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Evaluation of Antimicrobial activity and Genotoxic Potential of *Capparis spinosa* (*L.*) Plant Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Authors GMA and GIO designed the study, wrote the protocol, conducted experimental work, managed the analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: The aims of this study were to evaluate the antimicrobial activity and the genotoxic effect of both ethanolic and aqueous extracts of stem and leaf of *Capparis spinosa* (*C. spinosa*) plant on *Escherichia coli* (*E. coli*) ATCC 25922, *Staphylococcus aureus* (*S. aureus*) ATCC 6538P, clinical isolate of Methicillin-resistant *S. aureus* (MRSA) and *Klebsiella pneumoniae* (*K. pneumoniae*) and *Candida albicans* (*C. albicans*) ATCC 90028.
Materials and Methods: The antimicrobial activity was determined using microbroth dilution method, while the genotoxic effect was investigated using randomly amplified polymorphic DNA (RAPD)-PCR and enterobacterial repetitive intergenic consensus (ERIC)-PCR.
Results: The MIC values of both ethanolic and aqueous leaf and stem extracts of *C. spinosa* plant had a range 6.25 mg/ml to 100 mg/ml. In addition, it was found that ethanolic extract more effective than aqueous extract. The genotoxic activity of aqueous leaf extract, showed changes in both Random Amplified Polymorphic DNA (RAPD)-PCR and Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR profiles of *E. coli* strain treated with extract compared to untreated (negative) control. These changes included an alteration in the intensity, absence or appearance of new amplified fragments.

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Conclusions: Results of this study strongly show the genotoxic effect of aqueous leaf extract from *C. spinosa* plant on *E. coli*. The findings draw awareness to the possible toxic effect use of *C. spinosa* plant in traditional medicine and point out the capability of using *C. spinosa* to treat bacterial or fungal infections. More studies are needed to detect the exact ingredients of this plant as well as the mechanisms responsible for genotoxicity. Further *in vivo* genotoxicity studies are recommended to ensure and to evaluate the safety of using plants for therapeutic purposes. In addition, results of this study showed that molecular fingerprinting based on ERIC-PCR can be used to evaluate the genotoxic effect in the model bacterial species *E. coli*.

Keywords: Capparis spinosa; antimicrobial; genotoxic effect; ethanolic extract; aqueous extract.

1. INTRODUCTION

Plants are considered a rich source of medicinal and nutraceutical agents for centuries [1-2]. In the modern age, approximately 25% of the new drugs originated from plant sources. Among valuable flora, wild plants have gained much awareness in recent decades because of their functional food and potential health benefits [3-4]. *Capparis* L. is considered the largest genus of the family Capparaceae (or Capparidaceae). This genus includes 350 species and is distributed in many parts of the world, in arid and semi-arid regions of the tropical and subtropical world, many of them distributed in the Mediterranean regions [5]. The Caper (*Capparis spinosa* (*C. spinosa*)) is naturally widely distributed from the Atlantic coast of the Canary Island and Morocco to the Black Sea, in Crimea and Armenia, and to the east side of the Caspian Sea and Iran. It is also spread in Europe, North Africa, Australia, West Asia and Afghanistan. This plant might have emerged in the tropic areas, and then extended to other parts of the world such as the Mediterranean basin and Central Asia [5]. *Capparis spinosa* plant is a perennial shrub, thorny, 0.3–1 m tall and has deep roots, which can extend up to 6-10 m [6-7].

