Frontiers in Health InformaticsISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2025; Vol 14: Issue 6
Open Access

Evaluation of DNA Protective Properties among Various *Trigonella* Species

Shahrokh Mojarradgandoukmolla[1]*, Saman Muhsin Abdulkareem[2], Fakhir Najim K. Sabir[3], Nadir Mustafa Qadir Nanakali[4], Khabat A. Ali[5], Tanya Salam Salih[6], Mohammed M. Hussein M. Raouf[7] , Abdulrahman Ismael[8], Bakhtawar Ziad Omer[9], Zahraakhan Maaroof Taher[10], Abdullah Jawdat Zebari[11]

[1]Department of Biology, College of Education, Salahaddin University-Erbil, Iraq
[2]Department of Biology, College of Education, Salahaddin University-Erbil, Iraq
[3]Department of Biology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region – F.R. Iraq
[4]Department of Biomedical Sciences, College of Applied Sciences, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq
[4]Department of Biology, College of Education, Salahaddin University-Erbil, Iraq
[5]Department of Biology, College of Education, Salahaddin University-Erbil, Iraq
[6]Department of Biomedical Sciences, College of Applied Sciences, Cihan University Erbil, Erbil, Kurdistan Region, Iraq
[7]Department of Biomedical Sciences, College of Applied Sciences, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq
[8]Department of Community Health Nursing, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq
[9]Department of Biomedical Sciences, College of Applied Sciences, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq
[10]Department of Biomedical Sciences, College of Applied Sciences, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq
[11]Department of Medical Biochemical Analysis, College of Health Technology, Cihan University-Erbil, Erbil, Kurdistan Region, Iraq
*Corresponding Author

Cite this paper as: Shahrokh Mojarradgandoukmolla, Saman Muhsin Abdulkareem, Fakhir Najim K. Sabir, Nadir Mustafa Qadir Nanakali, Khabat A. Ali, Tanya Salam Salih, Mohammed M. Hussein M. Raouf , Abdulrahman Ismael, Bakhtawar Ziad Omer, Zahraakhan Maaroof Taher, Abdullah Jawdat Zebari (2025), Evaluation of DNA Protective Properties among Various Trigonella Species. *Frontiers in Health Informatics*, *14(6) 4765-4777*

ABSTRACT

Background/aim: *Trigonella strangulata* Boiss., *Trigonella filipes* Boiss., and *Trigonella uninata* Banks & Sol. are three widely used species that grow well in the northern suburbs of Iraq and are highly used in oral consumption and folk medicine. In this study, the effect of the different extracts from *Trigonella filipes, Trigonella strangulata*, and *Trigonella uncinata* plants with pBR322 plasmid DNA tertiary structure was investigated.

Materials and methods: In this study, the effect of the different extracts from *Trigonella filipes, Trigonella strangulata*, and *Trigonella uncinata* plants with pBR322 plasmid DNA tertiary structure was investigated by their ability to modify the supercoiling of closed circular pBR322 plasmid DNA.

Results: According to this protocol, the activity of the plant extracts to prevent DNA damage was increased in the presence of hydrogen peroxide (H_2O_2) and UV light, which are factors that cause damage to DNA. We used three different solvents for plant extraction (Hexane Extraction, Ethanol Extraction, and Water

Extraction), and we used the Soxhlet method for extraction. The DNA protective action of the water and ethanol extracts of Trigonella species was better than hexane extracts. It was found that hexane extracts of all 3 plant species showed lower activity in terms of DNA defensive activity potentials. It was observed that the DNA protective activity of the *T. strangulata* plant was high at all doses applied with water and ethanol extracts, and Form 1, 2, and 3 DNA bands of pBR322 plasmid DNA.

Conclusion: It was found that the water extract and ethanol extract of the *T. uncinata* plant showed a better level of DNA protective activity than the hexane extract. The pBR322 interaction results of these plants demonstrate that these plants have been able to cause moderately conformational changes in DNA**.**

Key words: DNA Protective Properties, Trigonella Species, Iraq, pBR322 plasmid DNA

INTRODUCTION

Trigonella is a genus of the Fabaceae family. The most well-known member of the plant is fenugreek. About 135 species of this genus are naturally found in the Canary Islands, southern Europe and subtropical Africa, western and central Asia, the Indian subcontinent, and Australia. Fenugreek seeds (*T. foenum-graecum*) have several medicinal properties, and the seeds are used to treat various diseases from the past to the present (Akan et al., 2020). *Trigonella's* biological and therapeutic properties are primarily related to the variety of its physiologically active chemical components, which serve as raw materials for the production of several therapeutic and hormonal medications (Srinivasa and Naidu, 2021).

Trigonella has a wide diversity of bioactive compounds and nutrients needed to improve the function of biological systems and health. Fenugreek seeds contain 58% carbohydrates, 26% proteins, 25% fiber, and 0.9% fats. Fenugreek leaves have 6% carbohydrates, 4.4% proteins, and 1.1% fiber (Wani and Kumar, 2018). In addition, fenugreek consists of various minerals like calcium, copper, magnesium, zinc, potassium, manganese, and iron. In addition, β-carotene and Vitamin C are considered significant fenugreek components (Al-Jasass and Al-Jasser, 2012).

