Original Research Article

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

Awni Abu-Hijleh[1*], Ghaleb Adwan[1] and Wafa Abdat[1]

[1]Department of Biology and Biotechnology, An-Najah National University, P.O.Box 7, Nablus, Palestine.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AA and GA designed the study, wrote the protocol. Author WA conducted experimental work. Authors AA and GA managed the analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aims: This 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 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 that 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 in 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, which 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 this 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 Materials

All chemicals used in plant extract preparation, determination of antibacterial activities, DNA extraction, ERIC-PCR, protein assay, SDS-PAGE and silver staining were purchased from Sigma Chemicals, St. Louis, USA.

2.2 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 possible loss of effective ingredients. After that, the fresh fruits (20g) were homogenized finely using blender after addition of 80 ml of absolute ethanol. The dried leaves were powdered finely using blender. Approximately 30-40g of dried leaf material was mixed thoroughly using 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, mixtures were centrifuged at 5,000 rpm for 15 min at 4℃, to remove fine particles. Then, the extracts were dried and concentrated using rotary evaporator at 50℃, and freeze dryer (lyophilizer). The obtained dried extract was stored at 4℃. 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.

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

2.3.1 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.

112 **2.4 Evaluation of the Genotoxic Potential of *Ecballium elaterium* Ethanolic Extracts on**
113 **DNA Extracts of *Escherichia coli***
114
115 **2.4.1 Inoculation of *Escherichia coli***
116
117 From 24h old colonies of both *E. coli* strains plated on Meuller-Hinton agar media, three to four
118 colonies were sub-cultured under sterile conditions into bottles containing 25 ml nutrient broth for 2h
119 with continuous shaking. In the next day, constant volumes of nutrient broth (25 ml) were inoculated
120 with 3.5 ml of *E. coli* strain (from the previously prepared bacterial suspension) and incubated at 37℃
121 for 1h with continuous shaking. Then different concentrations of ethanolic leaf extract and ethanolic
122 fruit extract of *E. elaterium* were added to bacterial broth culture into a final concentration 25mg/ml,
123 10mg/ml and 6 mg/ml. Samples of 3 and 6 ml size were taken from the bacterial culture treated with
124 plant extract after 2h, 6h, and 24h, centrifuged for 10 minutes at 14.000 rpm, then the supernatant
125 was discarded and the pellet was saved at -20℃. Pellet of 3 ml sample was used to isolate bacterial
126 DNA for ERIC-PCR and pellet of 6 ml sample was used to isolate bacterial protein for SDS-PAGE. A
127 broth sub-cultured with *E. coli* strain and treated with 10% Dimethyl sulfoxide (DMSO) was used as a
128 negative control.
129
130 **2.4.2 DNA extraction and ERIC-PCR assay**
131
132 *Escherichia coli* DNA genome was prepared for PCR according to method described previously [22].
133 Enterobacterial repetitive intergenic consensus PCR was performed using primer ERIC1: 5`-ATG TAA
134 GCT CCT GGG GAT TCA C-3` and primer ERIC2 : 5`-AAG TAA GTG ACT GGG GTG AGC G-3`,
135 described previously [23]. Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR
136 premix (ReadyMixTM Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.8 µM of each primer, 52 ng DNA
137 template, concentration of dNTPs was modified to 0.4 mM, $MgCl_2$ to 3 mM and Taq DNA polymerase
138 to 1.5 U. The reaction mixture was given a short spin for complete mixing of the components. DNA
139 amplification was then carried out using the thermal cycler (Mastercycler personal, Eppendorf)
140 according to the following thermal conditions: initial denaturation for 3 min at 94ºC, followed by 40
141 cycles of denaturation at 94ºC for 50 s, annealing at 50ºC for 1 min and extension at 72ºC for 2 min,
142 with a final extension step at 72ºC for 5 min [24]. The ERIC-PCR products were analyzed by
143 electrophoresis on 2% agarose gel. The amplified pattern was visualized on a UV trans-illuminator
144 and photographed. The changes in ERIC banding pattern profiles following plant extract treatments
145 including, variations in band intensity as well as gain or loss of bands, were invistigated [25,26].
146
147 **2.5 Protein Assay**
148
149 **2.5.1 Protein isolation and determination of protein concentration**
150
151 To each previously taken 6 ml *E. coli* sample, 300 µl of lysis buffer (25 mM Tris-HCL, 100 mM NaCl
152 [pH 8]) were added then vortexed. The samples were then left on ice for 15-20 minutes. After that,
153 each sample was sonicated using sonicator (Q55 Sonicator, QSonica, CT, U.S.A.) at 35% power for
154 10 minutes divided as 10 seconds on and 10 seconds off. This process was carried out on ice. After
155 that, samples were centrifuged for 10 minutes at 16100Xg. The resulting supernatant was then
156 aspirated and stored at -20℃. The protein content in the culture filtrates was estimated by the dye
157 binding method of Bradford [27]. The protein concentrations were calculated using bovine serum
158 albumin (BSA) as standard.
159
160 **2.5.2 SDS-PAGE procedure and Silver staining of SDS-PAGE gels**
161
162 Total extracted protein was separated by denatured polyacrylamide gel electrophoresis (SDS-PAGE)
163 with 8% stacking gel and 12% separating gel [28]. Vertical slab gel electrophoresis was performed
164 using the ENDURO PAGE System (E2010-P0, Labnet Int., Inc., NJ, U.S.A.) The SDS-PAGE gels
165 were stained using silver staining [29]. The protein profile was analyzed by SDS-PAGE. Changes
166 occurring in protein banding profiles, following plant extract treatments, including variation in band
167 intensity as well as gain or loss of bands were investigated [30].
168
169

