O_2 scavenging activities of *C. sativum* of leaf ethyl acetate, seed hexane, and flower ethyl acetate extracts were observed to be the highest activities as 25.21, 23.57, and 21.05%. In the literature researches, no data on the content of H_2O_2 scavenging activities could occur. The $OH^{\cdot}$ radical scavenging activities of *C. sativum* of seed hexane, leaf hexane, and flower hexane were calculated as 52.60, 60.25, and 55.79%. %. The free radical scavenging activities of *C. sativum* of flower methanol, seed methanol, and leaf methanol extracts were the most effective as 98.86, 97.25, and 98.11%. Wangensteen and co-workers[17] determined that $DPPH^{\cdot}$ scavenging activities of *C. sativum* leaf and seed ethanol extracts were as 510.00±12.00 and 389.00±5.00 µg/mL. Demir and Korukluoglu[40] determined that the free radical scavenging activities of *C. sativum* seed methanol and ethanol extracts were as 2.20±0.20 and 5.60±0.20 mg/mL. Harsha and Anilakumar,[43] free radical scavenging activities of *C. sativum* leaf ethanol extract was found as 217.20 µg/mL. In another study, Msaada and co-workers[44] observed that free radical scavenging activities of methanol extracts of C. sativum fruits obtained from Tunisia, Syria, and Egypt were the IC50 values as 27.00±6.57, 36.00±3.22, and 32.00±2.87 µg/mL. In our study, the free radical scavenging activity results were expressed as %. Therefore, it could not be compared with the data in the literature due to unit differences. In a different study, Gallo and co-workers [45] observed the $DPPH^{\cdot}$ activities of ethanol-aqueous extracts obtained by using *C. sativum* seed, via ultrasound and microwave extraction, as 74.38 and 25.56%, however, in our study, free radical scavenging activities for the *C. sativum* seed ethanol extracts were calculated to be higher. Sreelatha and Inbavalli[46] determined the IC_{50} values of free radical scavenging activities as 20.36±0.63 µg/mL and 25.32±0.54 µg/mL for the *C. sativum* seed and leaf ethanol extracts. Mathew and Subramanian[51] found the free radical scavenging activity as 0.21±0.10% of 0.1 mg/mL concentration of *C. sativum* leaf methanol extract. Ahmed and co-workers[52] observed the free radical scavenging activities for the *C. sativum* seed methanol (maceration), methanol (soxhlet), chloroform, and petroleum ether extracts at 1 mg/mL concentration as 40.60, 28.36, 48.30, and 32.80% while in our study, free radical scavenging activities for the *C. sativum* seed methanol extracts were calculated to be higher. Conversely, free radical scavenging activities of *C. sativum* seed, flower, and leaf methanol (soxhlet) extracts were observed to be higher in our study. Martins and co-workers[50] found the free radical scavenging of *C. sativum* seed hydro methanolic extract as 1930.00±24.00 µg/mL.

In this work, the metal chelating activity of C. sativum leaf ethyl acetate, seed ethyl acetate, and flower ethyl acetate was observed as 39.84±0.30, 35.37±0.61, and 30.90±1.41%, respectively. Harsha and Anilakumar,[43] metal chelating activities of *C. sativum* leaf ethanol extract was found as 368.12 µg/mL. The superoxide anion scavenging activities of *C. sativum* of leaf aqueous, seed aqueous, and flower aqueous extracts were exhibited the highest as 75.40, 66.01, and 60.95%, respectively. The lipid peroxidation inhibition capacities of C. sativum of leaf ethyl acetate, seed ethyl acetate, and flower ethyl acetate were found effective with high values of 93.88, 85.58, and 87.89%, respectively. Harsha and Anilakumar,[43] IC_{50} values of lipid peroxidation inhibition activities of *C. sativum* leaf ethanol extract was found as 518.60 µg/mL.

3.3. Urease, AChE and BChE inhibition activities

Three different enzyme inhibition activity tests were applied to the methanol, aqueous, ethanol, hexane, and ethyl acetate extracts of *C. sativum* leaf, seed, and flower parts. Their results were exhibited in Table 3.