Capparis spinosa is considered a future source of invaluable nutrient materials for human food and has been used in traditional medicine to treat several human infections [6]. Phytochemical analysis showed that this plant has high quantities of numerous bioactive ingredients and molecules, which are responsible for different pharmacological activities. These activities include antioxidant effect [6,8-10], antifungal effect [11], phytotoxic effect [11-12], anticancer [9,13-14], nephrotoxicity and hepatotoxicity effects [15], antibacterial effect [12,16-19], antimutagenic effect [20]. Other pharmacological effects have also been reported. Since *C. spinosa* has several beneficial health effects on human diseases, the adverse effects of using or consumption certain parts of this plant is not studied [3]. This study was conducted to evaluate the genotoxic potential of the aqueous extract from *C. spinosa* growing wild in Palestine on *Escherichia coli* (*E. coli*) ATCC 25922 strain using Random Amplified Polymorphic DNA (RAPD)-PCR and Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR as well as to determine the antimicrobial activity of both ethanolic and aqueous extracts of stem and leaf of *C. spinosa* plant.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The stem and leaf parts of *C. spinosa* plant were collected from a natural habitat in Tulkarm province, West Bank-Palestine, during summer, 2019. Identification of the plant was conducted by the plant taxonomist Dr. Ghadeer Omar, Department of Biology and Biotechnology, An-Najah National University, Palestine.

The collected stem and leaf parts of *C. spinosa* were washed with water to eliminate soil and dust particles, then they were dried. Light exposure was avoided to minimize or prevent possible loss of active molecules. To obtain a fine powder that was ready for ethanolic and aqueous extract preparation, the air dried stem and leaf parts were powdered using an electric blender.

2.2 Plant Extract Preparation

2.2.1 Ethanolic extract

Ethanolic extract was prepared as described previously [21-22] with some modifications. Briefly, approximately 30 g of dried plant powder was mixed thoroughly using a magnetic stirrer in

150 ml of 80% ethanol. The ethanol-plant powder part mixture was incubated on a shaker at room temperature for 48h. The mixture was filtered using muslin cloth to remove large insoluble particles. After that, the plant mixture was centrifuged at 5,000 rpm for 15 min at 4°C, to remove fine particles. Then, the supernatant extract was dried in an incubator at 40°C. The dried plant extract powder was kept in a refrigerator at 4°C. Before starting the assays, the dried plant extract powder was dissolved in 10% dimethyl sulfoxide (DMSO) to obtain a final concentration of 200 mg/ml and stored at 4°C for further experiments.

2.2.2 Aqueous extract

Aqueous extract was prepared as described previously [21,22] with some modifications. Briefly, approximately 30 g of dried plant powder was mixed thoroughly using a magnetic stirrer in 150-ml cold (room temperature) sterile distilled water. The water-plant powder part mixture was incubated on a shaker at room temperature for 48h. The mixture was filtered using muslin cloth to remove large insoluble particles. After that, the mixture was centrifuged at 5,000 rpm for 15 min at 4°C, to remove fine particles. Then, the supernatant extract was dried and concentrated by freeze dryer (lyophilizer). The dried plant extract powder was kept in a refrigerator at 4°C. Before starting the assays, the dried plant extract powder was dissolved in sterile distilled water to obtain a final concentration of 200 mg/ml and stored at 4°C for further experiments.

2.3 Determination of Antimicrobial Activity of *C. spinosa* Extracts

2.3.1 Determination of MIC for plant extracts by the broth microdilution method

MIC of plant extracts was determined by the broth microdilution method in sterile 96-well microtiter plates according to the CLSI instructions [23]. The plant extract (200 mg/ml of 10% DMSO, 200 mg/ml of sterile distilled water) and 10% DMSO (negative control) were two-fold-serially diluted in Mueller Hinton broth directly in the wells of the plates in a final volume of 100 µl. After that, a bacterial inoculum size of 10^5 CFU/ml (*Candida albicans* (*C. albicans*) inoculum size of 0.5 to 2.5 × 10^5 CFU/ml) was added to each well. Negative control wells containing either 100µl Mueller Hinton broth only, or 100 µl DMSO with microorganism inoculum, or plant extracts and Mueller Hinton broth without microorganism were also included in these experiments. Each plant extract was performed in duplicate. The microtiter plates were then covered and incubated at 37°C for 24h. The MIC was taken as the lowest concentration of plant extract, which inhibits the visible growth of the test microorganism.