170
171 ## 3. RESULTS
172
173 ### 3.1 MIC Assay
174
175 The MIC value of both ethanolic fruit and ethanolic leaf extracts of *E. elaterium* on both *E. coli* strains
176 were found to be 25 mg/ml. The highest percentage of DMSO (negative control) which had a
177 concentration of 5 %, showed no antibacterial activity against these strains.
178
179 ### 3.2 Genotoxic Potential of Ethanolic *E. elaterium* Extract on DNA of *E. coli* Strains
180
181 DNA genome that was extracted from each *E. coli* strain, was exposed to different concentrations of
182 ethanolic leaf and fruit extracts of *E. elaterium* for various time intervals. Changes in extracted DNA
183 genome from these strains were evaluated in comparison with untreated controls at the same time
184 interval. In the current study, only major obvious changes in DNA banding profile were taken into
185 consideration. The results of three DNA samples in this study were excluded, because of their low
186 DNA concentrations. These samples included *E. coli* ATCC 25922 treated with 10 mg of ethanolic fruit
187 extract/ml for 2 h (Fig. 1A lane 2); clinical *E. coli* strain treated with 25 mg of ethanolic leaf extract/ml
188 and 25 mg of ethanolic fruit extract/ml for 24 h (Fig. 2A lane 7 and Fig. 2B lane 7).
189
190 #### 3.2.1 Evaluation of the genotoxic potential of ethanolic extracts of *E. elaterium* on DNA of *E.*
191 #### *coli* reference strain
192
193 The effect of fruit ethanolic extract on *E. coli* reference strain was evaluated using different extract
194 concentrations at different time intervals. ERIC-PCR profile showed that a band with an amplicon
195 length of about 450-bp was more intense in *E. coli* reference strain treated with 6 mg/ml of fruit
196 ethanolic extract for 2h in (Fig. 1A lane 3) comparison with the same band appeared in un-treated
197 control (Fig. 1A C1). This band disappeared after 6 and 24h from reference strain treated with 6
198 mg/ml fruit extract (Fig. 1A lanes 6 and 9) in comparison with the non-treated controls (Fig. 1A lanes
199 C2 and C3), respectively. Moreover, two additional bands with amplicon lengths of approximately 800-
200 bp and 300-bp, appeared after 6h in the same strain treated with 10 mg/ml fruit ethanolic extract
201 (Fig. 1 well 5), while these bands were not detected in non-treated control C2. ERIC-PCR profiles for
202 treated and untreated *E. coli* reference strain under different conditions are shown in Fig.1A.
203
204 Regarding the effect of ethanolic leaf extract on *E. coli* reference strain, the absence of the band
205 which had an amplicon size close to 750-bp length was noticed in the DNA extracted after 24h from
206 *E. coli* reference strain treated with 25 mg/ml ethanolic leaf extract (Fig. 1B lane 7) compared with the
207 non-treated control (Fig. 1B lane C3). This band was also found in samples treated with extract
208 concentrations of 6 and 10 mg/ml and incubated for 24h (Fig. 1B lanes 9 and 8). Moreover, it was
209 found that the band which had an amplicon size of approximately 450-bp was more intense in DNA
210 extracted after 6h from the same strain treated with 10 and 6 mg/ml ethanolic leaf extract (Fig. 1B
211 lanes 5 and 6) compared to samples incubated with extract concentration of 25 mg/ml and with un-
212 treated control (Fig. 1B lanes 4 and C2). ERIC-PCR profile for treated and untreated *E. coli* tested
213 strains with different concentrations of ethanolic leaf extract of *E. elaterium* at different time intervals is
214 shown in Fig. 1B.
215
216 Fruit and leaf ethanolic extracts of *E. elaterium* showed different effects on genomic DNA of treated-
217 reference *E. coli* strain at the same time interval. For example, it was observed that after 24h
218 treatment with 25 mg/ml fruit extract (Fig. 1A lane 7), the band of about 450-bp amplicon size was
219 almost having the same intensity as the control C3 (Fig. 1A lane C3). On the other hand, the same
220 band was fainter after 24h treatment with 25 mg/ml leaf extract (Fig. 1B lane 7) compared with the
221 control C3 (Fig. 1B lane C3). Absence of bands with amplicon sizes of about 300 and 750-bp was
222 also observed upon 24h treatment with 25 mg/ml leaf extract (Fig. 1B lane 7) compared with control
223 C3 (Fig. 1B lane C3).