Today's, inhibition of significant enzymes of diseases relevant to community health such as Alzheimer's enter into vital significance.[53] AChE is concerned with the growth of cells and aids the maturing of neurons and regeneration of nerves.[54] This enzyme inhibition induces the continuous and excessive acetylcholine (ACh) collection in nerve synapses. AChE, first of all, pervades in the nervous tissue, quickly intervenes in the hydrolysis of the neurotransmitter ACh, and reasons the cancellation of the conduction of nerve impulse, thereby providing a normal physical function of the body. AChE is a vital member of the nervous system. Thus, adverse effects on AChE activity can induce neurotoxicity.[55] BChE is connected in many physical factors, the most distinct the hydrolysis of not only noncholine but also choline esters. Consequently, it has a significant role in neurotransmission and anesthesia.[56] A vital rise in the acetylcholinesterase activity is spied in Alzheimer's disease early phase.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

The activity of BChE progressively advances in Alzheimer's late phases. Therefore, both AChE and BChE are considerable medicinal goals to the improvement of the cholinergic explicit and idea the AD.[57] In the AChE inhibition activity test, it was determined the IC_{50} values of *C. sativum* of leaf ethanol, seed ethanol, and flower ethanol as 145.07±8.09, 112.83±10.75, and 187.38±5.77 µg/mL extracts had the highest activity. These extracts have higher AChE inhibitory activity than galantamine (IC_{50}, 418.20±9.55 µg/mL). It shows that ethanol extracts of leaves, seeds, and flowers can be used as drugs for this enzyme.

Mathew and Subramanian[51] determined the AChE inhibition activity of *C. sativum* leaf methanol extract was 36.25±5.30% at 0.1 mg/mL. In our study, the AChE inhibition activity results were expressed as IC_{50} value. In the BChE inhibition activity test, it was determined the IC_{50} values of *C. sativum* of leaf ethanol, seed ethanol, and flower aqueous as 553.45±11.55, 334.28±23.09, and 430.67±14.06 µg/mL extracts had more effective than that of alanthamine (IC50, 409.52±15.84 µg/mL).

Table 2. Antioxidant activities of *C. sativum* leaf, seed, and flower extracts at 500 µg/mL.

