Original Research Article

Biochemical and Molecular Evaluation of the Plant *Ecballium elaterium* Extract Effects on *Escherichia coli*

ABSTRACT

Aims: The present study was conducted to evaluate the genotoxic effects of fruit and leaf ethanolic extracts of *E. elateruim* on clinical and reference strains of *E. coli (E. coli* ATCC 25922). **Methodology:** the genotoxic effects of these extracts were determined using enterobacterial repetitive intergenic consensus ERIC-PCR and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). **Results:** results of this research showed alterations in DNA and protein profiles of both tested *E. coli* strains treated with fruit and leaf extracts compared with untreated control. The alterations ranged between decreased or increased intensity of some bands, absence or appearance of new amplified fragments. Moreover, increased concentrations of *E. elaterium* extracts and increased time intervals seems to yield a more profound increase in total protein concentrations in both tested *E. coli* strains. **Conclusions:** Such findings strongly indicate the genotoxic effects of *E. elaterium* extracts on both *E. coli* strains. The findings draw attention to the unsafe improper use of *E. elaterium* extracts in folkloric medicine and also point out the capability of using *E. elaterium* to treat *E. coli* infections. More studies are required to find out the exact mechanisms responsible for the observed genotoxicity.

Keywords: *Ecballium elaterium*, genotoxic effect, ERIC-PCR, SDS-PAGE

1. INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines. Bioactive compounds usually extracted from plants are used as medicines, food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals. In some countries, 80% of the population are depending on medicinal plants to maintain their health and to cure their diseases [1].

The plant *Ecballium elaterium* (*E. elaterium*), bears the common name squirting cucumber, is a weed which belongs to Cucurbitaceae family. It is perennial, fleshy, rough hairy with stems 30 - 100 cm long. The flowers are greenish-yellow and the fruit is large juicy berry, 3 - 4 cm, ovate-oblong, detaching itself explosively at maturity scattering seeds and juice and when unripe of a pale green color and covered with numerous hairs. It is a plant indigenous to the Mediterranean countries and cultivated in central Europe and England. This plant grows wild in many places including the roadsides and cultivated areas [2,3].

Bioactive compounds of *E. elaterium* juice have been reported to possess different pharmacological activities for example: purgatives, analgesics, hemorrhoids, varicose veins, and nose bleeding so a fresh juice is applied locally to treat these diseases [4-7]. The fruit juice is known to be rich in cucurbitacins, phenolics and glycosylated compounds, which have numerous bioactivities [6-8]. Cucurbitacins are of interest medicinally because of their cytotoxic, antitumor and anti-jaundice properties [9]. In addition, the leaves, fruits, and flowers of *E. elaterium* are rich with flavonoids (phytomelin), which may have antioxidant, anti-inflammatory, anticarcinogen, antithrombotic, cytoprotective and vasoprotective activities [7,10]. Several studies reported that *E. elaterium* extracts possess antimicrobial and antifungal effects [10-14].

Genotoxic effect of *E. elaterium* fruit juice based on different techniques using different types of cells was studied [15-18]. To the best of our knowledge, no previous studies evaluated *E. elaterium* genotoxicity using PCR assays, that are the most reliable and the most widely used tools for assessment of the genetic variation and detection of DNA damage [19,20]. Thus the aim of the

present study was to evaluate the genotoxic effect of these extracts on *Escherichia coli* using enterobacterial repetitive intergenic consensus ERIC-PCR and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2. MATERIALS AND METHODS

2.1 Plant Material

The ripe fruits and mature leaves of *E. elaterium* were collected from Nablus, West Bank-Palestine, during August and September 2017. Ethanolic fruit and leaf extracts of *E. elaterium* were prepared by cold maceration method. The collected fruits and leaves were washed with water to remove soil and dust particles, then they were dried. Exposure to light was avoided to prevent the loss of effective ingredients. After that, the fresh fruits (20g) were homogenized finely using blender after addition 80 ml of absolute ethanol. The dried leaves were powdered finely using blender. Approximately 30-40g of dried leaf material was mixed thoroughly with magnetic stirrer in 150 ml of 80% ethanol. Both ethanol-fruit mixture and ethanol-leaf mixture were left on shaker at room temperature for 48h. The mixtures then were filtered using muslin cloth to remove large particle and insoluble materials. After that, to remove fine particles mixtures were centrifuged at 5,000 rpm for 15 min at 4°C. Then, the extracts were dried and concentrated using rotary evaporator at 50°C, and freeze dryer (lyophilizer). The dried extract obtained was stored at 4°C. Before starting the experiments, plant extracts were dissolved in 10% Dimethyl sulfoxide (DMSO) to obtain a concentration of 200 mg/ml and stored at 4ºC for further assays.

2.2 Determination of Antibacterial Activity of *E. elaterium* Extracts

2.2.1 Determination of MIC by broth microdilution method

MIC of plant extracts was determined by the microbroth dilution method in sterile 96- wells microtiter plates according to CLSI [21]. The plant extract (200 mg/ml of 10% DMSO) and 10% DMSO (negative control) were two fold-serially diluted in nutrient broth directly in the wells of the plates in a final volume of 100µL. Bacterial inoculum (*E. coli* ATCC 25922 or a clinical strain) 10^5 CFU/ml was added to each well. Negative control wells containing either 100µL nutrient broth only, or 100µL DMSO with bacterial inoculum, or plant extracts and nutrient broth without bacteria were included in these experiments. Each plant extract was run in duplicate. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism by visual inspection.

