

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. elaterium* 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

56 present study was to evaluate the genotoxic effect of these extracts on *Escherichia coli* using
57 enterobacterial repetitive intergenic consensus ERIC-PCR and sodium dodecyl sulfate polyacrylamide
58 gel electrophoresis (SDS-PAGE).
59

60 2. MATERIALS AND METHODS

61 62 2.1 Plant Material

63
64 The ripe fruits and mature leaves of *E. elaterium* were collected from Nablus, West Bank-Palestine,
65 during August and September 2017. Ethanolic fruit and leaf extracts of *E. elaterium* were prepared by
66 cold maceration method. The collected fruits and leaves were washed with water to remove soil and
67 dust particles, then they were dried. Exposure to light was avoided to prevent the loss of effective
68 ingredients. After that, the fresh fruits (20g) were homogenized finely using blender after addition 80
69 ml of absolute ethanol. The dried leaves were powdered finely using blender. Approximately 30-40g
70 of dried leaf material was mixed thoroughly with magnetic stirrer in 150 ml of 80% ethanol. Both
71 ethanol-fruit mixture and ethanol-leaf mixture were left on shaker at room temperature for 48h. The
72 mixtures then were filtered using muslin cloth to remove large particle and insoluble materials. After
73 that, to remove fine particles mixtures were centrifuged at 5,000 rpm for 15 min at 4°C. Then, the
74 extracts were dried and concentrated using rotary evaporator at 50°C, and freeze dryer (lyophilizer).
75 The dried extract obtained was stored at 4°C. Before starting the experiments, plant extracts were
76 dissolved in 10% Dimethyl sulfoxide (DMSO) to obtain a concentration of 200 mg/ml and stored at
77 4°C for further assays.

78 79 2.2 Determination of Antibacterial Activity of *E. elaterium* Extracts

80 81 2.2.1 Determination of MIC by broth microdilution method

82
83 MIC of plant extracts was determined by the microbroth dilution method in sterile 96- wells microtiter
84 plates according to CLSI [21]. The plant extract (200 mg/ml of 10% DMSO) and 10% DMSO
85 (negative control) were two fold-serially diluted in nutrient broth directly in the wells of the plates in a
86 final volume of 100µL. Bacterial inoculum (*E. coli* ATCC 25922 or a clinical strain) 10⁵ CFU/ml
87 was added to each well. Negative control wells containing either 100µL nutrient broth only, or 100µL
88 DMSO with bacterial inoculum, or plant extracts and nutrient broth without bacteria were included in
89 these experiments. Each plant extract was run in duplicate. The MIC was taken as the minimum
90 concentration of the dilutions that inhibited the growth of the test microorganism by visual inspection.
91

92 93 2.3 Evaluation of the Genotoxic Potential of *Ecballium elaterium* Ethanolic Extracts on 94 DNA Extracts of *Escherichia coli*

95 96 2.3.1 Inoculation of *Escherichia coli*

97 From 24h old colonies of both *E. coli* strains plated on Mueller-Hinton agar media, three to four
98 colonies were sub-cultured under sterile conditions into bottles containing 25 ml nutrient broth for 2h
99 with continuous shaking. In the next day, constant volumes of nutrient broth (25 ml) were inoculated
100 with 3.5 ml of *E. coli* strain (from the previously prepared bacterial suspension) and incubated at 37°C
101 for 1h with continuous shaking. Then different concentrations of ethanolic leaf extract and ethanolic
102 fruit extract of *E. elaterium* were added to bacterial broth culture into a final concentration 25mg/ml,
103 10mg/ml and 6 mg/ml. Samples of 3 and 6 ml size were taken from the bacterial culture treated with
104 plant extract after 2h, 6h, and 24h, centrifuged for 10 minutes at 14.000 rpm, then the supernatant
105 was discarded and the pellet was saved at -20°C. The 3 ml sample pellet was used to isolate bacterial
106 DNA for ERIC-PCR and the 6 ml sample pellet to isolate bacterial protein for SDS-PAGE. A broth
107 sub-cultured with *E. coli* strain and treated with 10% Dimethyl sulfoxide (DMSO) was used as a
108 negative control.
109