Samples/ Standards	Total antioxidant	Reducing power	H_2O_2 scavenging	$OH^{\cdot}$ radical scavenging	Free radical scavenging	Metal chelating	Superoxide anion scavenging	Lipid peroxidation
	Abs., 695 nm	Abs., 700 nm	Activity, %					
CSLA	0.443±0.000^{o}	0.301±0.000^{f}	13.04±1.57^{a}	44.85±0.41^{ij}	95.32±0.28^{g}	35.40±0.33^{g}	75.40±0.58^{l}	63.79±0.24^{c}
CSLME	0.372±0.001^{m}	0.564±0.006^{h}	18.64±1.21^{cd}	36.31±0.93^{d}	98.11±0.22^{hi}	28.94±0.46^{d}	54.88±0.06^{h}	58.27±2.09^{b}
CSLET	0.303±0.001^{l}	0.201±0.000^{d}	18.96±2.35^{cd}	51.46±0.39^{kl}	95.34±0.14^{g}	18.63±0.37^{a}	62.88±0.07^{jk}	64.24±0.42^{c}
CSLEA	0.103±0.001^{d}	0.144±0.000^{ab}	25.21±1.49^{f}	22.31±0.58^{ab}	80.11±0.35^{b}	39.84±0.30^{h}	38.32±0.67^{def}	93.88±0.14^{h}
CLHE	1.043±0.000^{t}	0.169±0.000^{c}	20.72±1.84^{de}	60.25±0.03^{n}	92.57±0.56^{f}	25.81±0.24^{b}	40.27±2.05^{f}	83.87±0.22^{f}
CSSA	0.557±0.000^{r}	0.235±0.002^{e}	16.55±1.05^{abc}	43.53±0.27^{hi}	88.97±0.34^{d}	27.46±0.72^{c}	66.01±0.03^{k}	61.56±1.67^{c}
CSSME	0.245±0.001^{i}	0.226±0.000^{e}	17.10±0.47^{bcd}	41.60±0.10^{fghi}	97.25±0.25^{h}	31.04±0.33^{ef}	58.02±0.20^{hi}	54.50±1.34^{d}
CSSET	0.096±0.001^{c}	0.200±0.000^{d}	14.14±1.88^{ab}	50.76±0.36^{kl}	84.71±0.29^{c}	31.52±0.07^{f}	35.25±0.05^{d}	76.20±0.13^{de}
CSSEA	0.065±0.001^{a}	0.136±0.000^{a}	19.18±2.01^{cd}	37.87±0.29^{def}	89.51±0.10^{e}	35.37±0.61^{g}	37.10±1.05^{def}	85.58±0.37^{fg}
CSSHE	0.080±0.000^{b}	0.127±0.000^{a}	23.57±1.33^{ef}	52.60±0.68^{lm}	86.59±0.52^{d}	34.74±0.33^{g}	35.44±0.06^{d}	75.69±1.51^{de}
CSFA	0.272±0.000^{k}	0.370±0.000^{g}	17.76±1.53^{bcd}	43.04±0.07^{ghi}	85.24±0.93^{cd}	25.93±0.52^{b}	60.95±0.07^{ij}	54.82±0.29^{a}
CSFME	0.260±0.000^{j}	0.313±0.000^{f}	19.40±1.71^{cd}	40.50±0.19^{efgh}	98.86±0.07^{i}	30.03±0.59^{de}	38.71±0.14^{ef}	74.24±0.43^{d}
CSFET	0.233±0.001^{h}	0.200±0.000^{d}	17.87±1.04^{bcd}	48.18±0.33^{jk}	94.07±0.97^{g}	29.75±0.19^{de}	43.56±1.05^{g}	75.02±1.12^{de}
CSFEA	0.221±0.001^{g}	0.200±0.001^{d}	21.04±2.16^{de}	21.02±0.49^{a}	95.39±0.27^{g}	30.90±1.41^{ef}	31.33±3.88^{c}	87.89±0.24^{g}
CSFHE	0.167±0.000^{f}	0.145±0.029^{c}	21.05±1.51^{de}	55.79±0.51^{m}	91.26±0.28^{f}	25.72±0.85^{b}	35.88±0.30^{de}	83.61±2.79^{f}
BHA	0.449±0.001^{p}	0.131±0.000^{a}	17.65±0.22^{bcd}	37.49±0.15^{de}	77.37±0.40^{a}	NT	25.19±0.18^{a}	63.51±0.03^{c}
BHT	0.633±0.001^{s}	0.131±0.000^{a}	19.52±0.10^{cd}	39.17±5.21^{defg}	77.46±0.29^{a}	NT	22.35±0.45^{b}	76.95±0.33^{de}
Trolox	0.392±0.007^{n}	0.147±0.000^{ab}	17.44±0.77^{bcd}	42.55±2.73^{ghi}	78.24±0.44^{a}	NT	25.55±1.28^{a}	74.32±0.37^{d}
TBHQ	0.372±0.001^{m}	0.161±0.000^{bc}	18.86±0.10^{cd}	24.41±0.27^{ab}	78.19±0.47^{a}	NT	21.14±0.19^{a}	77.62±0.43^{e}
α-tocopherol	0.393±0.003^{n}	0.156±0.000^{bc}	20.39±0.19^{cde}	25.43±0.34^{b}	77.68±0.53^{a}	NT	19.49±0.62^{i}	77.31±0.25^{de}
Ascorbic acid	0.144±0.001^{e}	0.194±0.000^{d}	18.53±0.10^{cd}	31.41±0.04^{c}	91.40±0.82^{f}	NT	59.04±0.07^{i}	83.44±1.05^{f}
EDTA	NT	NT	NT	NT	NT	85.61±0.49^{i}	NT	NT

NT: not tested, Variance analysis ($p<0.05$)

Urease is an enzyme to provide the hydrolysis of urea to form carbon dioxide and ammonia. The most significant part is to preserve the bacteria in the acidic ambiance of the stomach.[58] These enzyme inhibitors can enter into a vital to oppose impact the negative effect of urease in living organisms. These inhibitors are efficient against a few acute infections induced by the secretion of urease by *Helicobacter pylori* that contain gastric tract