2.3 Evaluation of the Genotoxic Potential of *Ecballium elaterium* Ethanolic Extracts on DNA Extracts of *Escherichia coli*

2.3.1 Inoculation of *Escherichia coli*

From 24h old colonies of both *E. coli* strains plated on Meuller-Hinton agar media, three to four colonies were sub-cultured under sterile conditions into bottles containing 25 ml nutrient broth for 2h with continuous shaking. In the next day, constant volumes of nutrient broth (25 ml) were inoculated with 3.5 ml of *E. coli* strain (from the previously prepared bacterial suspension) and incubated at 37°C for 1h with continuous shaking. Then different concentrations of ethanolic leaf extract and ethanolic fruit extract of *E. elaterium* were added to bacterial broth culture into a final concentration 25mg/ml, 10mg/ml and 6 mg/ml. Samples of 3 and 6 ml size were taken from the bacterial culture treated with plant extract after 2h, 6h, and 24h, centrifuged for 10 minutes at 14.000 rpm, then the supernatant was discarded and the pellet was saved at -20°C. The 3 ml sample pellet was used to isolate bacterial DNA for ERIC-PCR and the 6 ml sample pellet to isolate bacterial protein for SDS-PAGE. A broth sub-cultured with *E. coli* strain and treated with 10% Dimethyl sulfoxide (DMSO) was used as a negative control.

2.3.2 DNA extraction and ERIC-PCR assay

Escherichia coli DNA genome was prepared for PCR according to method described previously [22]. Enterobacterial repetitive intergenic consensus 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`, described previously [23]. Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix (ReadyMixTM Taq PCR Reaction Mix with MgCl2, Sigma), 0.8 µM of each primer, 52 ng DNA template, concentration of dNTPs was modified to 0.4 mM, MgCl2 to 3 mM and Taq DNA polymerase to 1.5 U. The reaction mixture was given a short spin for complete mixing of the components. DNA amplification was then carried out using the thermal cycler (Mastercycler personal, Eppendorf) according to the following thermal conditions: initial denaturation for 3 min at 94ºC was followed by 40 cycles of denaturation at 94ºC for 50 s, annealing at 50ºC for 1 min and extension at 72ºC for 2 min, with a final extension step at 72ºC for 5 min [24]. The ERIC-PCR products were analyzed by electrophoresis on 2% agarose gel. The amplified pattern was visualized on a UV trans-illuminator and photographed. The changes occurring in ERIC banding pattern profiles following plant extract treatments including, variations in band intensity as well as gain or loss of bands, were taken into consideration [25,26].

2.4 Protein Assay

2.4.1 Protein isolation and determination of protein concentration

To each previously taken 6 ml *E. coli* sample, 300 µl of lysis buffer (25 mM Tris-HCL, 100 mM NaCl [pH 8]) were added then vortexed. The samples were then left on ice for 15-20 minutes. After that, each sample was sonicated using sonicator (Q55 Sonicator, QSonica, CT, U.S.A.) at 35% power for 10 minutes divided as 10 seconds on and 10 seconds off. This process was carried out on ice. After that, samples were centrifuged for 10 minutes at 16100Xg speed. The resulting supernatant was then aspirated and stored at -20°C. The protein content in the culture filtrates was estimated by the dye binding method of [27]. The protein concentrations were calculated using bovine serum albumin (BSA) as standard.

2.4.2 SDS-PAGE procedure and Silver staining of SDS-PAGE gels

Total extracted protein was separated by denatured polyacrylamide gel electrophoresis (SDS-PAGE) with 8% stacking gel and 12% separating gel [28]. The SDS-PAGE gels were stained using silver staining [29]. The protein profile was analyzed by SDS-PAGE. Changes occurring in protein banding profiles, following plant extract treatments, including variation in band intensity as well as gain or loss of bands were taken in consideration [30].

3. RESULTS

3.1 MIC Assay

The MIC value of both ethanolic fruit and ethanolic leaf extracts of *E. elaterium* on both *E. coli* strains were found to be 25 mg/ml. The highest percentage of DMSO (negative control) which had a concentration of 5 %, showed no antibacterial activity against these strains.

3.2 Genotoxic Potential of Ethanolic *E. elaterium* Extract on DNA of *E. coli* Strains

DNA genome that was extracted from each *E. coli* strain, was exposed to different concentrations of ethanolic leaf and fruit extracts of *E. elaterium* for various time intervals. Changes in extracted DNA genome from these strains were evaluated in comparison with untreated controls at the same time interval. In the current study, only major obvious changes in DNA banding profile were taken into consideration. The results of three DNA samples in this study were excluded, because of their low DNA concentrations. These samples included *E. coli* ATCC 25922 treated with 10 mg of ethanolic fruit extract/ml for 2 h (Fig 3.1 A lane 2); clinical *E. coli* strain treated with 25 mg of ethanolic leaf extract/ml and 25 mg of ethanolic fruit extract/ml for 24 h (Fig 3.2 A lane 1 and Fig 3.2 B lane 1).

3.2.1 Evaluation of the genotoxic potential of ethanolic extracts of E. elaterium on DNA of E. coli reference strain

The effect of fruit ethanolic extract on *E. coli* reference strain was evaluated using different extract concentrations at different time intervals. ERIC-PCR profile showed that a band with an amplicon length of about 450-bp was more intense in *E. coli* reference strain treated with 6 mg/ml of fruit ethanolic extract for 2h in (Fig. 3.1 A lane 3) comparison with the same band appeared in un-treated control (Fig. 3.1 A C1). This band disappeared after 6 and 24h from *reference strain* treated with 6 mg/ml fruit extract (Fig. 3.1 A lanes 6 and 9) in comparison with the non-treated controls (Fig. 3.1 A lanes C2 and C3), respectively. Moreover, two additional bands with an amplicon lengths of approximately 800-bp and 300-bp, appeared after 6h in the same strain treated with 10 mg/ml fruit ethanolic extract (Fig. 3.1 well 5), while these bands were not detected in non-treated control C2. ERIC-PCR profiles for treated and untreated *E. coli* reference strain under different conditions are shown in Fig.1A.