In addition, *Trigonella* is a source of major essential amino acids like phenylalanine, tyrosine, leucine, glutamic acid, and aspartic acid. Furthermore, it contains minor amounts of sulfur-containing amino acids that play a significant physiological role in the body. Research has shown that fenugreek proteins have better quality than other plants' proteins (Syed, et al. 2020). Feyzi *et al.* (2015) compared the quality of fenugreek proteins with soy protein, the results proved that fenugreek seeds have higher and better protein content than soy protein (Feyzi, et al. 2015).

The strongest antifungal action is displayed by cysteine-rich peptides. Tfgd1, a 225 bp complementary DNA produced from fenugreek that is rich in cysteine. Due to the presence of recombinant protein, *E. coli* exhibits antifungal activity (Olli and Kirti 2006). Because it kills 92% of *Meloidogyne javanica* larvae, the portion of *Trigonella* extract that is soluble in methanol that has been exposed as a nematicide indicates the possibility of utilizing it against nematodes (Zia, et al. 2001). It is among the most significant qualities of fenugreek. It has a good impact on the body's defenses against dangerous germs and may be utilized in many food items to combat microorganisms and fungi.

Trigonella strangulata Boiss., *Trigonella filipes* Boiss., and *Trigonella uninata* Banks & Sol. are three widely used species that grow well in the northern suburbs of Iraq and are highly used in oral consumption and folk medicine. Therefore, the present study was designed to evaluate DNA protective properties among various *Trigonella* species.

Materials and methods

Collection and identification of plant

The mature and healthy plants of *T. strangulata*, *T. filipes*, and *T. uncinata* were collected from Amadya,

Rowanduz, and Erbil, the north of Iraq. They were identified with Flora of Iraq (Guest and Townsend, 1966) and recorded with 6560, 6561, and 6562 reference numbers at the Harran University Herbarium, Turkey. The specimens are preserved at the HARRAN herbarium. Plants were collected in the Erbil Mountains in the first week of March 2021. The whole plant was cleaned and sun-dried for a week, then powdered after drying and stored in the refrigerator at + 4 °C until the extracts (Mohammad et al., 2023). The www.theplantlist.org database was used in this investigation to verify the correctness of the scientific names of plant species.

We have interviewed 20 people using plants in the Erbil region for the ethnobotany of plant species. During our trips to the region, we asked local people to show us the plants they used for medicinal purposes; we recorded the local names of the plants they used.

According to field trips and preliminary investigations, three taxa of *Trigonella*, including *Trigonella strangulata, Trigonella filipes*, and *Trigonella uncinata* mostly used in the North of Iraq for different purposes (Table 1).

Table 1. Our preliminary visit to the local people of North Iraq for traditional uses of some species of Trigonella.

Species	Location	Traditional uses for human	Traditional uses for animals
Trigonella strangulata	Shaqlawa Safeen Las Mirawa Hiran Siktan	anti-diabetic, antiinflammation, abdominal pain, and hepatic abnormalities	dried and kept for animal diet in winter because it increases animals' milk production.
Trigonella filipes		Swelling of the body, such as leg and abdomen, fever, and cough	
Trigonella uncinata		Decrease lipid profile, inflammation, fatigue, and itching skin.	

Extraction of plant samples

We used three different solvents for plant extraction, and we used the Soxhlet method for extraction. Ethanol, n-hexane, and water were added to a round bottom flask into a soxhlet extractor and condenser on an isomantle. The solvent was heated to reflux and evaporated. Then, the solvent vapor traveled up to the distillation arm that was insulated with glass wood and flooded into the chamber housing the thimble containing dried and ground plants. Once the solvent level reached the siphon, it was poured back into the flask, and the cycle started again. The process lasted for a total of 3 hours. Finally, hexane was evaporated by a rotary evaporator, and a small yield of extracted plant material (2 to 3ml) was left in the bottom of the glass flask. Afterward, the extract was collected in tightened vials and stored in a refrigerator for later analysis. For the study with experimental animals, we used water as a solvent because people traditionally use tap water for boiling plants. Before being applied to rats, boiled extracts were retained at + 4 °C refrigerator for a week (Pandiselvam et al., 2023).

2.2.1. Hexane Extraction

About 10 g of dried powder of plant was added to a round bottom flask in a Soxhlet extractor and a condenser on an isomantle heater. The hexane solvent was heated to reflux and evaporate. Then, the solvent vapor

traveled up to the distillation arm that was insulated with glass wood and flooded into the chamber housing the thimble containing dried and ground plants. When the solvent level reached the siphon, it was poured back into the flask, and the cycle started again. The process should run for a total of 3 hours. Finally, the hexane was evaporated by a rotary evaporator, leaving a small amount of the extracted plant material (2-3 ml) in the glass bottom flask. Afterward, the extract was collected in tightened vials and stored in a refrigerator for further analysis (Ramluckan, et al. 2014).