110 111 2.3.2 DNA extraction and ERIC-PCR assay

112 *Escherichia coli* DNA genome was prepared for PCR according to method described previously [22].
113 Enterobacterial repetitive intergenic consensus PCR was performed using

114 primer ERIC1: 5'-ATG TAA GCT CCT GGG GAT TCA C-3' and primer ERIC2 : 5'-AAG TAA GTG
115 ACT GGG GTG AGC G-3', described previously [23]. Each PCR reaction mix (25 µL) was
116 performed using 12.5 µL of PCR premix (ReadyMix™ Taq PCR Reaction Mix with MgCl₂, Sigma),
117 0.8 µM of each primer, 52 ng DNA template, concentration of dNTPs was modified to 0.4 mM, MgCl₂
118 to 3 mM and Taq DNA polymerase to 1.5 U. The reaction mixture was given a short spin for complete
119 mixing of the components. DNA amplification was then carried out using the thermal cycler
120 (Mastercycler personal, Eppendorf) according to the following thermal conditions: initial denaturation
121 for 3 min at 94°C was followed by 40 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 1
122 min and extension at 72°C for 2 min, with a final extension step at 72°C for 5 min [24]. The ERIC-PCR
123 products were analyzed by electrophoresis on 2% agarose gel. The amplified pattern was visualized
124 on a UV trans-illuminator and photographed. The changes occurring in ERIC banding pattern profiles
125 following plant extract treatments including, variations in band intensity as well as gain or loss of
126 bands, were taken into consideration [25,26].

127

128 **2.4 Protein Assay**

129

130 **2.4.1 Protein isolation and determination of protein concentration**

131

132 To each previously taken 6 ml *E. coli* sample, 300 µl of lysis buffer (25 mM Tris-HCL, 100 mM NaCl
133 [pH 8]) were added then vortexed. The samples were then left on ice for 15-20 minutes. After that,
134 each sample was sonicated using sonicator (Q55 Sonicator, QSonica, CT, U.S.A.) at 35% power for
135 10 minutes divided as 10 seconds on and 10 seconds off. This process was carried out on ice. After
136 that, samples were centrifuged for 10 minutes at 16100Xg speed. The resulting supernatant was then
137 aspirated and stored at -20°C. The protein content in the culture filtrates was estimated by the dye
138 binding method of [27]. The protein concentrations were calculated using bovine serum albumin
139 (BSA) as standard.

140

141 **2.4.2 SDS-PAGE procedure and Silver staining of SDS-PAGE gels**

142

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

148

149 **3. RESULTS**

150

151 **3.1 MIC Assay**

152

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

156

157 **3.2 Genotoxic Potential of Ethanolic *E. elaterium* Extract on DNA of *E. coli* Strains**

158

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

167

168 **3.2.1 Evaluation of the genotoxic potential of ethanolic extracts of *E. elaterium* on DNA of *E. coli* reference strain**

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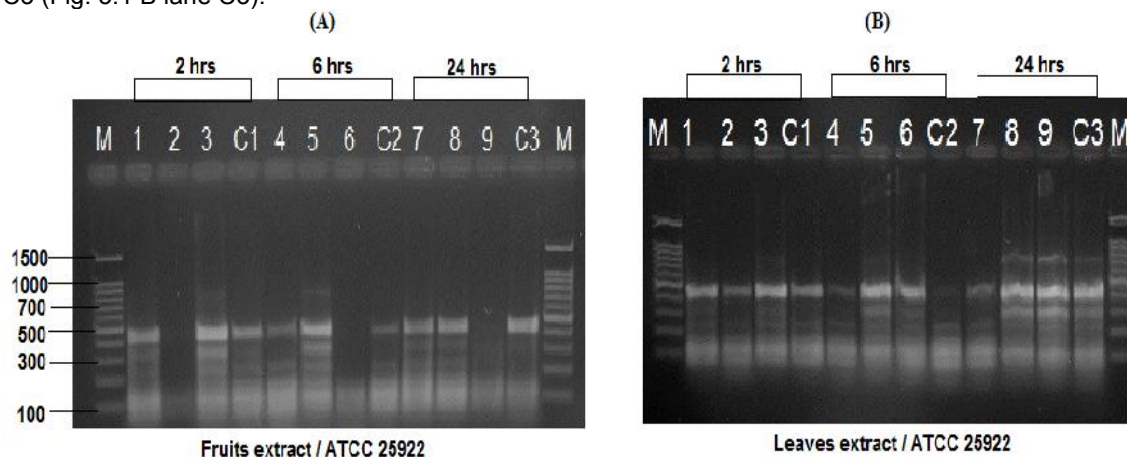
171 The effect of fruit ethanolic extract on *E. coli* reference strain was evaluated using different extract
 172 concentrations at different time intervals. ERIC-PCR profile showed that a band with an amplicon
 173 length of about 450-bp was more intense in *E. coli* reference strain treated with 6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

