JJBS

Volume 13, Number 1,March 2020
ISSN 1995-6673
Pages 123 - 126

Jordan Journal of Biological Sciences **Short Communication**

Evaluation of Antimicrobial and Genotoxic Activity of *Ephedra foeminea* Ethanolic and Aqueous Extracts on *Escherichia coli*

Shurooq M. Ismail*, Ghaleb M. Adwan, and Naser R. Jarrar

Department of Biology and Biotechnology, An-Najah National University,P.O. Box (7)-Nablus, Palestine

Received February 21, 2019; Revised April 23, 2019; Accepted May 7, 2019

Abstract

This study was conducted to evaluate the antimicrobial activity and the genotoxic effects of ethanolic and aqueous extracts of aerial parts of *Ephedra foeminea* (*E. foeminea*) plant on *Escherichia coli* (*E. coli* ATCC 25922). Antimicrobial activity was investigated using microbroth dilution method, while the genotoxic effect was determined using enterobacterial repetitive intergenic consensus (ERIC)-PCR. MIC value of both ethanolic and aqueous extracts of *E. foeminea* plant was found to be 50 mg/ml. Genotoxic effects of both extracts, showed an alteration in (ERIC)-PCR profiles of *E. coli* strain treated with extracts compared to untreated control. These alterations included a decreased intensity or absence of some amplified fragments. Such findings strongly indicate the genotoxic effects of both ethanolic and aqueous extracts from *E. foeminea* plant on *E. coli*. The findings draw attention to the unsafe, use of *E. foeminea* plant in folkloric medicine and point out the capability of using *E. foeminea* to treat bacterial infections. Future studies are required to know the exact molecules as well as the mechanisms responsible for the genotoxicity of this plant. *In vivo* genotoxicity studies are recommend for assessment of the safety of using *E. foeminea* plant for therapeutic purposes.

Keywords: *Ephedra foeminea*, Genotoxicity potential, Plant extracts, Antimicrobial activity.

1. Introduction

The family Ephedraceae consists of only one genus called *Ephedra* L. It has a group of approximately fifty species of perennials, evergreen, and dioecious sub-shrubs species growing up to four feet tall, with slender and joined stems. In general, species of this genus adapted to grow wild in arid and semiarid conditions and disseminated mainly in the moderate zones of Asia, Europe and North America (O'Dowd *et al*., 1998; Pirbalouti *et al*., 2013). Approximately 25 species of *Ephedra* are found in the drier regions of the Old World covering the area westwards from Central Asia across southwest Asia and into North Africa and Mediterranean Europe (Caveney *et al*., 2001). In the New World, about 24 species of *Ephedra* are found ranging from the southwestern United States to the central plateau of Mexico, and in South America occur in an area from Ecuador to Patagonia (Caveney et al., 2001). *Ephedra* grows widely in Palestine. In the flora Palestina, 5 species of *Ephedra* has been reported, included *E. foeminea*, *E. alata*, *E. aphyla*, *E. ciliata* and *E. fragilis* (Danin, 2018).

Approximately, all commercial applications of *Ephedra* extracts derived from the ephedrine alkaloids found in the stems in many Eurasian *Ephedra* species. These extracts are used in traditional medicine to treat several diseases such as bronchial asthma, coughs, chills, allergies, colds, edema, headaches, fever, flu and gastric disorders. In addition, *Ephedra* shows anticancer and antimicrobial activities (Parsaeimehr *et al*., 2010; Pirbalouti *et al*., 2013; Dehkordi *at al*., 2015; Dosari *et al*., 2016; Al-Rimawi *et al*., 2017; Mendelovich *et al*., 2017). Besides, it was shown that hydro-alcoholic extract of *E. pachyclada* was effective in experimentally healing rat ulcers (Pirbalouti *et al*., 2013).

Ephedra possesses a high antioxidant potential since it has been considered as a source of different phenolic compounds, as well as, a natural source of alkaloids such as ephedrine, pseudoephedrine, and other related compounds. (Eberhardt *et al*., 2000; Parsaeimehr *et al*. 2010; Amakura *et al*., 2013; Dehkordi *et al*., Ibragic and Sofić 2015; Al-Rimawi *et al*., 2017). The studies conducted on the cytotoxicity of *Ephedra* showed that ephedrine derivatives and ground ma-huang extracts were more cytotoxic than those of the whole herb extracts. A study on Neuro-2a cell line showed the cell line was more sensitive to the cytotoxicity (Lee *et al*., 2000). Ethanolic leaf extract and fruit juice of *E. foeminea* reduced viability of cancer cells *in vitro*, whereas the aqueous extract reduced the cytotoxicity in all cell lines (Mendelovich *et al*., 2017). Since there is no scientific report to date about the genetoxicity of *E. foeminea* on prokaryotes, the current study was performed to determine the antimicrobial effect of ethanolic and aqueous extracts from *E. foeminea* plant growing wild in Palestine as well as evaluate the genotoxic effect of these extracts on *E. coli* strain using enterobacterial repetitive intergenic consensus (ERIC)-PCR.