Regarding the effect of ethanolic leaf extract on *E. coli* reference strain, the absence of the band which had an amplicon size close to 750-bp length was noticed in the DNA extracted after 24h from *E. coli* reference strain treated with 25 mg/ml ethanolic leaf extract (Fig. 3.1 B lane 7) compared with the non-treated control (Fig. 3.1 B lane C3). This band was also found in samples treated with extract concentrations of 6 and 10 mg/ml and incubated for 24h (Fig. 3.1 B lanes 9 and 8). Moreover, it was found that the band which had an amplicon size of approximately 450-bp was more intense in DNA extracted after 6h from the same strain treated with 10 and 6 mg/ml ethanolic leaf extract (Fig. 3.1 B lanes 5 and 6) compared to samples incubated with extract concentration of 25 mg/ml and with un-treated control (Fig. 3.1 B lanes 4 and C2). ERIC-PCR profile for treated and untreated *E. coli* tested strains with different concentrations of ethanolic leaf extract of *E. elaterium* at different time intervals is shown in Fig. 1B.

Fruit and leaf ethanolic extracts of *E. elaterium* showed different effects on genomic DNA of treated-reference *E. coli* strain at the same time interval. For example, it was observed that after 24h treatment with 25 mg/ml fruit extract (Fig. 3.1 A lane 7), the band of about 450-bp amplicon size was almost having the same intensity as the control C3 (Fig. 3.1 A lane C3). On the other hand, the same band was fainter after 24h treatment with 25 mg/ml leaf extract (Fig. 3.1 B lane 7) compared with the control C3 (Fig. 3.1 B lane C3). Absence of bands with amplicon sizes of about 300 and 750-bp was also observed upon 24h treatment with 25 mg/ml leaf extract (Fig. 3.1 B lane 7) compared with control C3 (Fig. 3.1 B lane C3).

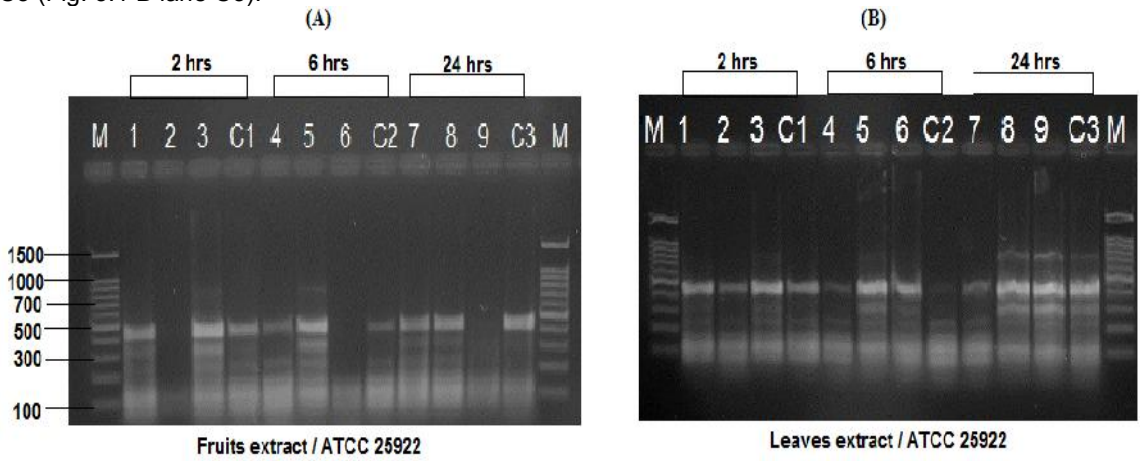


Fig. 1. ERIC-PCR profile of *E. coli* ATCC 25922 strain treated and untreated with different fruit extract concentrations (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml; Lanes 2, 5 and 8 treated with 10 mg/ml; Lanes 3, 6 and 9 treated with 6 mg/ml.

3.2.2 Evaluation of the genotoxic potential of ethanolic extracts of E. elaterium on DNA of clinical E. coli strain

Treatment of clinical *E. coli* strain with ethanolic fruit extract resulted in the loss of several bands from ERIC-PCR profile of this strain compared with the profile of untreated control samples. ERIC-PCR showed the absence of two major bands with amplicon sizes of about 1000-bp and 800-bp from clinical *E. coli* strain treated with 25 mg/ml, 10 mg/ml and 6 mg/ml ethanolic fruit extract for 2h (Fig. 3.2 A lanes 1, 2 and 3), compared with untreated control C1(Fig. 3.2 A lane C1). Another major band with approximately 550-bp amplicon size is also missing from the profile of the DNA extracted from the clinical strain treated with 25 mg/ml and 10 mg/ml after 2h (Fig. 3.2 A lanes 1 and 2), compared to the profile of the sample treated with 6 mg/ml (Fig. 3.2 A lane 3) and untreated control (Fig. 3.2 A lane C1). Moreover, the band which had an amplicon size of approximately 350-bp was faint and absent after 2h from the DNA profile of clinical *E. coli* strain treated with ethanolic fruit extracts of 25 mg/ml and 10 mg/ml (Fig. 3.2 A lanes 1 and 2), Compared with the sample treated with 6 mg/ml (Fig. 3.2 A lane 3) and untreated control (Fig. 3.2 A lane C1). The bands which had amplicon sizes of approximately 1500-bp and 600-bp, amplified from clinical *E. coli* strain treated with 25 mg/ml of fruit extract disappeared (Fig. 3.2 A lane 1), while the band which had amplicon size of about 100-bp length was faint in comparison with untreated control C2 after 2h (Fig. 3.2 A lane C2). Finally, ERIC-PCR showed that all bands were absent from clinical strain treated with 6 mg/ml and 10 mg/ml fruit extract for 24 h (Fig. 3.2 A lanes 8 and 9) compared with untreated control C3 (Fig. 3.2 A lane C3). ERIC-PCR profile for treated and untreated clinical *E. coli* strain with different concentrations of ethanolic fruit extract of *E. elaterium* at different time intervals is shown in Fig. 2A.