182

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

194

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



203

204
 205 **Fig. 1.** ERIC-PCR profile of *E. coli* ATCC 25922 strain treated and untreated with different fruit extract
 206 concentrations (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1,
 207 C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml; Lanes 2, 5 and 8
 208 treated with 10 mg/ml; Lanes 3, 6 and 9 treated with 6 mg/ml.

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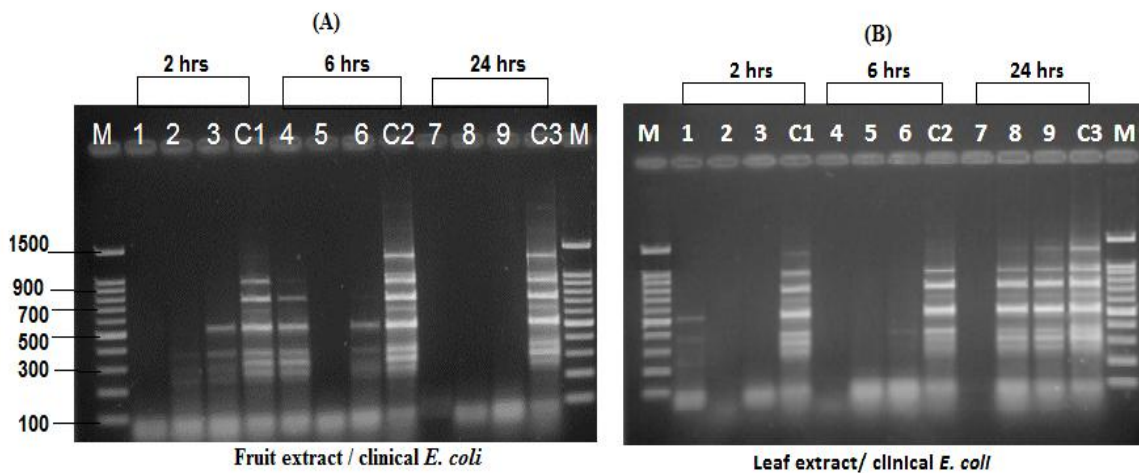
210 3.2.2 Evaluation of the genotoxic potential of ethanolic extracts of *E. elaterium* on DNA of 211 clinical *E. coli* strain

212

213 Treatment of clinical *E. coli* strain with ethanolic fruit extract resulted in the loss of several bands from
 214 ERIC-PCR profile of this strain compared with the profile of untreated control samples. ERIC-PCR
 215 showed the absence of two major bands with amplicon sizes of about 1000-bp and 800-bp from
 216 clinical *E. coli* strain treated with 25 mg/ml, 10 mg/ml and 6 mg/ml ethanolic fruit extract for 2h (Fig.
 217 3.2 A lanes 1, 2 and 3), compared with untreated control C1(Fig. 3.2 A lane C1). Another major band
 218 with approximately 550-bp amplicon size is also missing from the profile of the DNA extracted from
 219 the clinical strain treated with 25 mg/ml and 10 mg/ml after 2h (Fig. 3.2 A lanes 1 and 2), compared to
 220 the profile of the sample treated with 6 mg/ml (Fig. 3.2 A lane 3) and untreated control (Fig. 3.2 A lane
 221 C1). Moreover, the band which had an amplicon size of approximately 350-bp was faint and absent
 222 after 2h from the DNA profile of clinical *E. coli* strain treated with ethanolic fruit extracts of 25 mg/ml
 223 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
 224 lane 3) and untreated control (Fig. 3.2 A lane C1). The bands which had amplicon sizes of
 225 approximately 1500-bp and 600-bp, amplified from clinical *E. coli* strain treated with 25 mg/ml of fruit
 226 extract disappeared (Fig. 3.2 A lane 1), while the band which had amplicon size of about 100-bp
 227 length was faint in comparison with untreated control C2 after 2h (Fig. 3.2 A lane C2). Finally, ERIC-
 228 PCR showed that all bands were absent from clinical strain treated with 6 mg/ml and 10 mg/ml fruit
 229 extract for 24 h (Fig. 3.2 A lanes 8 and 9) compared with untreated control C3 (Fig. 3.2 A lane C3).
 230 ERIC-PCR profile for treated and untreated clinical *E. coli* strain with different concentrations of
 231 ethanolic fruit extract of *E. elaterium* at different time intervals is shown in Fig. 2A.