* Corresponding author e-mail: shurooq.ismail@najah.edu.

2. Materials and Methods

2.1. Plant collection and identification

The aerial parts of *E. foeminea* were collected from a natural habitat in Tulkarm province, West Bank-Palestine, during September, 2018. Identification of the plant was carried out by the plant taxonomist Dr. Ghadeer Omar, Department of Biology and Biotechnology, An-Najah National University, Palestine.

The collected aerial parts of *E. foeminea* were washed with water to remove soil and dust particles then dried. Exposure to light was avoided to prevent possible loss of effective ingredients. The dried aerial parts were powdered finely using a blender to make them ready for ethanolic and aqueous extract preparation.

2.2. Plant extract preparation

2.2.1. Ethanolic extract

Approximately 50 g of dried aerial parts powder were mixed thoroughly using magnetic stirrer in 200 mL of 80% ethanol. The ethanol-aerial parts mixture was incubated on a shaker at room temperature for 48h. The mixture was filtered using muslin cloth to remove large insoluble particles. After that, the mixture was centrifuged at 5,000 rpm for 15 min at 4°C to remove fine particles. Then, the supernatant extract was dried and concentrated by using rotary evaporator at 50°C. The obtained dried plant extract powder was stored in refrigerator at 4°C. Before starting the experiments, this material was dissolved in 10% Dimethyl Sulfoxide (DMSO) to obtain a concentration of 200 mg/mLand stored at 4ºC for further assays.

2.2.2. Aqueous extract

Aqueous aerial parts extract was prepared by mixing approximately 50 g of dried aerial parts powder thoroughly using magnetic stirrer in 200 ml of cold (room temperature) sterile distilled water. The water-aerial parts mixture was incubated on a shaker at room temperature for 48h. The mixture was filtered using muslin cloth to remove large insoluble particles. After that, the mixture was centrifuged at 5,000 rpm for 15 min at 4°C to remove fine particles. Then the supernatant extract was dried and concentrated by freeze dryer (lyophilizer). The obtained dried plant extract powder was stored in refrigerator at 4°C. Before starting the experiments, this dried plant extract powder was dissolved in a sterile distilled water to obtain a concentration of 200 mg/mL and stored at 4ºC for further assays.

2.3. Determination of MIC for plant extracts by broth microdilution method

MIC of plant extracts was determined by the broth microdilution method in sterile 96- wells microtiter plates according to the CLSI instructions (CLSI, 2017). 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. After that, a bacterial inoculum size of 10^4 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 this experiment. Each plant extract was run in duplicate. The microtiter plate was then covered and incubated at 37°C for 24h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism. MIC was determined by visual inspection.

2.4. Evaluation of the genotoxic potential of Ephedra foeminea aerial extracts on E. coli

Few colonies from a 24 hour old *E. coli* strain growth culture plated on EMB agar medium were sub-cultured under sterile conditions into a bottle containing 20-mL of nutrient broth, then incubated at 37°C for 1 hour with continuous shaking. After that, aseptically, 1 mL of one hour old *E. coli* culture was added to each of eight sterile bottles each containing 25 mL broth medium. These bottles were incubated at 37°C for 1 hour with continuous shaking. Then three different concentrations of ethanolic extract (3.5 mg/mL, 1.75 mg/mL and 0.875 mg/mL of 10% DMSO), and other three different concentrations of aqueous extract (3.5 mg/mL, 1.75 mg/mL and 0.875 mg/mL of distilled water) were added into six bottles of the *E. coli* broth culture. The other two bottles were considered as a negative or untreated control by adding a specific volume of 10% DMSO and distilled water into each bottle.