Loss of bands was also observed upon treatment of clinical *E. coli* strain with ethanolic leaf extract. Bands with amplicon sizes around 1000-bp, 800-bp and 550-bp lengths were found in controls C1 and C2, but disappeared after 2 and 6h in *E. coli* strain treated with 10 and 6 mg/ml ethanolic leaf extract (Fig. 3.2 B lanes 2, 3, 5 and 6), and after 6 h in strain treated with 25 mg/ml ethanolic leaf extract (Fig. 3.2 B lane 4). Moreover, ERIC-PCR showed that the band which had amplicon size about to 550-bp length was faint after 2h in clinical strain treated with 25 mg/ml ethanolic leaf extract in comparison with control C1 (Fig. 3.2 B lane 1). On the other hand, incubation for 24h for samples treated with 10 and 6 mg/ml leaf extract showed identical banding pattern compared with control C3 (Fig. 3.2 B lanes 8 and 9) with the exception of the presence of the band with amplicon size of about 1300 bp was faint in samples treated with 10 and 6 mg/ml leaf extract compared with control C3 (Fig. 3.2 B Lane 7). ERIC-PCR profile for treated and untreated clinical *E. coli* strain with different concentrations of ethanolic leaf extract of *E. elaterium* at different time intervals is shown in Fig. 2B.

Fruit and leaf ethanolic extracts of *E. elaterium* showed different effects on genomic DNA of clinical *E. coli* strain at the same time interval. For example, 24h treatment of clinical *E. coli* with 10 and 6mg/ml fruit extract (Fig. 3.2 A lanes 8 and 9) resulted in loss of all bands compared with the untreated control C3 (Fig. 3.2 A lane C3). On the other hand, 24h treatment of clinical *E. coli* with 10 and 6mg/ml leaf extract (Fig. 3.2 B lanes 8 and 9) resulted in neither loss nor appearance of extra bands compared with control C3 (Fig. 3.2 B lane C3), but the band with an amplicon size of about 1000-bp was faint upon treatment.

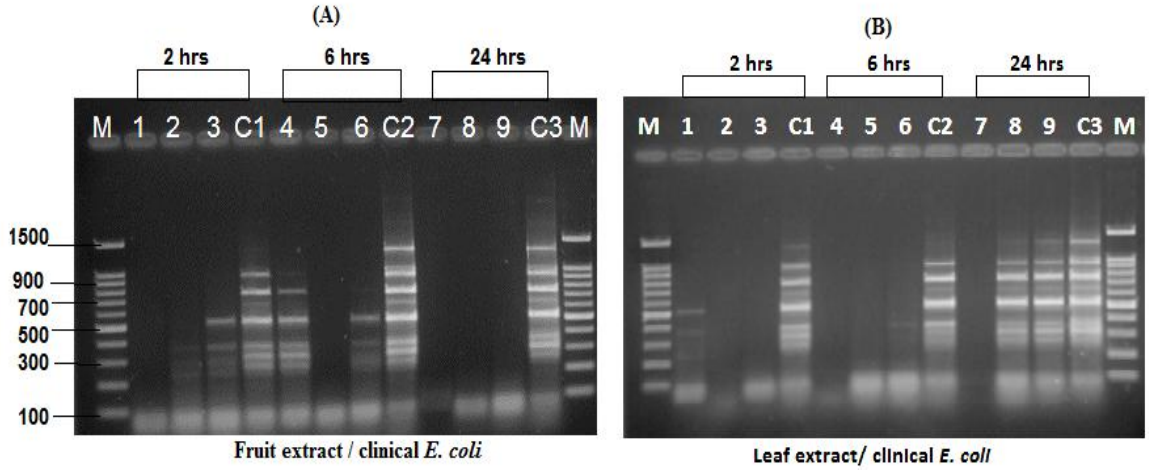


Fig. 2. ERIC-PCR profile of clinical *E. coli* strain treated and untreated with different concentrations of fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3

are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml; Lanes 2, 5 and 8 treated with 10 mg/ml; Lanes 3, 6 and 9 treated with 6 mg/ml.

3.3 Effect of Ethanolic Extracts of *E. elaterium* on Total Protein Concentration of Both *E. coli* Strains

Treatment of both *E. coli* strains with fruit and leaf ethanolic extracts of *E. elaterium* resulted in noticeable elevation in total protein concentration under different experimental conditions, especially in bacterial strains exposed to fruit extracts. This increase was not reported when *E. coli* ATCC 25922 reference strain was treated with 25, 10 and 6 mg/ml fruit extract for 24 h, and when clinical *E. coli* was treated for 24 h with 25 and 10 mg/ml fruit extract.

3.4 Effect of Ethanolic Extracts of E. elaterium on Protein Profile of E. coli Strains

Total protein was extracted from both *E. coli* strains that were exposed to different concentrations (6, 10 and 25 mg/ml) of ethanolic leaf and fruit extracts of *E. elaterium* for different time intervals (2, 6 and 24h). Changes in extracted protein from treated strains were evaluated in comparison with untreated control samples at the same interval time. In the present study, only major obvious changes were taken into consideration.