232
 233 Loss of bands was also observed upon treatment of clinical *E. coli* strain with ethanolic leaf extract.
 234 Bands with amplicon sizes around 1000-bp, 800-bp and 550-bp lengths were found in controls C1
 235 and C2, but disappeared after 2 and 6h in *E. coli* strain treated with 10 and 6 mg/ml ethanolic leaf
 236 extract (Fig. 3.2 B lanes 2, 3, 5 and 6), and after 6 h in strain treated with 25 mg/ml ethanolic leaf
 237 extract (Fig. 3.2 B lane 4). Moreover, ERIC-PCR showed that the band which had amplicon size about
 238 to 550-bp length was faint after 2h in clinical strain treated with 25 mg/ml ethanolic leaf extract in
 239 comparison with control C1 (Fig. 3.2 B lane 1). On the other hand, incubation for 24h for samples
 240 treated with 10 and 6 mg/ml leaf extract showed identical banding pattern compared with control C3
 241 (Fig. 3.2 B lanes 8 and 9) with the exception of the presence of the band with amplicon size of about
 242 1300 bp was faint in samples treated with 10 and 6 mg/ml leaf extract compared with control C3 (Fig.
 243 3.2 B Lane 7). ERIC-PCR profile for treated and untreated clinical *E. coli* strain with different
 244 concentrations of ethanolic leaf extract of *E. elaterium* at different time intervals is shown in Fig. 2B.

245
 246 Fruit and leaf ethanolic extracts of *E. elaterium* showed different effects on genomic DNA of clinical *E.*
 247 *coli* strain at the same time interval. For example, 24h treatment of clinical *E. coli* with 10 and 6mg/ml
 248 fruit extract (Fig. 3.2 A lanes 8 and 9) resulted in loss of all bands compared with the untreated control
 249 C3 (Fig. 3.2 A lane C3). On the other hand, 24h treatment of clinical *E. coli* with 10 and 6mg/ml leaf
 250 extract (Fig. 3.2 B lanes 8 and 9) resulted in neither loss nor appearance of extra bands compared
 251 with control C3 (Fig. 3.2 B lane C3), but the band with an amplicon size of about 1000-bp was faint
 252 upon treatment.



253
 254
 255 **Fig. 2.** ERIC-PCR profile of clinical *E. coli* strain treated and untreated with different concentrations of
 256 fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3

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

259 **3.3 Effect of Ethanolic Extracts of *E. elaterium* on Total Protein Concentration of Both** 260 ***E. coli* Strains**

261
 262 Treatment of both *E. coli* strains with fruit and leaf ethanolic extracts of *E. elaterium* resulted in
 263 noticeable elevation in total protein concentration under different experimental conditions, especially
 264 in bacterial strains exposed to fruit extracts. This increase was not reported when *E. coli* ATCC
 265 25922 reference strain was treated with 25, 10 and 6 mg/ml fruit extract for 24 h, and when clinical *E.*
 266 *coli* was treated for 24 h with 25 and 10 mg/ml fruit extract.