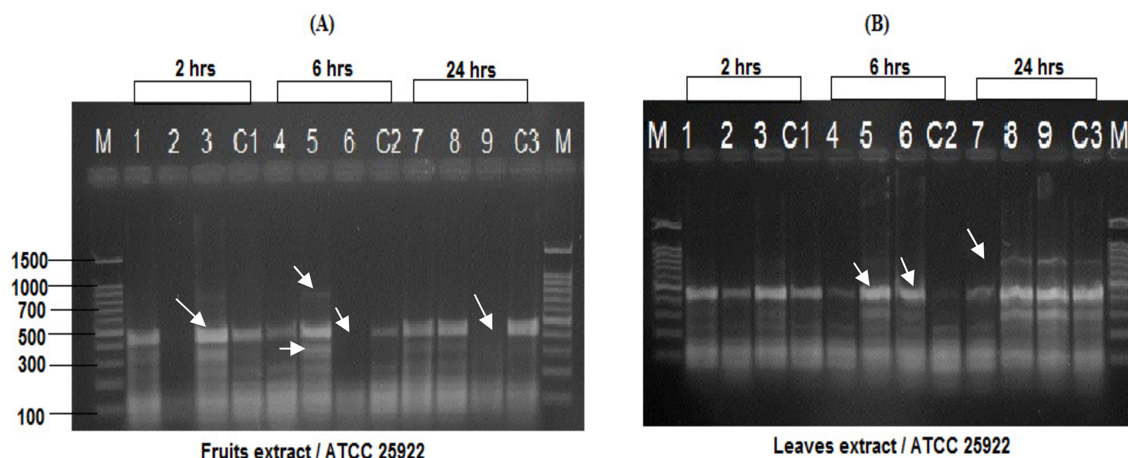


Fig. 1. ERIC-PCR profile of *E. coli* ATCC 25922 strain treated and untreated with different fruit extract concentrations (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml; Lanes 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. 2A lanes 1, 2 and 3), compared with untreated control C1(Fig. 2A 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. 2A lanes 1 and 2), compared to the profile of the sample treated with 6 mg/ml (Fig. 2A lane 3) and untreated control (Fig. 2A 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. 2A lanes 1 and 2), Compared with the sample treated with 6 mg/ml (Fig. 2A lane 3) and untreated control (Fig. 2A lane C1). The band which had an amplicon size of approximately 1500-bp, amplified from clinical *E. coli* strain treated with 25 mg/ml, 10 mg/ml and 6 mg/ml of fruit extract disappeared (Fig. 2A lanes 4, 5 and 6) in comparison with untreated control C2 after 6 h (Fig. 2A 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. 2A lanes 8 and 9) compared with untreated control C3 (Fig. 2A 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. 2B lanes 2, 3, 5 and 6), and after 6 h in strain treated with 25 mg/ml ethanolic leaf extract (Fig. 2B lane 4). Moreover, ERIC-PCR showed that the band which had amplicon size of about 550-bp length was faint after 2h in clinical strain treated with 25 mg/ml ethanolic leaf extract in comparison with control C1 (Fig. 2B 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. 