3.4.1 Effects of ethanolic extracts of E. elaterium on protein profile of reference E. coli strain

Several changes in reference *E. coli* protein profile were observed upon treatment with *E. elaterium* fruit extracts. Treatment with 25 mg/ml fruit extract for 6 h (Fig. 3.3 lane 4) caused several bands in untreated control C2 (Fig. 3.3 lane C2) to disappear including those with Rf values 0.1, 0.15, 0.25, 0.3 and 0.36. Moreover, the band at Rf 0.29 was very faint after 6 h treatment with 6 mg/ml fruit extract (Fig. 3.3 lane 6) and absent after 6 h treatment with 10 mg/ml fruit extract (Fig. 3.3 lane 5) compared with control C2 (Fig. 3.3 lane C2). Absence of bands with Rf values 0.04, 0.05 and 0.9 was observed at 24 h treatment with 25, 10 and 6 mg/ml fruit extract (Fig. 3.3 lanes 7, 8 and 9) compared with control C3 (Fig. 3.3 lane C3). Protein profile for treated and untreated reference *E. coli* strain with different concentrations of *E. elaterium* ethanolic fruit extract is shown in Fig. 3.

Leaf extract treatment of reference *E. coli* strain also resulted in several modifications in the protein profile. For example, upon 6 h treatment with 25 mg/ml leaf extract appearance of 2 new bands at Rf values of approximately 0.45 and 0.47 was recorded compared with untreated control C2. On the other hand, disappearance of bands with 0.14, 0.15, 0.24 and 0.65 Rf values was observed after 24 h treatment with 25 mg/ml leaf extract compared with control C3 (Figure is not shown).

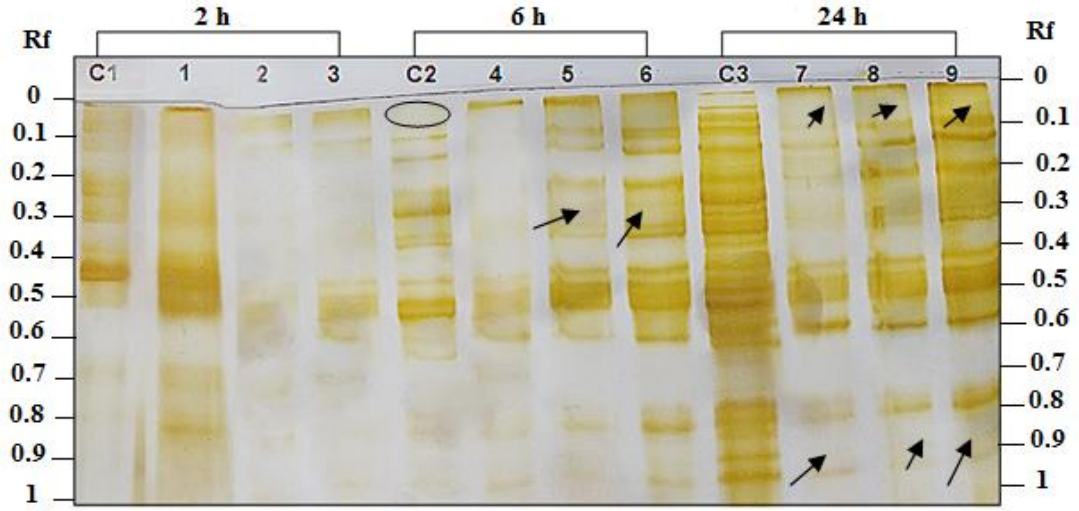
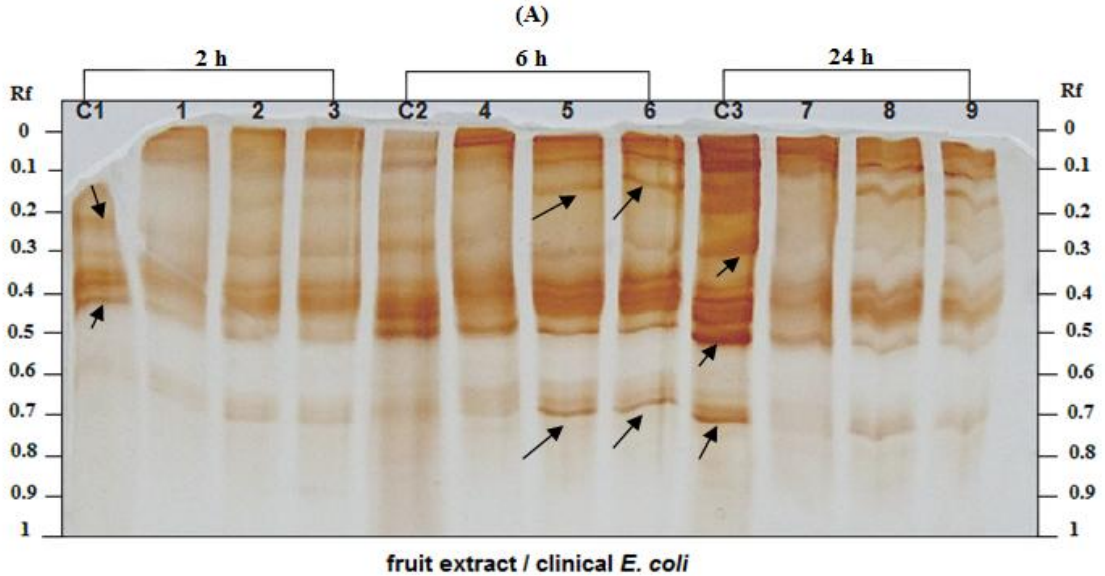


Fig. 3. Protein profile of *E. coli* ATCC 25922 reference strain treated and untreated with different concentrations of fruit ethanolic extract of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes 2, 5 and 8 are treated with 10 mg/ml; Lanes 3, 6 and 9 are treated with 6 mg/ml. Arrows and ovals indicate some of the treatment-affected bands mentioned in the text.