2.2.2. Ethanol Extraction

The amount of 1000 mL of ethanol was added to 100 g of dried plant powder on a Soxhlet apparatus, and the mixtures were subjected to the extraction process for 6 hours. After finishing the extraction process, the extract was filtered through the filter paper (Whatman blue band), and then solvent samples were evaporated in a rotary evaporator (Ishihara et al., 2010) at 40°C. The extracts were weighted to determine the yield efficiency of extracts (Vongsak et al., 2013).

2.2.3. Water Extraction

Water was then drawn out after the dried plants were homogenized in a Rondo homogenizer. In the water extraction procedure, 100 g of pulverized plant samples were combined with 1000 mL (80°C) distilled water and allowed to infuse for 24 hours. Filter paper (Whatman blue band) was used to filter after the period. Lyophilization was employed to separate the filtrate from the water that served as the solvent. By weighing the quantity of lyophilized plant powder obtained, the extraction method's yield was determined (Vongsak, et al. 2013).

Animal

In this study, 40 male albino rats, *Rattus norvegicus*, free of complications used. They were procured from the Laboratory Animal Center (College of Science, University of Zakho, Duhok Province, Iraq). All rats were 8-10 weeks old and weighed about 230-250 grams. The animals were bred and kept in the animal house of the Biology Department, College of Education, Salahaddin University-Erbil for a minimum of one week before the onset of the experiment, under observation to exclude any undercurrent infection and to acclimatize the laboratory conditions.

Determination of DNA Damage Protecting Activities of Plant Extracts

2.4.1. Electrophoresis Method

Molecules with natural positive or negative charges, such as DNA, protein, carbohydrate, RNA, or all charged compounds after various pretreatments, can be separated by electrophoresis. In the electrophoresis process, different buffers can be used to separate each molecule, and different buffers can be preferred for the same molecule (Takım, 2010). When working with natural double-stranded DNA, many buffers can be employed. EDTA, Tris-Acetate-EDTA (TAE), and Tris-Boric acid-EDTA (TBE) buffers are examples of these. The ionic strength and content of the electrophoresis buffer have an impact on the electrophoretic mobility of DNA. Typically, concentrated solutions of electrophoresis buffers are made and kept at room temperature (Takım, 2010).

2.4.2. Materials Needed for Electrophoresis

Necessary materials for electrophoresis include micropipette and pipette tips (1-20 µL), electrophoresis unit, power supply, agarose, electrophoresis buffer (TBE), Ethidium Bromide (10 mg/mL), loading buffer, sized DNA ladder (100bp), plasmid DNA samples, UV transilluminator for gel imaging (Victorino et al., 2020).

2.4.3. Detection of DNA Protective Activity

PBR322 plasmid DNA (Thermo) was used to determine the effectiveness of extracts to protect DNA against

UV and oxidative damage. H2O2 and UV damage plasmid DNA in the presence of extracts. The method modified by Russo et al. (2000) and optimized by Berk (2012) and Takım and Işık (2020) was used. Imaging was performed on 1.5% agarose gel. In preliminary trials before starting DNA protective activity tests, concentration values of 5.0% of the plant extracts were found to be insufficient. Therefore, a 7.0% stock of these extract concentrations was prepared. To prepare 7.0% of the stock concentration of extracts, 70 mg of the extracts were weighed, and then 1000 µL of DMSO was added to them. The dilutions of the extracts were carried out after the complete dissolution of the stock concentration (Berk, 2012; Takım, 2010).

2.4.4. Dilution Step of the 7% Stock Plant Solution

a) 62.86 µl of dH2O has been added on 7.14 µl of stock solution for the 1/10 diluted samples.
b) 55.72 µl of dH2O has been added to 14.28 µl of stock solution for the 1/5 diluted samples.
c) 41.43 µl of dH2O has been added to 28.57 µl of stock solution for the 1/2.5 diluted samples.
d) 34.29 µl of dH2O has been added to 35.71 µl of stock solution for the 1/ 1.25 diluted samples.

2.4.5. Preparation of Control and Extract Tubes

Each reaction tube was prepared with the following contents:

1. Control: Plasmid DNA (3 µl) + dH_2O (6 µl)
2. Control: Plasmid DNA (3 µl) + dH_2O (6 µl) + UV
3. Control: Plasmid DNA (3 µl) + dH_2O (6 µl) + UV+ H_2O_2 (1 µl)
4. Control: Plasmid DNA (3 µl) + dH_2O (6 µl) + H_2O_2 (1 µl)
5. Plasmid DNA (3 µl) + 1/10 diluted extracts Plant extract (5 µl) + UV+ H_2O_2 (1 µl)
6. Plasmid DNA (3 µl) + 1/5 diluted Plant extract (5 µl) + UV+ H_2O_2 (1 µl)
7. Plasmid DNA (3 µl) + 1/2.5 diluted Plant extract (5 µl) + UV+ H_2O_2 (1 µl)
8. Plasmid DNA (3 µl) + 1/1.25 diluted Plant extract (5 µl) + UV+ H_2O_2 (1 µl)
9. Plasmid DNA (3 µl) + Stock Plant extract (5 µl) + UV+ H_2O_2 (1 µl).

