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

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Authors' contributions

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

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ABSTRACT

Aims: This study was conducted to evaluate the genotoxic effects of fruit and leaf ethanolic extracts of *Ecaballium elateruim* on clinical and reference strains of *E. coli (E. coli* ATCC 25922).
Methodology: The genotoxic effects of fruit and leaf ethanolic extracts were determined by using enterobacterial repetitive intergenic consensus (ERIC-PCR) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).
Results: The results showed an alteration in DNA and protein profiles of both tested *Escherichia 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.

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Conclusions: Such findings strongly indicate the genotoxic effects of *E. elaterium* extracts on both *E. coli* strains. The results 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. Future studies should be needed 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 used as medicines, food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals. In some countries, 80% of the population is depending on medicinal plants to maintain their health and cure their diseases [1].

The plant *Ecballium elaterium* (common name squirting cucumber), is a weed that belongs to the Cucurbitaceae family. It is perennial, fleshy, rough hairy with 30-100 cm long stems. 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. During unripe condition, the fruit appears in pale green colour and covered with numerous hairs. It is an indigenous plant 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, haemorrhoids, varicose veins, and nose bleeding, therefore 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 medicinally important because of their cytotoxic, anti-tumor 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, anti-carcinogenic, anti-thrombotic, 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]. No previous studies were evaluated on *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 study aimed to evaluate the genotoxic effect of these extracts on *E. 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 (20 g) were homogenised finely using blender after addition of 80 ml of absolute ethanol. The dried leaves were powdered finely using a blender. Approximately 30-40 g 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 incubated on a shaker at room temperature for 48h. The mixtures were filtered using muslin a cloth to remove large particle and insoluble materials. After that, mixtures were centrifuged at 5,000 rpm for 15 min at 4°C, to remove fine particles. Then, the extracts were dried and concentrated by using rotary evaporator at 50°C, and freeze dryer

(lyophilizer). The obtained dried extract 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 4°C 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 to a final volume of 100µL. Bacterial inoculum (*E. coli* ATCC 25922 or a clinical strain) of 10^5 CFU/ml was added to each well. Negative control wells containing either 100 µL nutrient broth only, or 100 µL 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 tested microorganism by visual inspection.

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

2.4.1 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 to a final concentration of 25 mg/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, and centrifuged for 10 minutes at 14.000 rpm, then the supernatant was discarded and the pellet was stored at -20°C. Pellet of 3 ml sample was used to isolate the bacterial DNA for ERIC-PCR and pellet of 6 ml sample was used to isolate the 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.

2.4.2 DNA extraction and ERIC-PCR assay

Escherichia coli DNA genome was prepared for PCR according to the 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`, as described previously [23]. Each PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix (ReadyMixTM Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.8 µM of each primer, 52 ng DNA template, concentration of dNTPs was modified to 0.4 mM, $MgCl_2$ 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 94°C, followed by 40 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 1 min and extension at 72°C for 2 min, with a final extension step at 72°C for 5 min [24]. The ERIC-PCR products were analysed by electrophoresis on 2% agarose gel. The amplified pattern was visualised on a UV trans-illuminator and photographed. The changes in ERIC banding pattern profiles following plant extract treatments including, variations in band intensity as well as gain or loss of bands, were investigated [25,26].

2.5 Protein Assay

2.5.1 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 and vortexed. The samples were then left on ice for 15-20 minutes. After that, each sample was sonicated using a 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. The supernatant was then aspirated and stored at -

20°C. The protein content in the culture filtrates was estimated by the dye binding method of Bradford [27]. The protein concentrations were calculated using bovine serum albumin (BSA) as standard.

2.5.2 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]. Vertical slab gel electrophoresis was performed using the ENDURO PAGE System (E2010-P0, Labnet Int., Inc., NJ, U.S.A.). The SDS-PAGE gels were stained by using silver staining [29]. The protein profile was analysed by SDS-PAGE. The changes occurred in protein banding profiles, following plant extract treatments, including variation in band intensity as well as gain or loss of bands were investigated [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 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 compared with untreated controls at the same time interval. In the present study, only major obvious changes in DNA banding profile were taken into consideration. The results of three DNA samples 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. 1A 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. 2A lane 7 and Fig. 2B lane 7).