3.4.2 Effects of ethanolic extracts of E. elaterium on protein profile of clinical E. coli strain

Ethanolic fruit extract of *E. elaterium* resulted in differences in the protein profile of the treated clinical *E. coli*. The observed differences varied between either up regulation, down regulation or even complete disappearance of particular protein fractions. After 2 h of application of 25 mg/ml fruit extract (Fig. 3.4 A lane 1), bands at Rf values of about 0.22 and 0.42 were faint compared with control C1 (Fig. 3.4 A lane C1). The band at Rf 0.42 was also faint upon 2 h treatment with 10 and 6 mg/ml fruit extract compared with control C1 (Fig. 3.4 A lane C1). On the other hand, 6 h treatment with 10 and 6 mg/ml fruit extract (Fig. 3.4 A lanes 5 and 6) resulted in an increase in the intensity of bands at approximate Rf of 0.15 and 0.7 compared with untreated control C2 (Fig. 3.4 A lane C2). Decreasing the intensity of bands at Rf 0.53 and 0.7 was noticed after 24 h treatment with 25, 10 and 6 mg/ml fruit extract (Fig. 3.4 A lanes 7, 8 and 9) compared with control C3 (Fig. 3.4 A lane C3). Moreover, the band at Rf 0.3 was pale after 24 h treatment with 10 and 6 mg/ml fruit extract (Fig. 3.4 A lanes 8 and 9) and completely disappeared upon 24 h treatment with 25 mg/ml fruit extract (Fig. 3.4 A lane 7) compared with control C3 (Fig. 3.4 A lane C3). Protein profile for treated and untreated clinical *E. coli* strain with different concentrations of *E. elaterium* ethanolic fruit extract at different time intervals is shown in Fig. 4A.

Treatment of clinical *E. coli* strain with leaf extract also resulted in several modifications. The SDS-PAGE protein profile revealed that 6 h treatment with 10 mg/ml and 6 mg/ml leaf extract (Fig. 3.4 B lanes 5 and 6) resulted in increasing the intensity of bands at relative mobility (Rf) of 0.06 and 0.1 compared with the control C2 (Fig. 3.4 B lane C2). On the hand, 6h treatment with 25 mg/ml leaf extract resulted in the disappearance of band at Rf 0.06 compared with control C2 (Fig. 3.4 B lane C2). Moreover, appearance of new band with nearly 0.8 Rf value was recorded after 6 h treatment with 10 mg/ml leaf extract (Fig. 3.4 B lane 5) compared with control C2 (Fig. 3.4 B lane C2). Treatment with 25 mg/ml leaf extract for 24h (Fig. 3.4 B lane 7) caused the absence of band at approximate Rf value of 0.33, while the band at Rf 0.68 was very faint compared with the control C3 (Fig. 3.4 B lane C3). Protein profile for treated and untreated clinical *E. coli* strain with different concentrations of *E. elaterium* ethanolic leaf extract at different time intervals is presented in Fig. 4B.



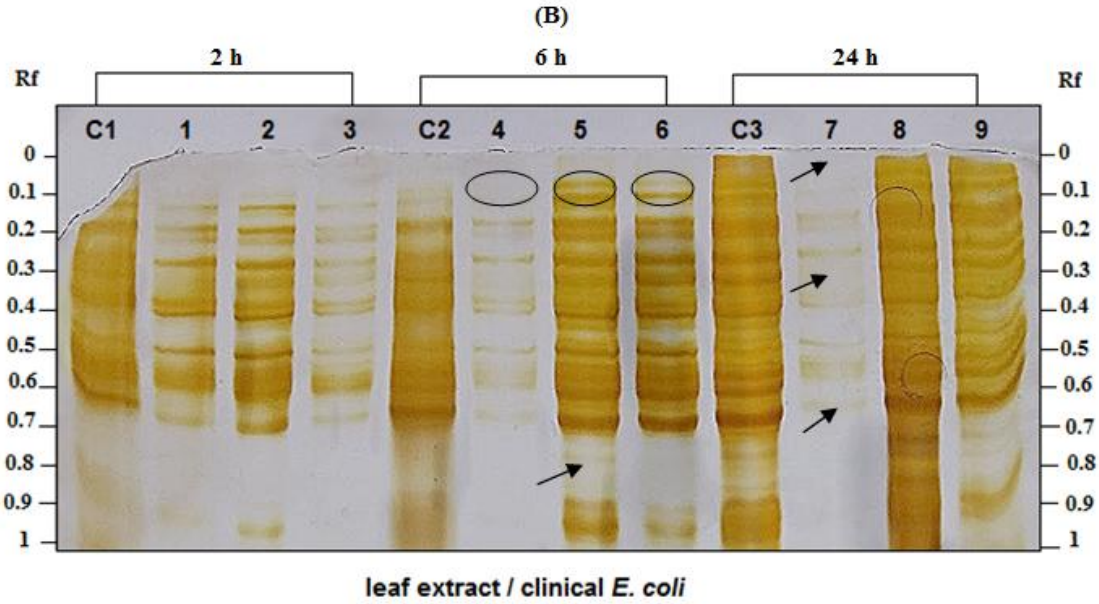


Fig. 4. Protein profile of clinical *E. coli* strain treated and untreated with different concentrations of fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes 2, 5 and 8 are treated with 10 mg/ml; Lanes 3, 6 and 9 are treated with 6 mg/ml. Arrows and ovals indicate some of the treatment-affected bands mentioned in the text.

4. DISCUSSION

In fact, drugs derived from natural products represent 70 % of these approved since 1981 [31]. Moreover, the traditional use of medicinal plants in treating diseases is increasing in many countries [15]. In spite of this, increasing evidence has shown that many of medicinal plant products may have toxic, mutagenic, and even carcinogenic effects [32]. Thus, investigating the genotoxicity of plants is of great importance for safe traditional use and safe use in drug formulations.