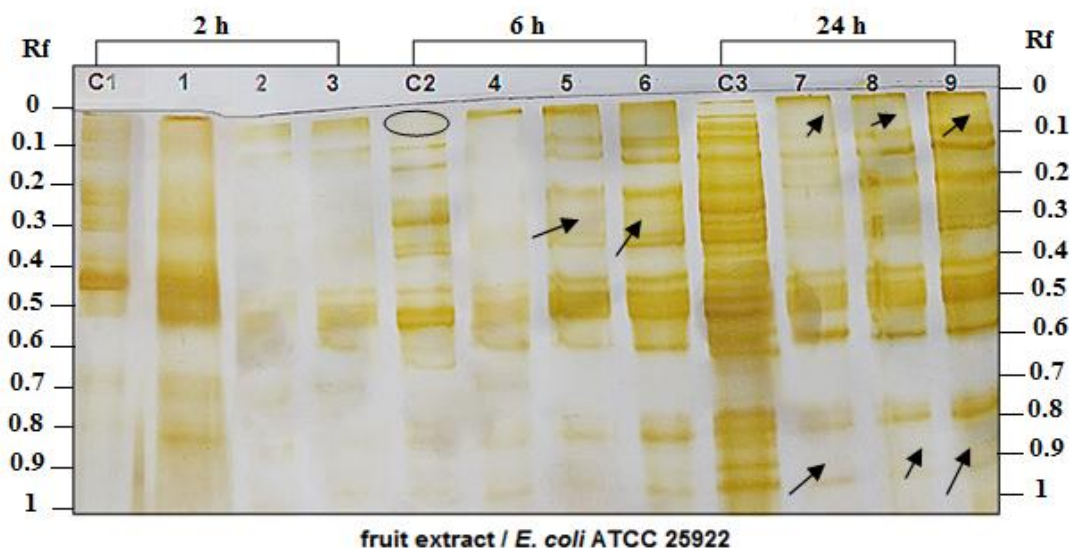
267 268 **3.4 Effect of Ethanolic Extracts of *E. elaterium* on Protein Profile of *E. coli* Strains**

269
 270 Total protein was extracted from both *E. coli* strains that were exposed to different concentrations (6,
 271 10 and 25 mg/ml) of ethanolic leaf and fruit extracts of *E. elaterium* for different time intervals (2, 6
 272 and 24h). Changes in extracted protein from treated strains were evaluated in comparison with
 273 untreated control samples at the same interval time. In the present study, only major obvious changes
 274 were taken into consideration.
 275

276 **3.4.1 Effects of ethanolic extracts of *E. elaterium* on protein profile of reference *E. coli* strain**

277
 278 Several changes in reference *E. coli* protein profile were observed upon treatment with *E. elaterium*
 279 fruit extracts. Treatment with 25 mg/ml fruit extract for 6 h (Fig. 3.3 lane 4) caused several bands in
 280 untreated control C2 (Fig. 3.3 lane C2) to disappear including those with Rf values 0.1, 0.15, 0.25, 0.3
 281 and 0.36. Moreover, the band at Rf 0.29 was very faint after 6 h treatment with 6 mg/ml fruit extract
 282 (Fig. 3.3 lane 6) and absent after 6 h treatment with 10 mg/ml fruit extract (Fig. 3.3 lane 5) compared
 283 with control C2 (Fig. 3.3 lane C2). Absence of bands with Rf values 0.04, 0.05 and 0.9 was observed
 284 at 24 h treatment with 25, 10 and 6 mg/ml fruit extract (Fig. 3.3 lanes 7, 8 and 9) compared with
 285 control C3 (Fig. 3.3 lane C3). Protein profile for treated and untreated reference *E. coli* strain with
 286 different concentrations of *E. elaterium* ethanolic fruit extract is shown in Fig. 3.
 287