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

syndromes and urinary tract infections.[59] It is now commonly accepted that duodenal ulcers and gastric are usually caused by *H. pylori* that grow and survive in an acidic medium.[60,61] This organism releases urease that changes urea into ammonia, and the excretion of ammonia defends it from the acidic medium of the stomach.[62] World Health Organization have classified *H. pylori* as a first-class carcinogen.[63] Several enzyme inhibitors were artificially developed for pharmacology. However, they could command some side effects like liver damage and gastrointestinal disturbances. Therefore, it increased significant interest in exploring safe and new inhibitors from natural sources.[64-66]

The urease inhibition activities of the leaf methanol, seed methanol, and flower methanol extracts were exhibited the highest activity as 68.75±0.97, 80.30±0.20, and 69.64±0.68%, respectively. These extracts have higher urease inhibition activity than thiourea (38.76±6.13%).

Table 3. Enzyme inhibition activities of leaf, seed, and flower extracts of C. sativum.

Samples/ Standards	AChE	BChE	Urease
	IC_{50}, µg/mL		
CSLA	247.36±11.55^{d}	NA	35.66±0.02^{b}
CSLME	NA	743.07±20.82^{e}	68.75±0.97^{e}
CSLET	145.07±8.09^{b}	553.45±11.55^{c}	33.65±0.02^{b}
CSLEA	NA	NA	41.84±0.02^{c}
CLHE	NA	NA	35.39±0.05^{b}
CSSA	440.91±0.00^{f}	367.63±23.09^{a}	32.62±0.11^{b}
CSSME	NA	NA	80.30±0.20^{f}
CSSET	112.83±10.75^{a}	334.28±23.09^{a}	33.81±0.19^{b}
CSSEA	NA	NA	32.31±0.09^{b}
CSSHE	648.21±28.17^{h}	NA	16.14±0.05^{a}
CSFA	533.22±102.63^{g}	430.67±14.06^{b}	34.31±0.05^{b}
CSFME	767.44±80.83^{i}	573.14±46.19^{e}	69.64±0.68^{e}
CSFET	187.38±5.77^{c}	940.47±28.28^{f}	33.79±0.17^{b}
CSFEA	NA	NA	56.75±0.05^{d}
CSFHE	634.45±40.62^{h}	640.83±24.38^{d}	15.19±0.26^{a}
Galantamine	418.20±9.55^{e}	409.52±15.84^{b}	NT
Thiourea	NT	NT	38.76±6.13^{c}

NA: not activity, NT: not tested, Variance analysis ($p<0.05$)

3.4.DNA protection activity

The leaf, seed, and flower methanol, aqueous, ethanol, hexane, and ethyl acetate extracts of *C. sativum* were analyzed in an anaerobic medium to observe the transformations in forms I, II, and III of plasmid DNA, to determine DNA protection activity (Figures 1 and 2). Two forms were observed in the gel image and these forms; form I, which is the super-coiled circular DNA, where plasmid DNA walks fast, and form II, which is the truncated DNA form, where it migrates slowly. Also, controls, extracts, and quercetin were observed to have higher DNA protection activity in form I than in form II. It was noted that when UV and H_2O_2 were applied together (Lane 2), it would cause DNA damage in form II. This damage can be reduced in the presence of the quercetin (1000 µg/mL) (Lane 18).[67] Further, the addition of *C. sativum* extracts to the H_2O_2 reaction mixture provided significant protection from damage to super-coiled circular DNA (Lane 3-17). While the form I DNA protection activity of the standard and the extracts was observed over 70% of the leaf hexane and flower ethyl acetate extracts, also, it was determined that the form II DNA protection activity was above 60% of the flower acetone and flower hexane extracts. In their study, Harsha and Anilakumar[43] determined that *C. sativum* leaf ethanol extract has DNA protection activity. In another study, Divya and co-worker[68] observed that carotenoids isolated from *C. sativum* fractions had DNA protection activity. In our study, the DNA protection activity results of *C. sativum* extract obtained from different polar and nonpolar solvents were comparable to that of quercetin and expressed as %.