In the present study, the potential genotoxic effect of the ethanolic leaf and fruit extracts of *E. elaterium* against two different strains of *E. coli* was examined using ERIC-PCR, and SDS-PAGE analytical methods. In literature, many plants were tested by different genotoxicity methods showed genotoxicity potential. Examples include *Curcuma longa*, *Melia azedarach*, *Rhazya stricta*, *Urtica dioica*, *Salvia triloba*, *Arctium minus*, *Plantago major*, *Momordica charantia*, *Thermopsis turcica*, *Moringa peregrina* [1,15,25,30,33].

To the best of our knowledge, this is the first study of an analysis of genomic alteration that tested on *E. coli* strains treated with different concentrations of ethanolic leaf and fruit extracts of *E. elaterium* using ERIC-PCR assay. ERIC-PCR banding pattern showed significant differences between the treated and untreated *E. coli* strains used in this study. The main changes in the treated *E. coli* included the disappearing or appearing of certain bands in comparison with untreated control, as well as differences in band intensity. The loss of bands in treated *E. coli* strains in comparison with that found in control samples may be due to the effect of the genotoxins present in the plant extracts. These genotoxins can induce DNA damage such as single and/or double strand breaks, point mutations and/or chromosomal rearrangements. These damages in the DNA may have a potential effect on the primer annealing sites and or inter-priming distances [1,25,33]. Point mutations, large deletions, and/or homologous recombination are considered as mechanisms that can produce new primer annealing sites, thus resulting in the appearance of extra new bands or change the amplicon size [33]. However, understanding and determining the specific mechanisms that lead to differences in ERIC-PCR profile is difficult. Other techniques can support and assist in understanding the proposed mechanisms such as analysis of amplicons using DNA sequencing or probing [25].

Results of the current study support the evidences reported previously that extracts of *E. elaterium* have genotoxic and mutagenic potential [15,17,18].

The observed genotoxicity of *E. elaterium* possibly were mediated by cucurbitacins. Different types of cucurbitacins such as B, D, E, I, L and R have been identified in the juice of *E. elaterium* [18]. The fruits juice of *E. elaterium* is especially rich with cucurbitacins that represent between 20-30% of the juice [7]. It was reported that cucurbitacins B, D, E, and I possess strong anticancer activities [34]. In addition, the genotoxic potential of cucurbitacin B was reported, as it induced DNA damage in A594 and MCF-7 cells using the comet assay [35,36]. The damage mediated by cucurbitacin B is due to induction of reactive oxygen species (ROS) formation, which results in double-stranded breakage and subsequently G2/M phase arrest in A594 cells.

The effect of fruit and leaf ethanolic extracts of *E. elaterium* on total protein profile of both *E. coli* strains was assessed using SDS-PAGE gels, where several alterations in the protein profile of *E. elaterium* treated *E. coli* compared to the untreated control was observed. These changes included increase or decrease in band intensity, absence or appearance of bands. The absence of protein bands indicates the interruption of protein synthesis pathways possibly stimulated by *E. elaterium* extract bioactive constituents [37]. The appearance of new bands and increasing the intensity of some bands can be explained by the ability of *E. elaterium* extract to apply a stress on treated *E. coli*. The stressed *E. coli* may respond under these conditions by up-regulation (increasing the expression) of some proteins and stimulating the expression of others [30]. Another explanation for this proposes the happening of frame shift mutations due to stress [30].

5. CONCLUSION

Our results showed that *E. elaterium* fruit and leaf ethanolic extracts possess genotoxic and mutagenic potential. The results also point out the capability of using *E. elaterium* to treat *E. coli* infections. More studies are required to find out the exact mechanism responsible for the observed genotoxicity.

REFERENCES

1. Hajar AS, Gumgumjee NM. Antimicrobial activities and evaluation of genetic effects of *Moringa peregrina* (forsk) fiori using molecular techniques. International Journal of Plant and Animal Environmental Sciences. 2014;4(1):65-72.
2. Abu-Irmeileh B. *Weeds of Jordan (weeds of crop fields)*. Amman, Jordan: University of Jordan; 1982.
3. Brouzas D, Oanta M, Loukianou E, Moschos M. Keratoconjunctivitis and Periorbital Edema due to *Ecballium elaterium*. Case reports in ophthalmology. 2012;3(1):87-90.
4. Lavi D, Willner D. The constituent of *Ecballium elatrrium* L. III. Elatrecein A and B. Journal of American Socity. 1985;80(3):710-714.
5. Yesilada E, Tanaka S, Sezik E, Tabata M. Isolation of Anti-Inflammatory Principle from the Fruit Juice of *Ecballium elaterium*. Journal of Natural Products. 1988;51(3):504-508.
6. Greige-Gerges H, Khalil RA, Mansour EA, Magdalou J, Chahine R, Ouaini N. Cucurbitacins from *Ecballium elaterium* juice increase the binding of bilirubin and ibuprofen to albumin in human plasma. *Chemico-Biological Interactions*. 2007;169(1):53-62.
7. Jaradat N, Jodeh S, Rinno T, Kharoof M, Zaid, AN, Hannon M. Determination the presence of phytomelin in *Ecballium elaterium* to approve its flok uses. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(2):233-237.
8. Rao MM, Meshulam H, Lavie D. The constituents of *Ecballium elaterium L.* Part XXIII. Cucurbitacins and hexanorcucurbitacins. Journal of the Chemical Society, Perkin Transactions 1. 1974;0: 2552-2556.
9. Salhab AS. Human exposure to *Ecballium elaterium* fruit juice: fatal toxicity and possible remedy. Pharmacology & Pharmacy. 2013;4(05):447-450.
10. El-Haci IA, Bekkara FA. Antioxidant activity of stems and leaves organic fractions of *Ecballium elaterium* L. Annals of Biological Research. 2011;2(3):327-332.
11. Oskay M, Sari D. Antimicrobial screening of some Turkish medicinal plants. Pharmaceutical Biology. 2007;45(3):176-181.
12. Oskay M, Oskay D, Kalyoncu F. Activity of some plant extracts against multi-drug resistant human pathogens. Iranian Journal of Pharmaceutical Research. 2009;8(4):293-300.