2.3.2 Evaluation of the genotoxic potential of *C. spinosa* aqueous leaf extract on *E. coli* ATCC 25922 strain

2.3.2.1 Inoculation of Escherichia coli ATCC 25922 strain

Few colonies from a 24h old *E. coli* strain growth culture plated on Eosin Methylene Blue (EMB) agar medium were subcultured under sterile conditions into a bottle containing 20 mL of nutrient broth, then incubated at 37°C for 1h with continuous shaking. After that, aseptically, 1 ml of *E. coli* culture was added to each of the four sterile bottles each containing 25 ml nutrient broth medium. These bottles were incubated at 37°C for 1h with continuous shaking. Then, three concentrations of aqueous leaf extract (250 µg/ml, 125 µg/ml and 62.5 µg/ml of distilled water) were added to three bottles of the *E. coli* broth culture. The fourth bottle was considered a negative or untreated control by adding 1 ml of sterile distilled water.

2.3.2.2 DNA extraction

The DNA genome of *E. coli* was prepared for randomly amplified polymorphic DNA (RAPD)-PCR and enterobacterial repetitive intergenic consensus (ERIC) PCR according to the method described previously [24]. Three ml samples were taken from the *E. coli* growth culture after 2 h, 5 h, and 24 h, centrifuged for five minutes at 14,000 x g where the supernatant of each sample was discarded. Then, each bacterial sample pellet was re-suspended in 0.8 ml of Tris-EDTA (10 mM Tris-HCl, 1 mM EDTA [pH 8]), centrifuged for 5 min at 14,000 x g; after that, the supernatant was discarded. The pellet of each bacterial sample was re-suspended in 300 µl of sterile distilled water and boiled for 15 min. Then, the mixture was incubated in ice for 10 min. The samples were pelleted by centrifugation at 14,000 x g for 5 min, and each sample supernatant was transferred into a new Eppendorf tube. The DNA concentration for each sample was determined using nanodrop spectrophotometer (GenovaNano, Jenway) and

the DNA samples were kept at -20°C for RAPD-PCR and ERIC-PCR- based DNA fingerprinting techniques.

2.3.2.3 RAPD-PCR assay and ERIC-PCR assay

The RAPD-PCR was conducted using RAPD primer 208 5'-ACG GCC GAC C-3' [25], while ERIC-PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3` and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3` [26]. Each PCR reaction mix (25 µL) was composed of 10 mM PCR buffer pH 8.3; 3 mM $MgCl_2$; 0.4 mM of each dNTP; 0.8 µM primer; 1.5 U of Taq DNA polymerase and fixed amount of DNA template (30 ng). Then, DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions for RAPD-PCR: initial denaturation for 3 min at 94°C; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 32°C for 1 min and extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min. The thermal conditions for ERIC-PCR were initial denaturation for 3 min at 94°C; followed by 40 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 1 min and extension at 72 °C for 1 min, followed by a final extension step at 72°C for 5 min. The PCR products were analyzed by electrophoresis through 1.8% agarose gel. The PARD-PCR and ERIC-PCR profiles were visualized using UV trans-illuminator and photographed. Changes in PARD-PCR or ERIC-PCR banding pattern profiles following plant extract treatments, including variations in band intensity as well as gain or loss of bands, were taken into consideration [21,22,27-28].

3. RESULTS

3.1 Antimicrobial Activity of *C. spinosa* Extracts

Results of this study showed that both ethanolic and aqueous extracts of stem and leaf of *C. spinosa* plant had antimicrobial activity. The MIC value of both aqueous and ethanolic extracts of *C. spinosa* on different bacterial strains had a range 6.25 mg/ml to 100 mg/ml. However, the MIC value of both aqueous and ethanolic extracts of *C. spinosa* on *C. albicans* had a range 25 mg/ml to 50 mg/mL. The MIC profile of both ethanolic and aqueous extracts of stem and leaf of *C. spinosa* plant against different microorganisms is shown in Table 1.