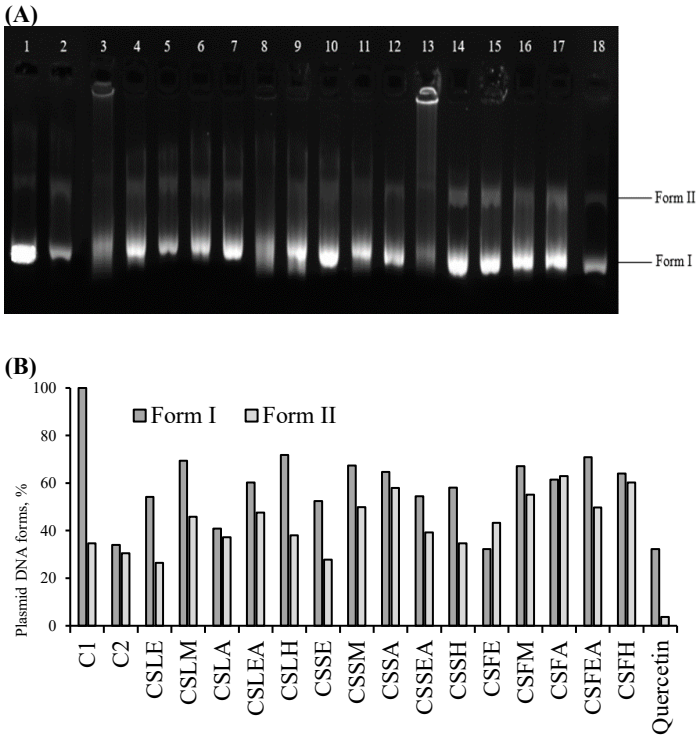

Figure 1. The effect of DNA protection activity of crude *C. sativum* leaf, seed and flower extracts.

(A) Agarose gel electrophoresis; Lane 1: Negative control (C1); Lane 2: Positive control (C2); Lane 3: CSLE; Lane 4: CSLM; Lane 5: CSLA; Lane 6: CSLEA; Lane 7: CSLH; Lane 8: CSSE; Lane 9: CSSM; Lane 10: CSSA; Lane 11: CSSEA; Lane 12: CSSH; Lane 13: CSFE; Lane 14: CSFM; Lane 15: CSFA; Lane 16: CSFEA; Lane 17: CSFH; Lane 18: Quercetin

(B) The % intensity of Form I and Form II in agarose gel electrophoresis image

4. CONCLUSIONS

Five different extracts (aqueous, methanol, ethanol, ethyl acetate, and hexane) of *C. sativum* (leaf, flower, and seed parts) were obtained by using the soxhlet method and the boiling method. The chemical content analysis (total phenol, flavonoid, and anthocyanins), antioxidant activities (total antioxidant, reducing power, metal chelating, lipid peroxidation inhibition, free radical, H_2O_2, $OH^{\cdot}$, and superoxide anion scavenging), enzyme

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

inhibition (AChE, BChE, and urease) and DNA protective activities were applied to *C. sativum* extracts. Although there were studies on the different activities of the *C. sativum* leaf and seed extracts in the literature, no study has been found on *C. sativum* flower extracts. According to their chemical contents and antioxidant activities, results showed that hexane, ethyl acetate, and aqueous extracts have higher chemical content and activity. In enzyme inhibition tests, ethanol extracts had high activity in AChE and BChE inhibition activities, while methanol extracts had high activity in urease inhibition activity. In addition, ethanol extracts are shown to have higher activity than galantamine in AChE inhibition activity. Due to its effects on different forms of plasmid DNA (Form I and Form II), the DNA protection potentials of extracts of *C. sativum* have been demonstrated. Further, by looking at all results, leaf extracts have higher activities than other extracts in general. Flower extracts have high activity in some activity tests but low activity. The different effective properties of this plant in medicine and food can also be examined by applying other methods and techniques, as a medicine or as a food supplement.

ACKNOWLEDGEMENTS

OMÜ-BAP supported this work with the projects numbered PYO.FEN.1904.13.003 and PYO.FEN.1904.13.006.

Conflict of interests

The authors declared no conflict of interest with any person, institute, company, etc.

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