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 by 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 (Fig. 1A lane 3) in comparison with the same band appeared in un-treated control (Fig. 1A C1). This band disappeared after 6 and 24h from reference strain treated with 6 mg/ml fruit extract (Fig. 1A lanes 6 and 9) in comparison with the non-treated controls (Fig. 1A lanes C2 and C3), respectively. Moreover, two additional bands with 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. 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. 1B lane 7) when compared with the non-treated control (Fig. 1B lane C3). This band was also found in samples treated with extract concentrations of 6 and 10 mg/ml and incubated for 24h (Fig. 1B lanes 9 and 8). Moreover, it was recorded 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. 1B lanes 5 and 6) compared to samples incubated with extract concentration of 25 mg/ml and with un-treated control (Fig. 1B lanes 4 and C2). ERIC-PCR profile for treated and un-treated *E. coli* tested strains with different concentrations of ethanolic leaf extract of *E. elaterium* at different time intervals (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. 1A lane 7), the band of about 450-bp amplicon size almost had same intensity as the control C3 (Fig. 1A lane C3). In contrast, the same band was

fainter after 24h treatment with 25 mg/ml leaf extract (Fig. 1B lane 7) compared with the control C3 (Fig. 1B lane C3). The 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. 1B lane 7) in compared with the control C3 (Fig. 1B lane C3).

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 un-treated 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) when compared with un-treated 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 un-treated 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) when compared with the sample treated with 6 mg/ml (Fig. 2A lane 3) and un-treated 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 un-treated 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 un-treated control C3 (Fig. 2A lane C3). ERIC-PCR profile for treated and un-treated 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 an 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). In contrast, incubation of 24h for samples treated with 10 and 6 mg/ml leaf

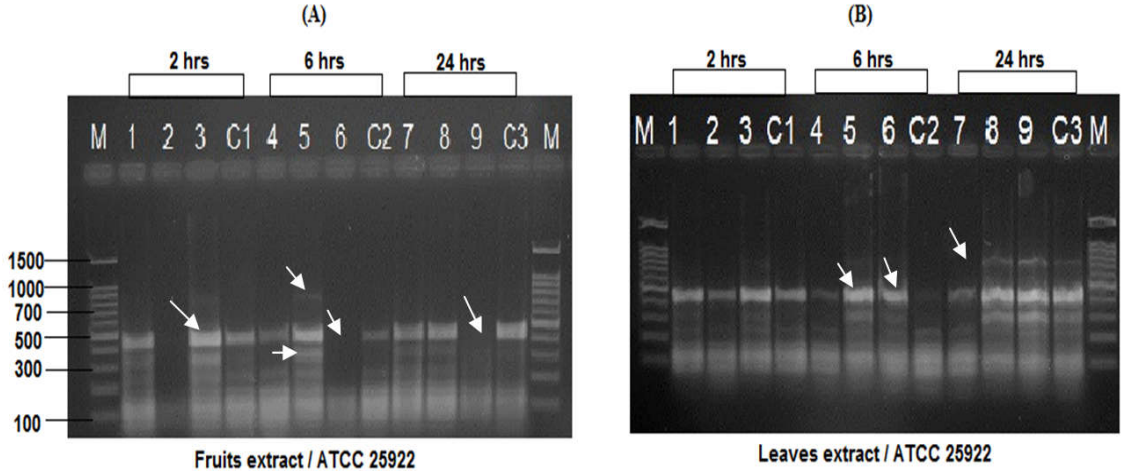

Fig. 1. ERIC-PCR profile of *E. coli* ATCC 25922 strain treated and un-treated with different fruit extract concentrations (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml;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

extract showed an identical banding pattern in compared with control C3 (Fig. 2B lanes 8 and 9) with the exception of the band with amplicon size of about 1300bp that was faint in samples treated with 10 and 6 mg/ml leaf extract when compared with control C3 (Fig. 2B Lane C3). ERIC-PCR profile for treated and un-treated 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.

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 a 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 when compared with un-treated 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. A treatment with 25 mg/ml fruit extract for 6 h (Fig. 3 lane 4) caused several bands in un-treated control C2 (Fig. 3 lane C2) to disappear including those with Rf values of 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)