2B lanes 8 and 9) with the exception of the band with amplicon size of about 1300 bp that was faint in samples treated with 10 and 6 mg/ml leaf extract compared with control C3 (Fig. 2B Lane C3). 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. 2A lanes 8 and 9) resulted in loss of all bands compared with the untreated control C3 (Fig. 2A lane C3). On the other hand, 24h treatment of clinical *E. coli* with 10 and 6mg/ml leaf extract (Fig. 2B lanes 8 and 9) resulted in neither loss nor appearance of extra bands compared with control C3 (Fig. 2B lane C3), but the band with an amplicon size of about 1000-bp was faint upon treatment.

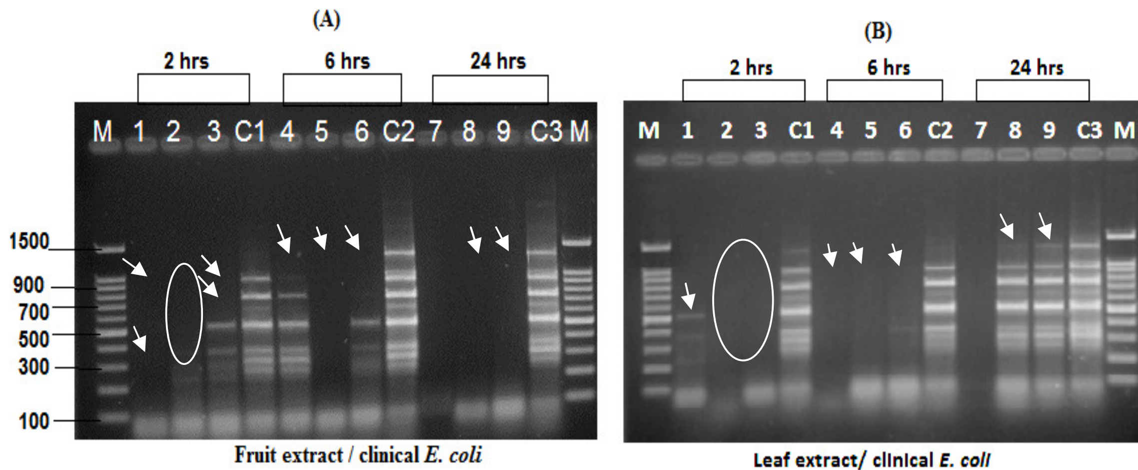


Fig. 2. ERIC-PCR profile of clinical *E. coli* strain treated and untreated with different concentrations of fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml; Lanes 2, 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 lane 4) caused several bands in untreated control C2 (Fig. 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 lane 6) and absent after 6 h treatment with 10 mg/ml fruit extract (Fig. 3 lane 5) compared with control C2 (Fig. 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 lanes 7, 8 and 9) compared with control C3 (Fig. 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).