In addition, 5.0 µl of diluted plant extracts (1/10 diluted extract in tube 5, 1/5 diluted extract in tube 6, 1/2.5 diluted extract in tube 7, and 1/1.25 diluted extract in tube 8) were added. Then, 5.0 µl of the stock extract solution was added directly to the 9th tube without dilution. 3.0 µl of pBR322 plasmid DNA (100 ng.µl-1) and 1.0 µl of 30% H_2O_2 reactive were placed in each tube.

Tubes containing plant extracts (tubes 5 to 9) and tubes 3 and 4 were exposed to UV rays for 5 minutes. At room temperature, an intense light transilluminator device (DNR-IS) was used as a light source of 302 nm wavelengths and 8000 µW/cm2. Its purpose was to decompose H_2O_2 in the environment into OH radicals by exposure to UV light, to cause damage to plasmid DNA, and to reveal the protective properties of plant extracts. After exposure to UV, 2.0 µl of loading buffer was added to each tube, and then the contents of the tubes were loaded on a 1.5% agarose gel. The gels were photographed in a gel imaging system (SynGene). As a control in this experimental setup, pBR322 plasmid DNA without UV and H_2O_2 exposure treatment was used (Berk, 2012).

2.4.6. Preparation of Agarose Gel

1. A mold was formed with the plastic plate of the electrophoresis device, and the plate was placed in a flat place in a horizontal position. Comb plastic was placed by the number of samples.
2. Agarose (1.5 g) was added to 100 mL of 1x Tris Boric acid EDTA (TBE) buffer in a 250 mL flask, and the flask was kept in a microwave oven until the agarose melted.

3. The agarose solution was cooled to 50-60ºC. Then 1.5 µl of ethidium bromide (10 mg/mL) was added and mixed.
4. The agarose solution was poured into the plastic plate.
5. The agarose gel was left at room temperature for 15 to 30 minutes to solidify completely. After the gel had solidified, the combs and the mold attachments on the sides of the plate were carefully removed.
6. The frozen solidified gel was placed in the electrophoresis tank in the appropriate direction, and the inside of the tank was filled with 1x TBE buffer until it reached the maximum line (Davis, 2012).

2.4.7. Loading and Running Samples on Agarose Gel

1. To Eppendorf tubes that had been UV-excluded, 5 µl of loading buffer was added. The gel loading buffer increases the density of the DNA samples to be investigated and ensures that the DNA settles properly into the well. At the same time, it makes the loading process visible by coloring the samples to be investigated. It provides convenience to the researchers by making it visible that the DNA samples move toward the anode in the applied electrical field during the electrophoresis process. Gel loading buffers are usually prepared as 6x concentrated solutions.
2. It was ensured that the gel wells formed by removing the comb apparatus were in good contact with the electrophoresis buffer. Care should be taken during this process to add 1x TBE buffer to coat the gel.
3. The prepared DNA samples were carefully loaded into buffer-filled wells with an appropriate pipette.
4. The lid of the tank was closed, and electrical connections were made. DNA moves from the cathode (black end) to the anode (red end). Since the DNA molecule is negative (-) due to the phosphate groups that it carries, it will move toward the positive pole (+) in the applied electric field. Electrophoresis was carried out for 3 hours by applying 500 mA current at 40 V.
5. The electric current was cut off, and the electrophoresis cover was removed.
6. Agarose gel was taken on the UV transilluminator device to examine the DNA bands by photographing it. Here, ultraviolet light was given to the gel from the bottom so that the DNA fragments to which ethidium bromide was attached could be seen visually. Ethidium bromide is a toxic and carcinogenic chemical, and it binds tightly by entering between the double-chain bonds of DNA, which is the genetic material. Also, ethidium bromide is a fluorescent substance that appears to glow under ultraviolet (UV) light. In this step, since ethidium bromide forms chemical bonds between the DNA fragments, it is possible to see the DNA on the gel when examined under UV light (Davis, 2012).

3. Results and Discussion

Although there are many ways to study DNA molecules, gel electrophoresis is one of the most basic techniques that is frequently employed in labs. The mobility of these molecules in an electric field is influenced by the net electric charges of the molecules. This idea also serves as the foundation for the electrophoresis method. Agarose gel electrophoresis is the most widely used technique for detecting, purifying, and separating nucleic acid fragments. Because of this, the agarose gel electrophoresis technique is a crucial experimental system in the field of molecular genetics for the identification of DNA and RNAs isolated for various purposes, determining what form they are in, determining their size, and especially for the examination of new forms obtained after changes in DNA structure with genetic engineering techniques. The samples in an agarose gel are run horizontally in an electric field with constant power and direction. Because of its sugar-phosphate backbone, DNA has a negative charge, which causes it to migrate from the anode to the cathode in an electrical field (Reineke and Schlömann, 2023).