288 Leaf extract treatment of reference *E. coli* strain also resulted in several modifications in the protein
 289 profile. For example, upon 6 h treatment with 25 mg/ml leaf extract appearance of 2 new bands at Rf
 290 values of approximately 0.45 and 0.47 was recorded compared with untreated control C2. On the
 291 other hand, disappearance of bands with 0.14, 0.15, 0.24 and 0.65 Rf values was observed after 24 h
 292 treatment with 25 mg/ml leaf extract compared with control C3 (Figure is not shown).
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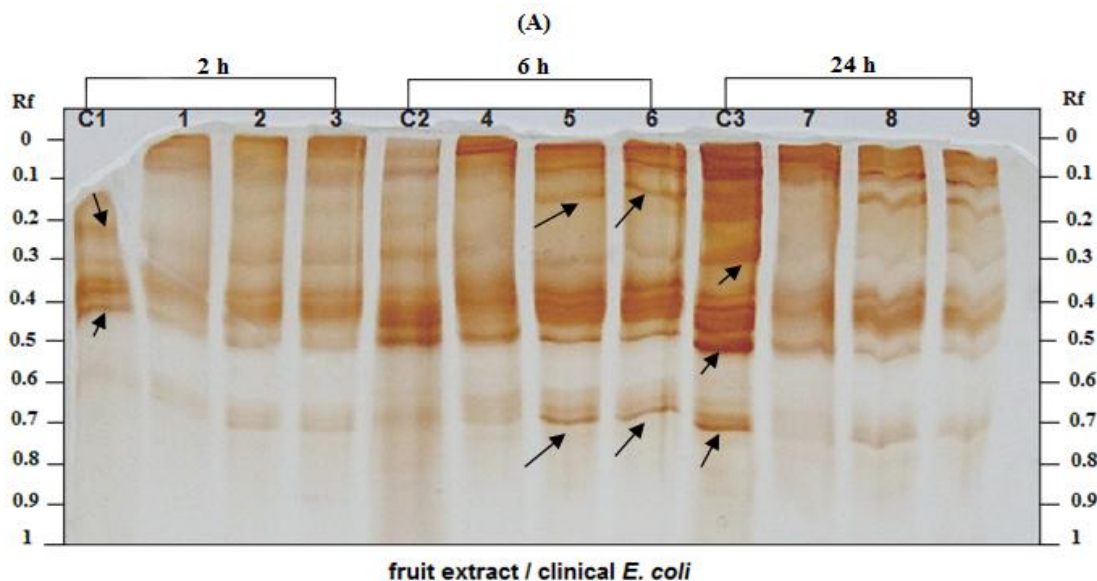
294
 295

296 **Fig. 3.** Protein profile of *E. coli* ATCC 25922 reference strain treated and untreated with different
 297 concentrations of fruit ethanolic extract of *E. elaterium* at different time intervals. Lanes C1, C2 and
 298 C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes 2, 5 and 8 are
 299 treated with 10 mg/ml; Lanes 3, 6 and 9 are treated with 6 mg/ml. Arrows and ovals indicate some of
 300 the treatment-affected bands mentioned in the text.
 301

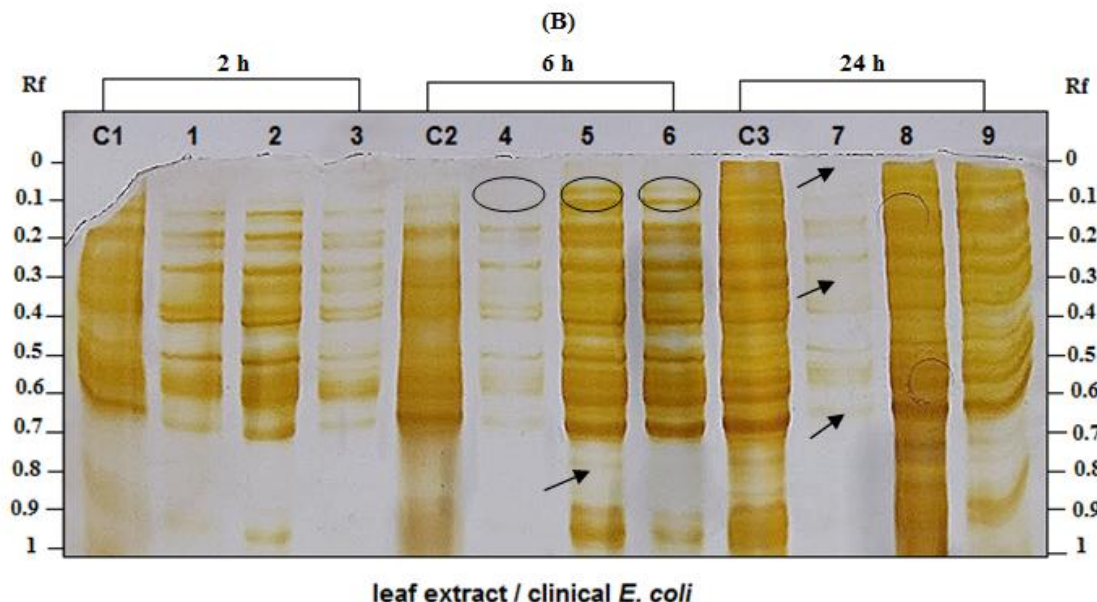
302 **3.4.2 Effects of ethanolic extracts of *E. elaterium* on protein profile of clinical *E. coli* strain**