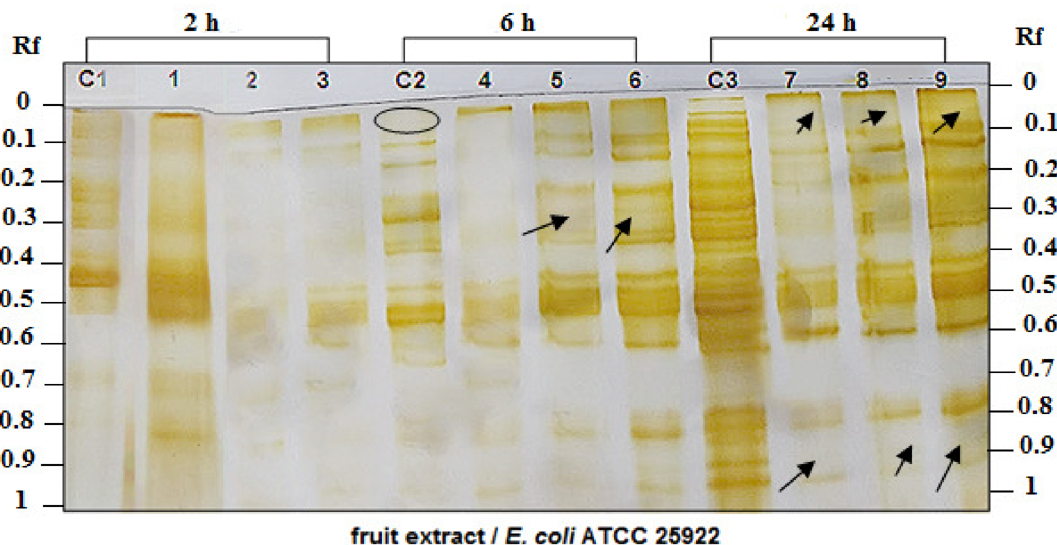


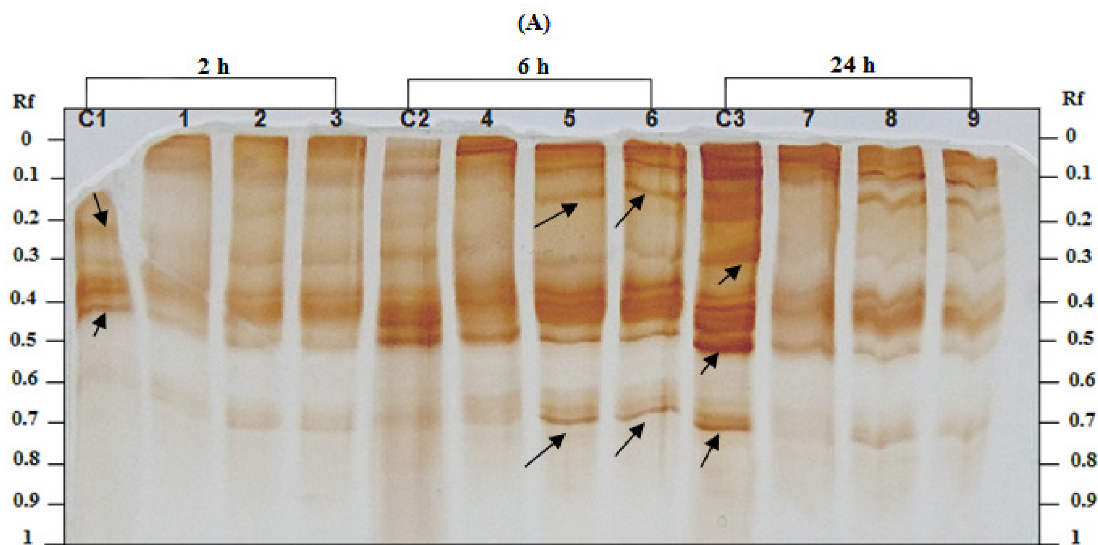
Fig. 3. Protein profile of *E. coli* ATCC 25922 reference strain treated and untreated with different concentrations of fruit ethanolic extract of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes 2, 5 and 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. 4A lane 1), bands at Rf values of about 0.22 and 0.42 were faint compared with control C1 (Fig. 4A 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. 4A lane C1). On the other hand, 6 h treatment with 10 and 6 mg/ml fruit extract (Fig. 4A 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. 4A 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. 4A lanes 7, 8 and 9) compared with control C3 (Fig. 4A lane C3). Moreover, the band at Rf 0.3 was pale after 24 h treatment with 10 and 6 mg/ml fruit extract (Fig. 4A lanes 8 and 9) and completely disappeared upon 24 h treatment with 25 mg/ml fruit extract (Fig. 4A lane 7) compared with control C3 (Fig. 4A 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. 4B 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. 4B 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. 4 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. 4B lane 5) compared with control C2 (Fig. 4B lane C2). Treatment with 25 mg/ml leaf extract for 24h (Fig. 4B 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. 4B 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.