There are three forms of plasmid DNA:

1) Supercoiled (There is no broken chain on the DNA, and it is a very condensed circular complex, Form I): Supercoil conformation can only be sustained if both polynucleotide chains are intact. The technical name for this conformation is the covalently closed circular form (covalently closed circular –ccc) (Takım, 2010).
2) Open circular (there is a broken chain on one of the DNA chains, and it is still circular DNA, Form II): If only one of the polynucleotide chains is broken, the plasmid adopts its alternate shape when the double helix resumes its naturally relaxed condition: open circular –oc (open circular form) (Takım, 2010).
3) Linear (there are two broken chains on the DNA duplex chain, so it is not circular, it turns linear double strained DNA, Form III) (Takım, 2010).

In Agarose Gel Electrophoresis, these forms move at different speeds (Takım, 2010). Form I moves the fastest in the gel because of its low charge density and low volume. Since Form II is less dense, it moves more slowly. Form III has a faster speed than Form I and Form II (Figure 1).

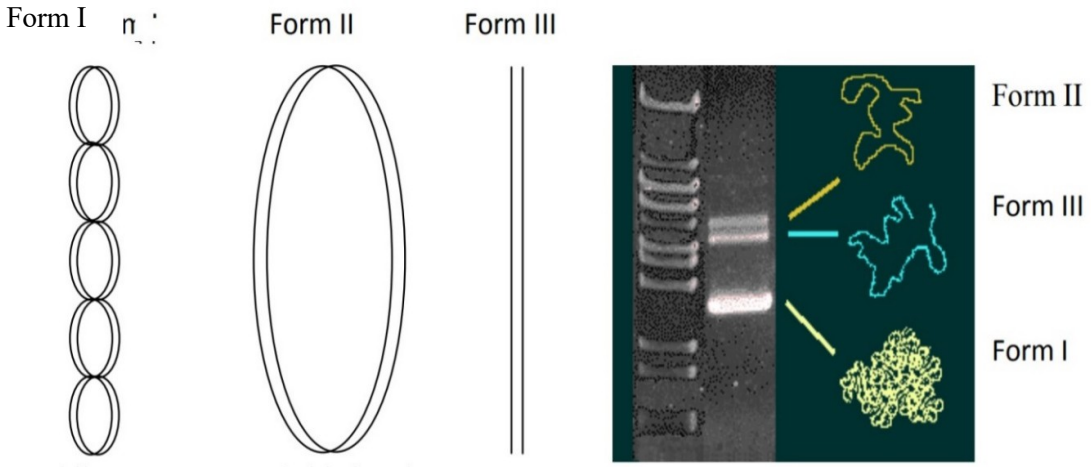

Figure 1. The different forms of plasmid DNA.

Generally, it is well acknowledged that the drug's interaction with cellular DNA underlies its antineoplastic effect. If the cell cannot remove the DNA damage, by the time the cells die by one of the metabolic pathways (Boğatarkan et al., 2015; Wang and Lippard, 2005). In this study, the effect of the different extracts from *Trigonella filipes, Trigonella strangulata*, and *Trigonella uncinata* plants with pBR322 plasmid DNA tertiary structure was investigated by their ability to modify the supercoiling of closed circular pBR322 plasmid DNA. According to this protocol, the activity of the plant extracts to prevent DNA damage was increased in the presence of hydrogen peroxide (H_2O_2) and UV light, which are factors that cause damage to DNA. This electrophoretic visualization pattern of pBR322 plasmid DNA forms is consistent with previous reports (Boğatarkan et al., 2015; Takım, 2010; Wang and Lippard, 2005).

There are normally 2 forms of pBR322 plasmid DNA. Form I is a complete, covalently closed, and swift-moving circular shape. The supercoiled shape will relax to produce the open circular form II, which moves more slowly if modification only affects one chain. A linear form III that transitions between form I and form II will be seen if both double chains are cleaved by any factor. Within the scope of this study, pBR322 plasmid DNA was treated with different doses of plant extracts of *Trigonella* species at 37 °C for 24 h. No interaction between plasmid DNA bands was detected at low extract doses. In the visualization of these samples, it was seen that the form I and form II bands' density decreased, while the form III band appeared between these 2 band places on the gel in Figures 2-4. Columns 2-5 in the gel images contain control samples.

In the first column, a DNA ladder sample is loaded to compare the size of the DNA bands. The plant extract

was not applied in the control samples, and there was plasmid DNA. In different columns, applications such as hydrogen peroxide and UV, which will cause damage to plasmid DNA, were made. The components applied in the columns are indicated in the figure caption. In the gel column indicated with C1 marking, it was possible to see all forms of plasmid DNA in both gels since the factors that would cause damage to the plasmid DNA were not used. It is seen that the plasmid DNA is eliminated in column (C3), where only UV damage is applied to the plasmid DNA (C2), and both UV damage and hydrogen peroxide damage are applied together (Figures 2-4). In this case, the damaging effects of UV light and UV light + hydrogen peroxide effects on DNA are understood. In column (C4), on which only hydrogen peroxide was applied to the plasmid DNA, the DNA bands were weakened and faded (Figures 2-4).