3.2 Evaluation of the Genotoxic Potential of *C. spinosa* Aqueous Leaf Extract

DNA genome was extracted from each *E. coli* strain, which was treated with different concentrations of aqueous leaf extract of *C. spinosa* at various time intervals. Changes in the extracted DNA genome from treated *E. coli* strain were evaluated and compared with negative (untreated) controls at the same time intervals. The effect of aqueous leaf extract on *E. coli* genome was evaluated using molecular fingerprinting based on PARD-PCR and ERIC-PCR techniques. RAPD-PCR profile showed that a band with an amplicon length of about 700-bp was less intense in *E. coli* strain treated with 3 doses (250 µg/ml, 125 µg/ml and 62.5 µg/ml) of aqueous leaf extract for 2h (Fig. 1, lanes 1, 2 and 3), compared with the same band that appeared in the negative control. However, the profile showed that a band with an amplicon length of about 1500-bp was more intense in *E. coli* strain treated with 3 doses for 2h of the same extract (Fig. 1, lanes 1, 2 and 3), compared with the same band that appeared in the negative control. The bands with an amplicon length of about 300-bp and 400-bp were less intense in *E. coli* strain treated with 250 µg/mL and 125 µg/ml of the aqueous extract for 2h (Fig. 1, lanes 1 and 2), compared with the same bands that appeared in the negative control. In addition, a band with an amplicon length of about 900-bp was less intense in *E. coli* strain treated with 250 µg/ml for 2h (Fig. 1, lane 1), compared with the same band that appeared in the negative control. Besides, a band with an amplicon length of about 200-bp appeared in *E. coli* strain treated with 250 µg/ml of aqueous leaf extract for 2h (Fig. 1, lane 1), compared with negative control. In addition, RAPD-PCR profile showed that a band with an amplicon size of more than 1500-bp appeared in *E. coli* strain treated with 3 doses of the same extract for 2h (Fig. 1, lanes 1, 2 and 3), compared with negative control. Results of RAPD-PCR showed that bands with an amplicon length of about 1500-bp, 700-bp, 400-bp and 300-bp were less intense in *E. coli* strain treated with 250 µg/ml of aqueous leaf extract for 5h (Fig. 1, lane 4), compared with the same bands that appeared in the negative control. Additionally, a band with an amplicon length of about 1200-bp was less intense in *E. coli* strain treated with 3 doses of aqueous leaf extract for 5h (Fig. 1, lane 4, 5 and 6), compared with the same band that appeared in the negative control. Besides, a new band with an amplicon size of about 200-bp appeared in *E. coli* strain treated

with 250 µg/ml and 125 µg/ml of the same extract for 5h (Fig. 1, lanes 4 and 5), compared with negative control. Results of RAPD-PCR also showed that bands with an amplicon length of about 700-bp and 300-bp were less intense in *E. coli* strain treated with 3 doses of the aqueous leaf extract for 24h (Fig. 1, lanes 7, 8 and 9), compared with the same bands that appeared in the negative control. RAPD-PCR profiles of *E. coli* strain treated with different concentrations of aqueous leaf extract of *C. spinosa* and negative control at the different time intervals are shown in Fig. 1.