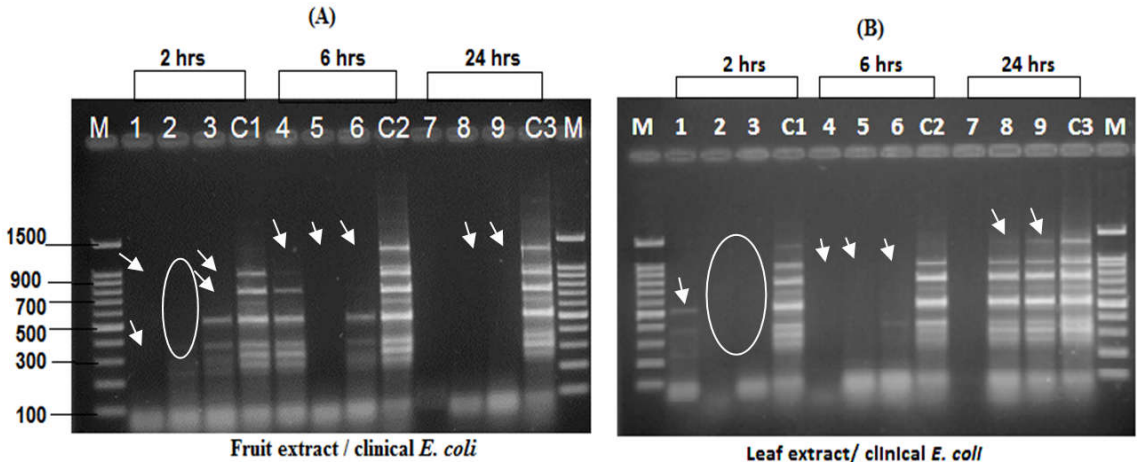

Fig. 2. ERIC-PCR profile of clinical *E. coli* strain treated and untreated with different concentrations of fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 25 mg/ml; Lanes 2,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

when compared with control C2 (Fig. 3 lane C2). The 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 respectively (Fig. 3 lanes 7, 8 and 9) in compared with control C3 (Fig. 3 lane C3). Protein profile for treated and un-treated 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. In contrast, the disappearance of bands with Rf values of 0.14, 0.15, 0.24 and 0.65 were observed after 24 h treatment with 25 mg/ml leaf extract in compared with control C3. (Figure is not shown).

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 when 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). In contrast, 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 approximately Rf 0.15 and 0.7 compared with un-treated 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) when 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 un-treated clinical *E. coli* strain with different concentrations of *E. elaterium* ethanolic fruit extract at different time intervals has been depicted in Fig. 4A.

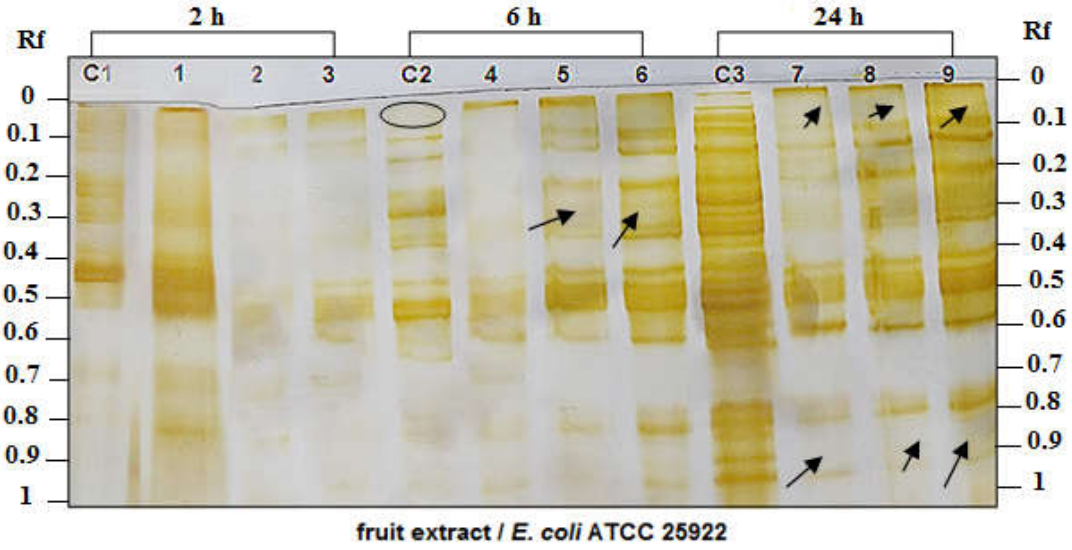

Fig. 3. Protein profile of *E. coli* ATCC 25922 reference strain treated and untreated with different concentrations of fruit ethanolic extract of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes 2, 5 and 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.

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).In contrast, 6h treatment with 25 mg/ml leaf extract resulted in the disappearance of band at Rf 0.06 when 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) in compared to control C2 (Fig. 4B lane C2). Treatment with 25 mg/ml leaf extract for 24h (Fig. 4B lane 7) caused the absence of a band at approximate Rf value of 0.33, while the band at Rf 0.68 was very faint when compared with the control C3 (Fig. 4B lane C3). Protein profile for treated and un-treated clinical *E. coli* strain with different concentrations of *E. elaterium* ethanolic leaf extract at different time intervals are presented in Fig. 4B.