The findings regarding the DNA protective activity of different extracts of *Trigonella* sp. plants are given in Figures 2-4. The effects of ethanol, hexane, and water extracts of *Trigonella filipes* against DNA damage are shown in Figure 2. DNA defensive activities of ethanol extract, hexane extract, and water extracts of *Trigonella strangulata* plant are given in Figure 3. The DNA defensive activities of ethanol, hexane, and water extracts of *Trigonella uncinata* are shown in Figure 4, respectively. They are gel wells in which plant extracts are used between the 5th and 10th wells in all gel images. Therefore, the DNA protective activities of the plants we investigated in our study are displayed.

As seen from the gel images in Figures 2-4, the DNA protective action of the water and ethanol extracts of Trigonella species was better than hexane extracts. It was found that hexane extracts of all 3 plant species showed lower activity in terms of DNA defensive activity potentials. It was resolute that almost all of the pBR322 plasmid DNA was damaged and destroyed in the column marked as number 1, where ethanol and hexane extracts of the *T. filipes* plant were used at the lowest concentration (Figure 2). It has been determined that the linear DNA (Form III) band and Form II, which we call open circular, are quite clearly evident in columns 2-4, in the samples where higher doses of plant extracts were applied. It has been determined that the form I band, which we call supercoiled DNA, which is specific to plasmid DNA, can generally be seen in the samples marked as 5th and 6th columns, where high doses of plant extracts are applied (Figures 2-4).

The samples in which the water extract of the *T. filipes* plant was applied in high doses appeared to carry all 3 forms of plasmid DNA on the gel. (Columns 3-6 in Figure 2). It was determined that the samples to which the ethanol extract of the *T. filipes* plant was applied carried open circular and linear DNA, which we called form 2 and form 3, of the plasmid DNA on the gel (columns 2-6 in Figure 2). In the samples where the hexane extract of *T. filipes* plant was applied at stock concentrations, it was determined that only plasmid DNA carried open circular and supercoiled DNA, which we called form2 and form 1, on the gel. The protective activity of plasmid DNA was not detected in plant extract applications at other concentrations (columns 5-6 in Figure 2).

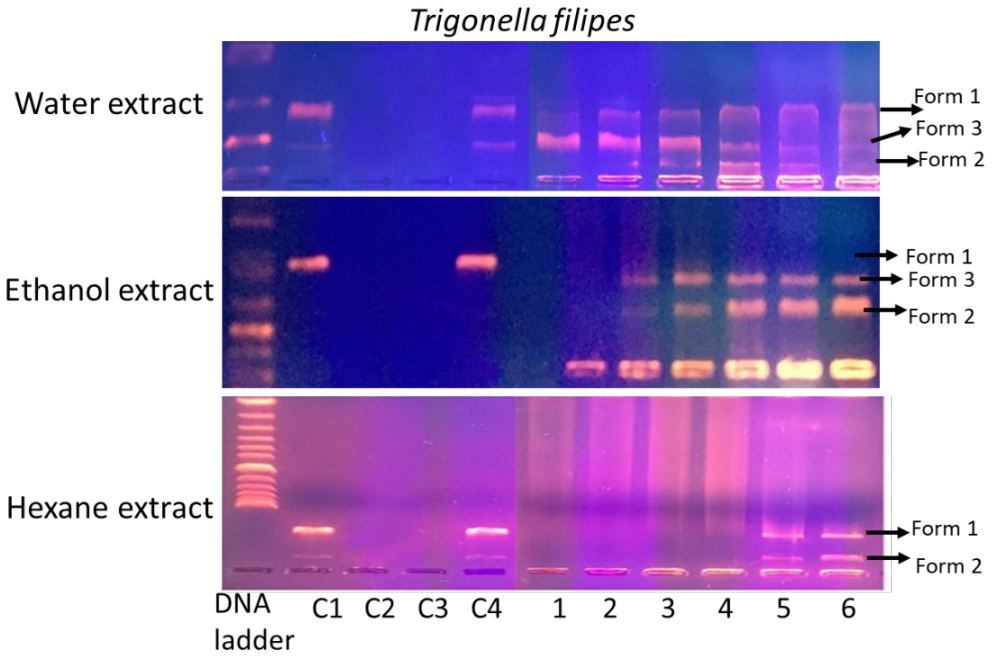

Figure 2. Imaging of DNA protective activity of *Trigonella filipes* plants on an agarose gel. [1. column is the DNA ladder. C1 column: Plasmid DNA + distilled water, C2 column: Plasmid DNA + distilled water+ UV, C3 column: Plasmid DNA + distilled water + UV+ H_2O_2, C4 column: Plasmid DNA + dH_2O + H_2O_2, The column 1-4: Plasmid DNA + *Trigonella filipes* plant extracts (from most diluted sample to the most concentrated extract were added sequentially) + UV+ H_2O_2. Column 5. and 6.: Plasmid DNA + directly stock *Trigonella filipes* extract + UV+ H_2O_2]

It was observed that the DNA protective activity of the *T. strangulata* plant was high at all doses applied with water and ethanol extracts, and Form 1, 2, and 3 DNA bands of pBR322 plasmid DNA were present on the gel (Figure 3). It was observed that the hexane extract of the *T. strangulata* plant showed a weaker DNA protective activity. It was determined that the Form 1 and Form 2 bands of the plasmid DNA were seen on the gel, and the Form 3 band was seen as faintly in the samples where only a high dose of stock concentration was applied (Columns 5 and 6 in Figure 3).