ERIC-PCR profile showed that two bands with an amplicon fragment size of than 1500-bp appeared in *E. coli* strain treated with 250 µg/ml and 125 µg/ml of aqueous leaf extract of *C. spinosa* for 2h (Fig. 2, lanes 1 and 2), compared with negative control. Additionally, the band with an amplicon fragment size of about 700-bp was more intense, while the band with an amplicon fragment size of about 300-bp was less intense in *E. coli* strain treated with both doses 125 µg/ml and 62.5 µg/ml of the same extract for 2h (Fig. 2, lanes 2 and 3), compared with the same bands that appeared in the negative control. Besides, the bands with an amplicon fragment length of about 300-bp and 700-bp disappeared in *E. coli* strain treated with 125 µg/ml of aqueous leaf extract of *C. spinosa* for 2 h (Fig. 2, lane 1), compared with the same bands that appeared in the negative control. Results of ERIC-PCR showedthat band with an amplicon fragment size of more than 1500-bp was less intense in *E. coli* strain treated with 3 doses of aqueous leaf extract for 5 h (Fig. 1, lane 4, 5 and 6), compared with the same band that appeared in the negative control. Besides, a new band with an amplicon fragment size of about 1500-bp appeared in *E. coli* strain treated with 250 µg/ml of the same extract for 5 h (Fig. 1, lane 4), compared with negative control. Additionally, the band with an amplicon fragment size of about 700-bp was more intense in *E. coli* strain treated with both doses 125 µg/ml and 62.5 µg/ml of aqueous leaf extract, while the same band that disappeared in *E. coli* strain treated with both doses 250 µg/ml of the same extract for 5 h (Fig. 2, lanes 4, 5 and 6), compared with the same bands that appeared in the negative control. The band with an amplicon fragment length of about 450-bp was less intense in *E. coli* strain treated with 250 µg/ml and 125 µg/ml for 5 h (Fig. 2, lanes 5 and 6), compared with the same band that appeared in the negative control. In addition, a new band with an amplicon fragment size of about 100-bp appeared in *E. coli* strain treated with 3 doses for 5h of the same extract (Fig. 2, lanes 4, 5 and 6), compared with negative control. ERIC-PCR profile also showed that the band with an amplicon fragment length more than 1500-bp was more intense in *E. coli* strain treated with 3 doses of aqueous leaf extract for 24 h (Fig. 2, lane 7, 8 and 9), compared with the same band that appeared in the negative control. Besides, the band with an amplicon fragment length of about 1300-bp appeared in *E. coli* strain treated with 250 µg/ml and 125 µg/ml for 24h (Fig. 2, lanes 7 and 8), compared with negative control. Additionally, the band with an amplicon fragment length of about 450-bp was less intense, while the same band was more intense in *E. coli* strain treated with 250 µg/ml and 62.5 µg/ml of the same extract for 24 h, respectively (Fig. 2, lanes 7 and 9), compared with the same bands that appeared in the negative control. Also, the band with an amplicon fragment size of about 100-bp was less intense in *E. coli* strain treated with 250 µg/ml and 125 µg/ml for 24 h (Fig. 2, lanes 7 and 8), compared with the same band that appeared in the negative control. ERIC-PCR profiles of *E. coli* strain treated with different concentrations of aqueous leaf extract of *C. spinosa* and negative control at the different time intervals are shown in Fig. 2.

Table 1. MIC profile of both ethanolic and aqueous extracts of leaf and stem of *C. spinosa* plant against different microorganisms

Microorganism	MIC (mg/ml)			
	Aqueous extract		Ethanolic extract	
	Leaf	Stem	Leaf	Stem
E. coli ATCC 25922	6.25	25	6.25	12.5
S. aureus ATCC 6538P	12.5	25	12.5	25
MRSA	50	100	12.5	6.25-12.5
K. pneumoniae	50	100	25	50
C. albicans ATCC 90028	25	50	25	50