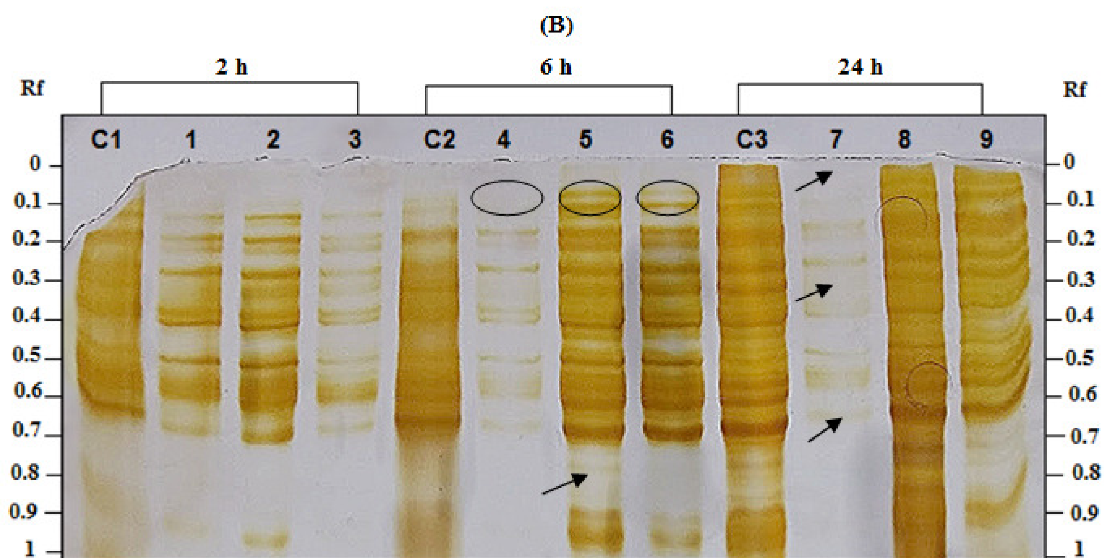


Fig. 4. Protein profile of clinical *E. coli* strain treated and untreated with different concentrations of fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes 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.