303
 304 Ethanolic fruit extract of *E. elaterium* resulted in differences in the protein profile of the treated clinical
 305 *E. coli*. The observed differences varied between either up regulation, down regulation or even
 306 complete disappearance of particular protein fractions. After 2 h of application of 25 mg/ml fruit extract
 307 (Fig. 3.4 A lane 1), bands at Rf values of about 0.22 and 0.42 were faint compared with control C1
 308 (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
 309 extract compared with control C1 (Fig. 3.4 A lane C1). On the other hand, 6 h treatment with 10 and 6
 310 mg/ml fruit extract (Fig. 3.4 A lanes 5 and 6) resulted in an increase in the intensity of bands at
 311 approximate Rf of 0.15 and 0.7 compared with untreated control C2 (Fig. 3.4 A lane C2). Decreasing
 312 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
 313 extract (Fig. 3.4 A lanes 7, 8 and 9) compared with control C3 (Fig. 3.4 A lane C3). Moreover, the
 314 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
 315 9) and completely disappeared upon 24 h treatment with 25 mg/ml fruit extract (Fig. 3.4 A lane 7)
 316 compared with control C3 (Fig. 3.4 A lane C3). Protein profile for treated and untreated clinical *E. coli*
 317 strain with different concentrations of *E. elaterium* ethanolic fruit extract at different time intervals is
 318 shown in Fig. 4A.
 319

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

340 4. DISCUSSION

341

342 In fact, drugs derived from natural products represent 70 % of these approved since 1981 [31].
343 Moreover, the traditional use of medicinal plants in treating diseases is increasing in many countries
344 [15]. In spite of this, increasing evidence has shown that many of medicinal plant products may have
345 toxic, mutagenic, and even carcinogenic effects [32]. Thus, investigating the genotoxicity of plants is
346 of great importance for safe traditional use and safe use in drug formulations.

347

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

354

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

370

371 Results of the current study support the evidences reported previously that extracts of *E. elaterium*
372 have genotoxic and mutagenic potential [15,17,18].
373

374 The observed genotoxicity of *E. elaterium* possibly were mediated by cucurbitacins. Different types of
375 cucurbitacins such as B, D, E, I, L and R have been identified in the juice of *E. elaterium* [18]. The
376 fruits juice of *E. elaterium* is especially rich with cucurbitacins that represent between 20-30% of the
377 juice [7]. It was reported that cucurbitacins B, D, E, and I possess strong anticancer activities [34]. In
378 addition, the genotoxic potential of cucurbitacin B was reported, as it induced DNA damage in A594
379 and MCF-7 cells using the comet assay [35,36]. The damage mediated by cucurbitacin B is due to
380 induction of reactive oxygen species (ROS) formation, which results in double-stranded breakage and
381 subsequently G2/M phase arrest in A594 cells.
382

383 The effect of fruit and leaf ethanolic extracts of *E. elaterium* on total protein profile of both *E. coli*
384 strains was assessed using SDS-PAGE gels, where several alterations in the protein profile of *E.*
385 *elaterium* treated *E. coli* compared to the untreated control was observed. These changes included
386 increase or decrease in band intensity, absence or appearance of bands. The absence of protein
387 bands indicates the interruption of protein synthesis pathways possibly stimulated by *E. elaterium*
388 extract bioactive constituents [37]. The appearance of new bands and increasing the intensity of some
389 bands can be explained by the ability of *E. elaterium* extract to apply a stress on treated *E. coli*. The
390 stressed *E. coli* may respond under these conditions by up-regulation (increasing the expression) of
391 some proteins and stimulating the expression of others [30]. Another explanation for this proposes the
392 happening of frame shift mutations due to stress [30].
393

394 5. CONCLUSION

395
396 Our results showed that *E. elaterium* fruit and leaf ethanolic extracts possess genotoxic and
397 mutagenic potential. The results also point out the capability of using *E. elaterium* to treat *E. coli*
398 infections. More studies are required to find out the exact mechanism responsible for the observed
399 genotoxicity.
400

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