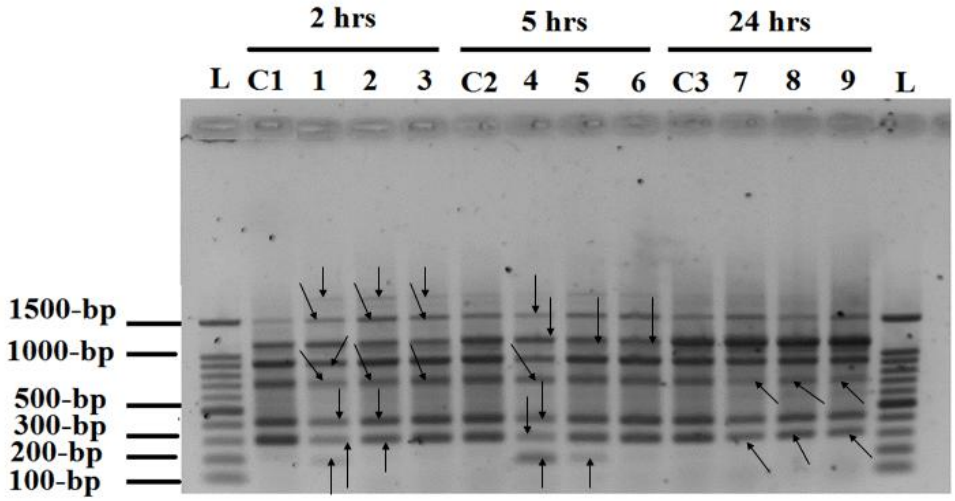

Fig. 1. RAPD-PCR profile of *E. coli* strain untreated (negative control) and treated with different concentrations of *C. spinosa* leaf aqueous extract at different time intervals. Lanes C1, C2 and C3 are negative controls; lanes 1, 4 and 7 treated with 250 µg/ml; Lanes 2, 5 and 8 treated with 125 µg/ml; Lanes 3, 6 and 9 treated with 62.5 µg/ml of plant extract; lanes L (ladder)

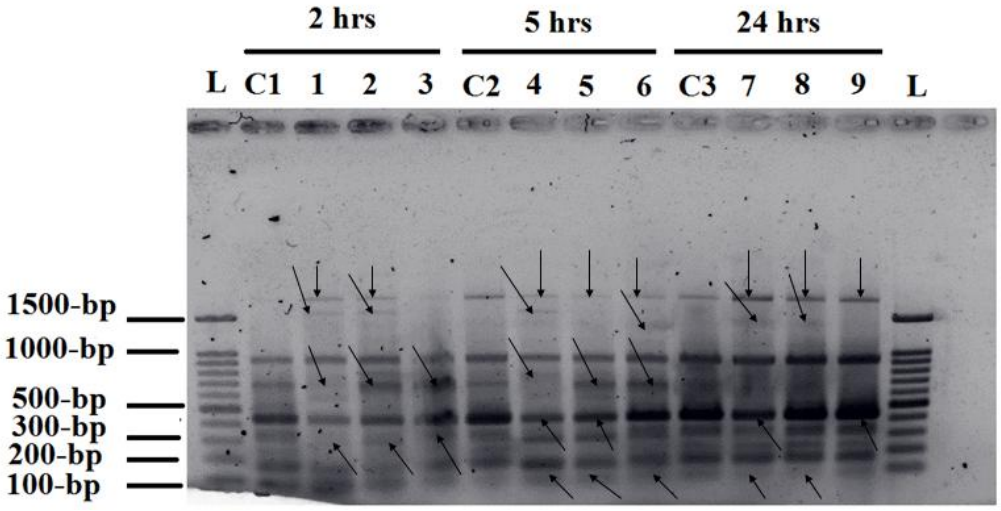

Fig. 2. ERIC-PCR profile of *E. coli* strain untreated (negative control) and treated with different concentrations of *C. spinosa* leaf aqueous extract at different time intervals. Lanes C1, C2 and C3 are negative controls; lanes 1, 4 and 7 treated with 250 µg/ml; Lanes 2, 5 and 8 treated with 125 µg/ml; Lanes 3, 6 and 9 treated with 62.5 µg/ml of plant extract; lanes L (ladder).

Results of the current study showed that molecular fingerprinting based on ERIC-PCR can be used to evaluate the genotoxic effects to estimate the chemical compounds or molecules risk connected with their potential mutagenic effects in the model bacterial species *E. coli*. Molecular fingerprinting based on ERIC-PCR has sensitivity to evaluate genotoxicity as well as molecular fingerprinting based on RAPD-PCR Figs. 1 and 2.