425 2. Abu-Irmeileh B. *Weeds of Jordan (weeds of crop fields)***.** Amman, Jordan: University of Jordan;
426 1982.
427 3. Brouzas D, Oanta M, Loukianou E, Moschos M. Keratoconjunctivitis and Periorbital Edema due to
428 *Ecballium elaterium*. Case reports in ophthalmology. 2012;3(1):87-90.
429 4. Lavi D, Willner D. The constituent of *Ecballium elatrrium* L. III. Elatrecein A and B. Journal of
430 American Socity. 1985;80(3):710-714.
431 5. Yesilada E, Tanaka S, Sezik E, Tabata M. Isolation of Anti-Inflammatory Principle from the Fruit
432 Juice of *Ecballium elaterium*. Journal of Natural Products. 1988;51(3):504-508.
433 6. Greige-Gerges H, Khalil RA, Mansour EA, Magdalou J, Chahine R, Ouaini N. Cucurbitacins from
434 *Ecballium elaterium* juice increase the binding of bilirubin and ibuprofen to albumin in human
435 plasma. *Chemico-Biological Interactions*. 2007;169(1):53-62.
436 7. Jaradat N, Jodeh S, Rinno T, Kharoof M, Zaid, AN, Hannon M. Determination the presence of
437 phytomelin in *Ecballium elaterium* to approve its flok uses. International Journal of Pharmacy
438 and Pharmaceutical Sciences. 2012;4(2):233-237.
439 8. Rao MM, Meshulam H, Lavie D. The constituents of *Ecballium elaterium L.* Part XXIII.
440 Cucurbitacins and hexanorcucurbitacins. Journal of the Chemical Society, Perkin
441 Transactions 1. 1974;0: 2552-2556.
442 9. Salhab AS. Human exposure to *Ecballium elaterium* fruit juice: fatal toxicity and possible
443 remedy. Pharmacology & Pharmacy. 2013;4(05):447-450.
444 10. El-Haci IA, Bekkara FA. Antioxidant activity of stems and leaves organic fractions of *Ecballium*
445 *elaterium* L. Annals of Biological Research. 2011;2(3):327-332.
446 11. Oskay M, Sari D. Antimicrobial screening of some Turkish medicinal plants. Pharmaceutical
447 Biology. 2007;45(3):176-181.
448 12. Oskay M, Oskay D, Kalyoncu F. Activity of some plant extracts against multi-drug resistant human
449 pathogens. Iranian Journal of Pharmaceutical Research. 2009;8(4):293-300.
450 13. Adwan G, Salameh Y, Adwan K. Effect of ethanolic extract of *Ecballium elaterium* against
451 *Staphylococcus aureus* and *Candida albicans*. Asian Pacific Journal of Tropical Biomedicine.
452 2011;1(6):456-460.
453 14. Abbassi F, Ayari B, Mhamdi B, Toumi L. Phenolic contents and antimicrobial activity of squirting
454 cucumber (*Ecballium elaterium*) extracts against food-borne pathogens. Pakistan journal of
455 pharmaceutical sciences. 2014;27(3):475-479.
456 15. Basaran AA, Yu TW, Plewa MJ, Anderson D. An investigation of some Turkish herbal medicines
457 in *Salmonella typhimurium* and in the COMET assay in human lymphocytes. Teratogenesis,
458 carcinogenesis, and mutagenesis. 1996;16(2):125-138.
459 16. Shabbar I, Maslat AO. Genotoxicity of *Ecballium Elaterium* (L) A Rich Cucurbitaceae Fruit juice
460 Using Micronucleus Assay & DNA Single Strand Break Techniques. The Internet Journal of
461 Health. 2006;6(2):10-12.
462 17. Rencüzogullari E, Ila HB, Kayraldiz A, Diler SB, Yavuz A, Arslan M, Funda Kaya F, Topaktas M.
463 The mutagenic and anti-mutagenic effects of *Ecballium elaterium* fruit juice in human
464 peripheral lymphocytes. Genetika. 2006;42(6):768-772.
465 18. Çelik TA, Aslantürk ÖS. Investigation of cytotoxic and genotoxic effects of *Ecballium elaterium*
466 juice based on Allium test. Methods and findings in experimental and clinical
467 pharmacology. 2009;31(9):591-596.
468 19. Kumari S, Rastogi RP, Singh KL, Singh SP, Sinha RP. DNA damage: detection strategies. EXCLI
469 Journal. 2008;7:44-62.
470 20. Qari SH, Abdel-Fattah NA. Genotoxic studies of selected plant oil extracts on *Rhyzopertha*
471 *dominica* (Coleoptera: Bostrichidae). Journal of Taibah University for
472 Science. 2017;11(3):478-486.
473 21. CLSI (Clinical Laboratory Standards Institute). Performance standards for antimicrobial
474 susceptibility testing. Twenty second informational supplement. 2015; Wayne, PA, USA:
475 CLSI: M100-S25.
476 22. Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. Prevalence of seg, seh and sei Genes among
477 Clinical and Nasal Staphylococcus aureus Isolates in Palestine. *British* Microbiology
478 Research Journal. 2013;3(2):139-149.
479 23. Meacham KJ, Zhang L, Foxman B, Bauer RJ, Marrs CF. Evaluation of genotyping large numbers
480 of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. Journal of
481 clinical microbiology. 2003;41(11):5224-5226.
482 24. Adwan G, Shatayah A, Adwan K, Al-Sheboul S, Othman S. Prevalence and molecular
483 characterization of *P. aeruginosa* isolates in the West Bank-Palestine for ESBLs, MBLs and
484 interogens. Journal of Applied Life Sciences International. 2016;8(2):1-11.