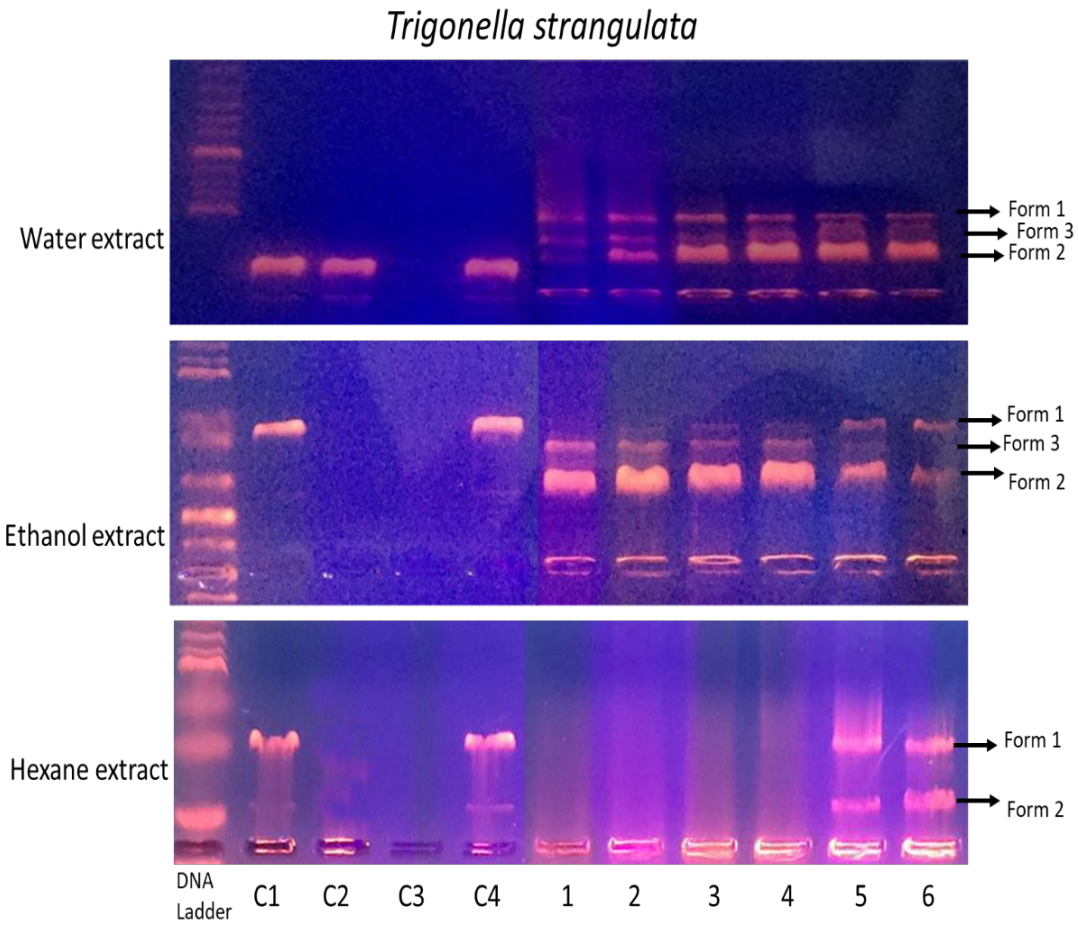

Figure 3. Imaging of DNA protective activity of *Trigonella strangulata* plant on an agarose gel. [1. column is the DNA ladder. C1 column: Plasmid DNA + distilled water, C2 column: Plasmid DNA + distilled water + UV, C3 column: Plasmid DNA + distilled water + UV+ H_2O_2, C4 column: Plasmid DNA + dH2O + H_2O_2, The column 1-4: Plasmid DNA + *Trigonella strangulata* plant extracts (from most diluted sample to the most concentrated extract were added sequentially) + UV+ H_2O_2. Coulumn 5. and 6.: Plasmid DNA + directly stock *Trigonella strangulata* extracts + UV+ H2O2].

It was found that the water extract and ethanol extract of the *T. uncinata* plant showed a better level of DNA protective activity than the hexane extract. It was observed that Form 2 and Form 3 bands of plasmid DNA were found on the gel at all doses of ethanol and water extract of the *T. uncinata* plant. However, it is seen that the supercoiled DNA band, which we call the form 1 band, has become very faint. Considering that this plant gives the same band image in all dose groups, it is predicted that supercoiled DNA form 1 band can also be detected on the gel if higher doses of plant extract are applied. It is seen that hexane extracts of the *T. uncinata* plant do not have DNA protective activities in low plant dose applications, but when plant extract is applied at stock concentrations, pBR322 plasmid DNA carries Form I and Form 2 bands (Figure 4).