4. DISCUSSION

In this study broth microdilution method was used to detect the potential antimicrobial effect of both ethanolic and aqueous leaf and stem

extracts of *C. spinosa* against different species of microorganisms. These species included *E. coli*, *S. aureus*, MRSA, *K. pneumoniae* and *C. albicans*. The results of the current confirmed that both ethanolic and aqueous leaf and stem extracts of *C. spinosa* showed antimicrobial effect on these microorganisms. Antimicrobial activity of *C. spinosa* has been reported previously using different types of extracts and plant parts against different types of microorganisms [11,12,16-19]. According to the previously conducted studies, diverse phytochemical compounds are the active ingredients of *C. spinosa* plant [6-10,13].

Most nutraceutical plants are used without any standard safety and toxicological trials. The common hypothesis that the products of these plants are nontoxic. However, this hypothesis is incorrect, so toxicological tests should be conducted for herbal drugs [29]. Recently advances in machine learning and artificial intelligence immensely decoded and empowered, the herbal drug discovery and modeling, which gave medicine modern tool to predict the biosafety and efficacy [30-31] and in-silico methods [32-33] to potentially decipher the quantitative nanostructure activity-relationship (Nano-QSAR).

In this study, the potential genotoxic effect of the aqueous extract of *C. spinosa* plant against *E. coli* was tested using molecular fingerprinting based on ERIC-PCR and RAPD-PCR techniques. Reviewing the scientific literature showed that this study is the first of its kind that studied the genotoxicity of *C. spinosa* extract on prokaryotes using molecular fingerprinting based on ERIC-PCR and RAPD-PCR techniques. Besides, many plants were previously examined to investigate their genotoxic potential using different techniques [21,22,28,34-39]. In this study, RAPD-PCR and ERIC-PCR profiles showed many significant differences between the treated and untreated *E. coli* strain. The alterations in the treated *E. coli* strain with aqueous leaf extract at different time intervals included the appearance and disappearance of certain bands and the alteration in the band intensity compared with negative control. These alterations in both the RAPD-PCR and ERIC-PCR profiles of the treated *E. coli* strain compared with the negative control could be explained due to the effect of the genotoxic ingredients that were present in the aqueous leaf extract. These ingredients can induce different alterations and changes such as point mutations and/or rearrangements in chromosomes, damage and chromosomal aberrations. These alterations in the DNA might have a potential change on the primer binding sites and/or inter-priming distances [21,22]. Using other techniques such as DNA sequencing or probing can help understand the correct mechanisms that lead to such differences in RAPD-PCR and ERIC-PCR profiles [7,18,24]. Findings of the current study were in contrast to study published previously [20], which showed that *C. spinosa* buds aqueous extract is non-genotoxic and their study reveals that *C. spinosa* aqueous extract had antimutagenic potential against Ethyl Methane sulfonate induced chromosomal aberrations in *A. cepa* root meristem cells. This may be due to differences in plant parts and the techniques used to evaluate genotoxicity. In a literature survey, it is also showed that plant extracts can be mutagenic and antimutagenic depending on the test system used. This indicates that a group of assays is needed before any conclusion can be reached about the genotoxic effect [34].

Results of the current study showed that molecular fingerprinting based on ERIC-PCR is an effective and sensitive technique that can be used to evaluate the genotoxic effects to estimate the chemical compounds or molecules risk connected with their potential mutagenic effects in the model bacterial species *E. coli*.

5. CONCLUSION

The results of the current study showed that aqueous leaf extract of *C. spinosa* possesses genotoxic and mutagenic potential effects on *E. coli*. In addition, the results also point out the capability of using *C. spinosa* to treat and prevent infections caused by several microorganisms. Further studies are recommended to determine the specific ingredients in this plant as well as the correct mechanisms responsible for that genotoxicity. In addition, findings of this study showed that molecular fingerprinting based on ERIC-PCR is an effective and sensitive technique that can be used to evaluate the genotoxic effect in the model bacterial species *E. coli.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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