13. Adwan G, Salameh Y, Adwan K. Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. Asian Pacific Journal of Tropical Biomedicine. 2011;1(6):456-460.
14. Abbassi F, Ayari B, Mhamdi B, Toumi L. Phenolic contents and antimicrobial activity of squirting cucumber (*Ecballium elaterium*) extracts against food-borne pathogens. Pakistan journal of pharmaceutical sciences. 2014;27(3):475-479.
15. Basaran AA, Yu TW, Plewa MJ, Anderson D. An investigation of some Turkish herbal medicines in *Salmonella typhimurium* and in the COMET assay in human lymphocytes. Teratogenesis, carcinogenesis, and mutagenesis. 1996;16(2):125-138.
16. Shabbar I, Maslat AO. Genotoxicity of *Ecballium Elaterium* (L) A Rich Cucurbitaceae Fruit juice Using Micronucleus Assay & DNA Single Strand Break Techniques. The Internet Journal of Health. 2006;6(2):10-12.
17. Rencüzogullari E, Ila HB, Kayraldiz A, Diler SB, Yavuz A, Arslan M, Funda Kaya F, Topaktas M. The mutagenic and anti-mutagenic effects of *Ecballium elaterium* fruit juice in human peripheral lymphocytes. Genetika. 2006;42(6):768-772.
18. Çelik TA, Aslantürk ÖS. Investigation of cytotoxic and genotoxic effects of *Ecballium elaterium* juice based on Allium test. Methods and findings in experimental and clinical pharmacology. 2009;31(9):591-596.
19. Kumari S, Rastogi RP, Singh KL, Singh SP, Sinha RP. DNA damage: detection strategies. EXCLI Journal. 2008;7:44-62.
20. Qari SH, Abdel-Fattah NA. Genotoxic studies of selected plant oil extracts on *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Taibah University for Science. 2017;11(3):478-486.
21. CLSI (Clinical Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement. 2015; Wayne, PA, USA: CLSI: M100-S25.
22. Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. Prevalence of seg, seh and sei Genes among Clinical and Nasal Staphylococcus aureus Isolates in Palestine. *British* Microbiology Research Journal. 2013;3(2):139-149.
23. Meacham KJ, Zhang L, Foxman B, Bauer RJ, Marrs CF. Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. Journal of clinical microbiology. 2003;41(11):5224-5226.
24. Adwan G, Shatayah A, Adwan K, Al-Sheboul S, Othman S. Prevalence and molecular characterization of *P. aeruginosa* isolates in the West Bank-Palestine for ESBLs, MBLs and interogens. Journal of Applied Life Sciences International. 2016;8(2):1-11.
25. Lalrotluanga, Kumar NS, Gurusubramanian, G. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage in mosquito larvae treated with plant extracts. Science Vision. 2011;11(3):155-158.
26. Atienzar FA, Venier P, Jha AN, Depledge MH. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2002;521(1-2):151-163.
27. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein was utilizing the principle of protein–dye binding. Analytical Biochemistry. 1976;72:248-254.
28. Baeshin NA, Qari SH, Sabir JSM, ALhejin AM. Biochemical and Molecular Evaluation of Genetic Effects of *Rhazya stricta* (Decne) Leafs Extract on *Aspergillus terreus*. Saudi Journal of Biological Sciences. 2008;15(1):25-33.
29. Blum H, Beier H, Gross HJ. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis. 1987;8(2):93-99.
30. El-Tarras AA, Hassan MM, El-Awady MA. Evaluation of the genetic effects of the in vitro antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning electron microscope. African journal of Biotechnology. 2013;12(21):3171-3180.
31. Jafargholizadeh N, Zargar SJ, Yassa N, Tavakoli S. Purification of Cucurbitacins D, E, and I from *Ecballium Elaterium* (L.) A. Rich Fruits and Study of Their Cytotoxic Effects on the AGS Cell Line. Asian Pacific journal of cancer prevention. 2016;17(10):4631.
32. Celik TA. (2012). Potential Genotoxic and Cytotoxic Effects of Plant Extracts. A Compendium of Essays on Alternative Therapy. Arup Bhattacharya (Ed.), Edition:1, Chapter: 11, Publisher: InTech.
33. Ciğerci, İ. H., Cenkci, S., Kargıoğlu, M., Konuk, M. Genotoxicity of *Thermopsis turcica* on *Allium cepa L.* roots revealed by alkaline comet and random amplified polymorphic DNA assays. Cytotechnology. 2016;68(4):829-838.

34. Lee DH, Iwanski GB, Thoennissen NH. Cucurbitacin: Ancient Compound Shedding New Light on Cancer Treatment. The Scientific World Journal. 2010;10:413–418.
35. Guo J, Wu G, Bao J, Hao W, Lu J, Chen X. Cucurbitacin B induced ATM-mediated DNA damage causes G2/M cell cycle arrest in a ROS-dependent manner. PLOS one. 2014;9(2):e88140.
36. Ren G, Sha T, Guo J, Li W, Lu J, Chen X. Cucurbitacin B induces DNA damage and autophagy mediated by reactive oxygen species (ROS) in MCF-7 breast cancer cells. Journal of natural medicines. 2015;69(4):522-530.
37. Othman AS, Ahmed NA. Antibacterial Effect of the Ethanol Leaves Extract of *Moringa oleifera* and *Camellia sinensis* against Multi Drug Resistant Bacteria. International Journal of Pharmacology. 2017;13(2):156-165.