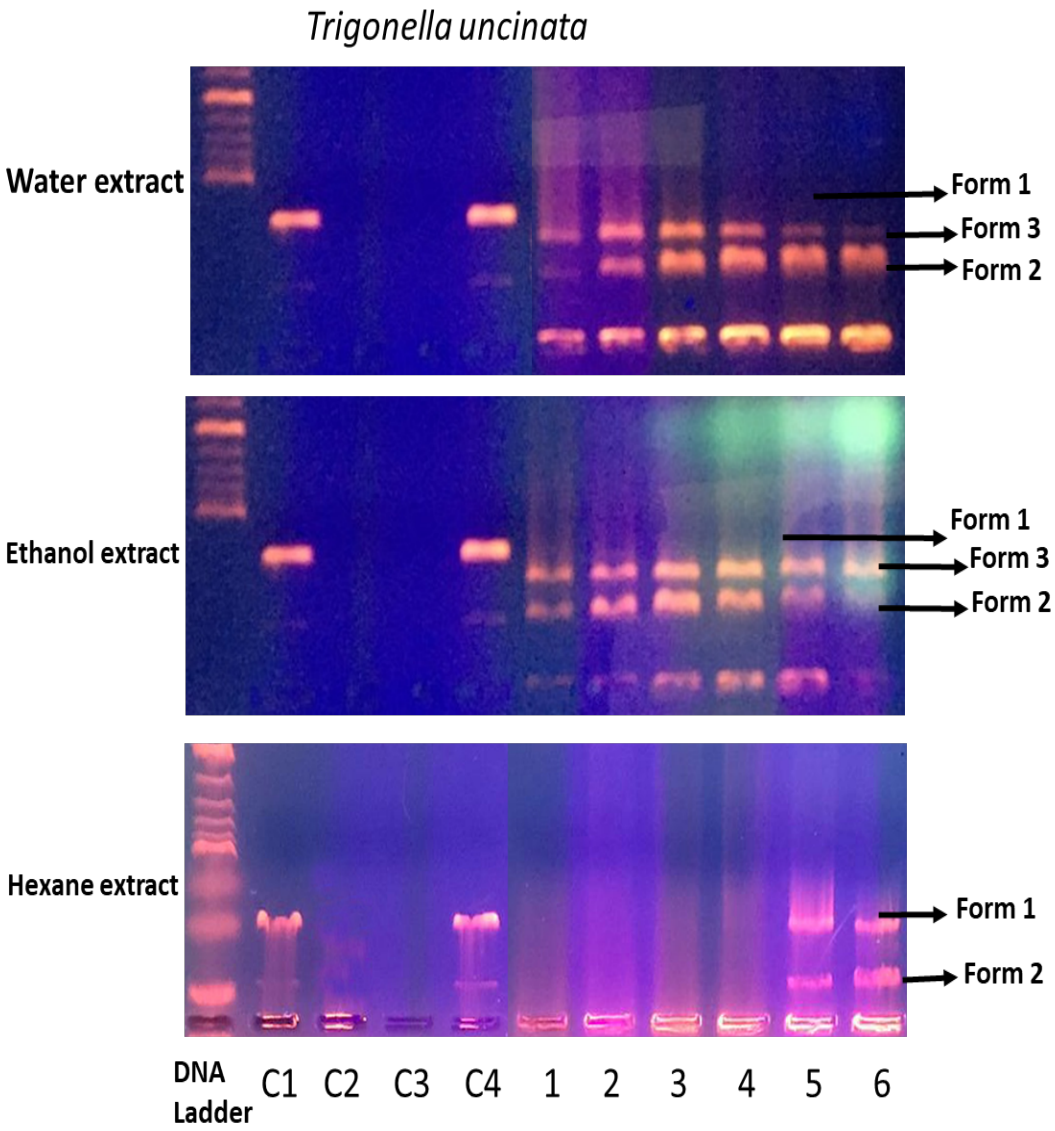

Figure 4. Imaging of DNA protective activity of *Trigonella uncinata* plant on an agarose gel. [1. column is the DNA ladder. C1 column: Plasmid DNA + distilled water, C2 column: Plasmid DNA + distilled water+ UV, C3 column: Plasmid DNA + distilled water + UV+ H_2O_2, C4 column: Plasmid DNA + dH2O + H_2O_2, The column 1-4: Plasmid DNA + *Trigonella uncinata* plant extracts (from most diluted sample to the most concentrated extract were added sequentially) + UV+ H_2O_2. Columns 5. and 6.: Plasmid DNA + directly stock *Trigonella uncinata* extract + UV+.

In general, the clarity and brightness of the plasmid DNA bands observed in all samples are also of very good quality (Figures 2-4). It can be said that the protective activities of the water and ethanol extracts of the *T. strangulata* plant on DNA are of the best quality among all 3 plant species. It has been observed that all 3 plant species reduce the destructive effect of H_2O_2+UV damage, have very good protective potentials against DNA damage at high doses, and stabilize the DNA well (Figures 2-4).

4. Conclusions

The pBR322 interaction results of these plants demonstrate that these plants have been able to cause moderately conformational changes in DNA. This is considered to be originating from covalent inter-strand binding. We think that these plants may be drug candidates in the future with the support of various in vitro and in vivo studies.

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