485 25. Lalrotluanga, Kumar NS, Gurusubramanian, G. Evaluation of the random amplified polymorphic
486 DNA (RAPD) assay for the detection of DNA damage in mosquito larvae treated with plant
487 extracts. Science Vision. 2011;11(3):155-158.
488 26. Atienzar FA, Venier P, Jha AN, Depledge MH. Evaluation of the random amplified polymorphic
489 DNA (RAPD) assay for the detection of DNA damage and mutations. Mutation
490 Research/Genetic Toxicology and Environmental Mutagenesis. 2002;521(1-2):151-163.
491 27. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein was
492 utilizing the principle of protein–dye binding. Analytical Biochemistry. 1976;72:248-254.
493 28. Baeshin NA, Qari SH, Sabir JSM, ALhejin AM. Biochemical and Molecular Evaluation of Genetic
494 Effects of *Rhazya stricta* (Decne) Leafs Extract on *Aspergillus terreus*. Saudi Journal of
495 Biological Sciences. 2008;15(1):25-33.
496 29. Blum H, Beier H, Gross HJ. Improved silver staining of plant proteins, RNA and DNA in
497 polyacrylamide gels. Electrophoresis. 1987;8(2):93-99.
498 30. El-Tarras AA, Hassan MM, El-Awady MA. Evaluation of the genetic effects of the in vitro
499 antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning
500 electron microscope. African journal of Biotechnology. 2013;12(21):3171-3180.
501 31. Jafargholizadeh N, Zargar SJ, Yassa N, Tavakoli S. Purification of Cucurbitacins D, E, and I from
502 *Ecballium Elaterium* (L.) A. Rich Fruits and Study of Their Cytotoxic Effects on the AGS Cell
503 Line. Asian Pacific journal of cancer prevention. 2016;17(10):4631.
504 32. Celik TA. (2012). Potential Genotoxic and Cytotoxic Effects of Plant Extracts. A Compendium of
505 Essays on Alternative Therapy. Arup Bhattacharya (Ed.), Edition:1, Chapter: 11, Publisher:
506 InTech.
507 33. Ciğerci, İ. H., Cenkci, S., Kargıoğlu, M., Konuk, M. Genotoxicity of *Thermopsis turcica* on *Allium*
508 *cepa L.* roots revealed by alkaline comet and random amplified polymorphic DNA
509 assays. Cytotechnology. 2016;68(4):829-838.
510 34. Lee DH, Iwanski GB, Thoennissen NH. Cucurbitacin: Ancient Compound Shedding New Light on
511 Cancer Treatment. The Scientific World Journal. 2010;10:413–418.
512 35. Guo J, Wu G, Bao J, Hao W, Lu J, Chen X. Cucurbitacin B induced ATM-mediated DNA damage
513 causes G2/M cell cycle arrest in a ROS-dependent manner. PLOS one. 2014;9(2):e88140.
514 36. Ren G, Sha T, Guo J, Li W, Lu J, Chen X. Cucurbitacin B induces DNA damage and autophagy
515 mediated by reactive oxygen species (ROS) in MCF-7 breast cancer cells. Journal of natural
516 medicines. 2015;69(4):522-530.
517 37. Othman AS, Ahmed NA. Antibacterial Effect of the Ethanol Leaves Extract of *Moringa oleifera* and
518 *Camellia sinensis* against Multi Drug Resistant Bacteria. International Journal of
519 Pharmacology. 2017;13(2):156-165.