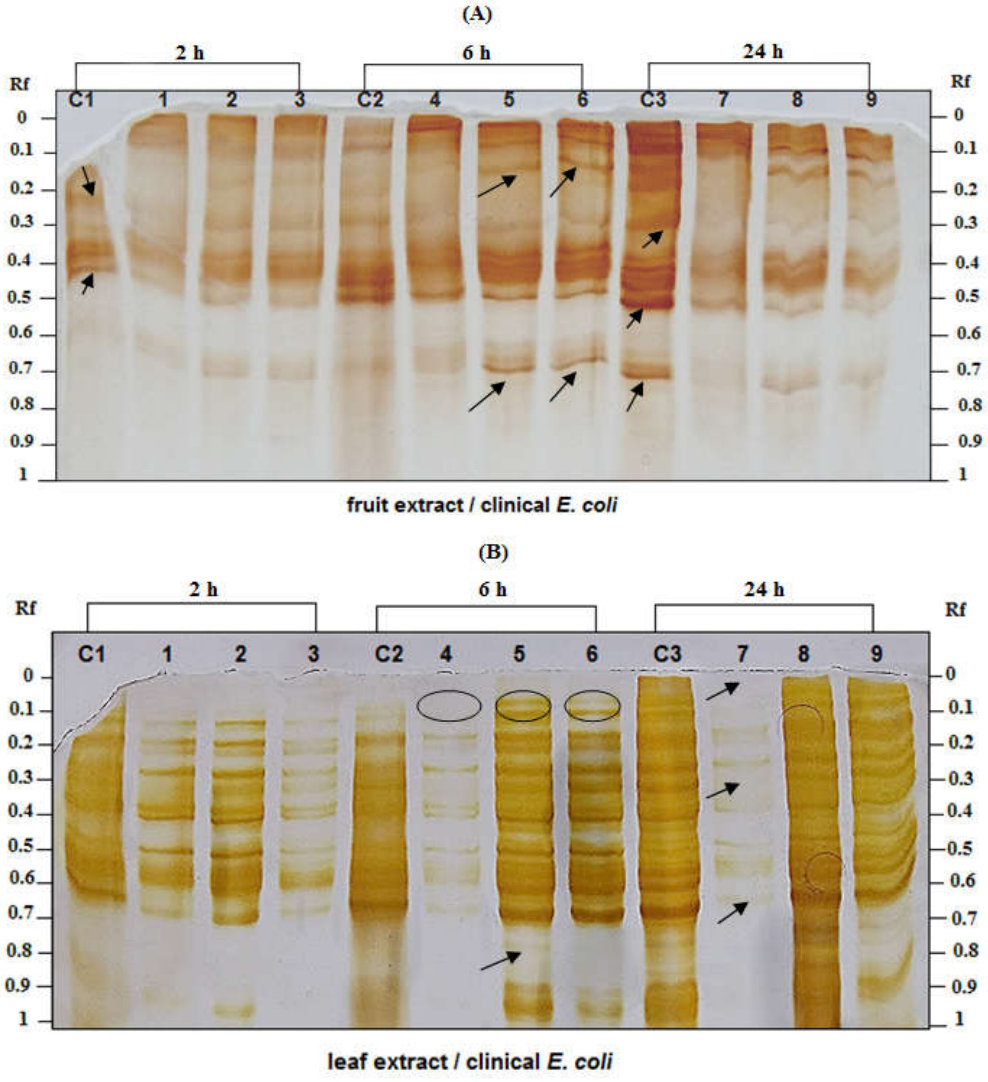

Fig. 4. Protein profile of clinical *E. coli* strain treated and un-treated with different concentrations of fruit (A) and leaf (B) ethanolic extracts of *E. elaterium* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); Lanes 1,4 and 7 are treated with 25 mg/ml; Lanes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

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 several countries [15]. In spite of this, increasing evidence has shown that many of the medicinal plant products might 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].

The study is the first of its kind for analysis of genomic alteration 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 un-treated *E. coli* strains used in this study. The primary changes in the treated *E. coli* include the disappearance or appearance of certain bands in compared with un-treated control, as well as differences in band intensity. The loss of bands in treated *E. coli* strains in comparison with the control samples might 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 might 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 by using DNA sequencing or probing [25].

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

The observed genotoxicity of *E. elaterium* was possibly 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 fruit 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 by using SDS-PAGE gels, where several alterations in the protein profile of *E. elaterium* treated *E. coli* in compared to the un-treated control was observed. These changes included an 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 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 is the happening of frameshift mutations due to stress [30].

5. CONCLUSION

The 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. Further studies are needed to find out the exact mechanism responsible for the observed genotoxicity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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