Genome of *E. coli* was prepared for enterobacterial repetitive intergenic consensus (ERIC) PCR according to the method described previously (Adwan *et al*., 2013). Three mL samples were taken from the *E. coli* growth culture after 2 hours, 6 hours, and 24 hours, centrifuged for five minutes at 14,000 rpm where the supernatant of each sample was discarded. Then, each bacterial sample pellet was re-suspended in 0.8 mL of Tris-EDTA (10 mM Tris-HCl, 1 mM EDTA [pH 8]), centrifuged for five minutes at 14,000 rpm; after that, the supernatant was discarded. The pellet of each bacterial sample was re-suspended in a 300 µL of sterile distilled water and boiled for 15 minutes. Then the mixture was incubated in ice for 10 minutes. The samples were pelleted by centrifugation at 14,000 rpm for five minutes, and each sample supernatant was transferred into a new Eppendorf tube. The DNA concentration for each sample was determined by using nanodrop spectrophotometer (GenovaNano, Jenway) and the DNA samples were stored at -20ºC for ERIC-PCR analysis. The ERIC-PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3` and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3`. Each PCR reaction mix (25 µL) was composed of 10 mM PCR buffer pH 8.3; 3 mM $MgCl_2$; 0.4 mM of each dNTP; 0.8 µM primer; 1.5U of Taq DNA polymerase and fixed amount of DNA template (60 ng). Then, DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94 ºC; followed by 40 cycles of denaturation at 94 ºC for 50 s, annealing at 50 ºC for 1 min and extension at 72 ºC for 1 min, followed by a final extension step at 72ºC for 5 min. The PCR products were analyzed by electrophoresis through 1.8% agarose gel. The ERIC-PCR profile was visualized using UV trans-illuminator and photographed. Changes in ERIC-PCR banding pattern profiles following plant extracts treatments, including variations in band intensity as well as gain or loss of bands, were taken into consideration (Lalrotluanga *et al*., 2011; Atienzar *et al*., 2002).

3. Results

Results of this study showed that both aqueous and ethanolic aerial parts extracts of *E. foeminea* had an antibacterial activity. The MIC value of both aqueous and ethanolic aerial parts extracts of *E. foeminea* on *E. coli* strain were found to be 50 mg/ml.

DNA genome which was extracted from each *E. coli* strain which was treated with different concentrations of both aqueous and ethanolic aerial parts extracts of *E. foeminea* at various time intervals. Changes in extracted DNA genome from *E. coli* strain were evaluated and compared with untreated controls at the same time intervals.

The effect of aqueous aerial parts extract on genome of *E. coli* strain was evaluated by using ERIC-PCR. ERIC-PCR profile showed that a band with an amplicon length of about 800-bp was less intense in *E. coli* strain treated with 3.5 mg/mL and 1.75 mg/mL (Figure 1A, lanes 1 and 2) of aqueous aerial parts extract for 2h. Besides, this band disappeared in *E. coli* strain treated with 0.875 mg/ml of the same extract (Figure 1A, lane 3), in comparison with the same band appeared in un-treated control. Moreover, all bands disappeared after 6h in the *E. coli* strain treated with 3.5 mg/mL aqueous aerial parts extract (Figure 1A, lane 4). The band with an amplicon length of about 800-bp was less intense in *E. coli* strain treated with 1.75 mg/mL (Figure 1A, lane 5) disappeared in *E. coli* strain treated with 0.875 mg/mL of the same extract for 6h (Figure 1A, lane 6), in comparison with the same band appeared in the un-treated control. The band with an amplicon length of about 800-bp disappeared in *E. coli* strain treated with 3.5 mg/ml, 1.75 mg/mL and 0.875 mg/mL aqueous aerial parts extract for 24h (Figure 1A, lanes 7,8 and 9). Moreover, the band with an amplicon length of about 300-bp was less intense (Figure 1A, lanes 7, 8 and 9) in comparison with the same band appeared in un-treated control. It was observed that in lane number four most bands disappeared when treated with aqueous aerial parts extract of *E. foeminea* of 3.5 mg/mL concentration. ERIC-PCR profiles for *E. coli* strain untreated and treated with different concentrations of aqueous aerial parts extract of *E. foeminea* at the different time intervals are shown in Figure 1A.

Comparing the ERIC-PCR profile of untreated control samples with the profile of the *E. coli* treated with ethanolic aerial parts extract showed decreasing of intensity or loss of some bands from the profile. ERIC-PCR profile showed that a band with an amplicon length of about 800-bp was less intense in *E. coli* strain treated with 3.5 mg/mL (Figure 1B, lane 2 and 5) and disappeared in *E. coli* strain treated with 1.75 mg/mL (Figure 1B, lane 3 and 6) of ethanolic aerial parts extract for 2h and 6h. The band with an amplicon length of about 800-bp was less intense in *E. coli* strain treated with 3.5 mg/mL (Figure 1B, lane 7), and disappeared in *E. coli* strain treated with 1.75 mg/mL and 0.875 mg/mL (Figure 1B, lane 8 and 9) aqueous aerial parts extract for 24h. In addition, the band with an amplicon length of about 300-bp was less intense (Figure 1B, lanes 8 and 9) in comparison with the same band appeared in untreated control. ERIC-PCR profiles for *E. coli* strain untreated and treated with different concentrations of ethanolic aerial parts extract of *E. foeminea* at the different time intervals are shown in Figure 1A.

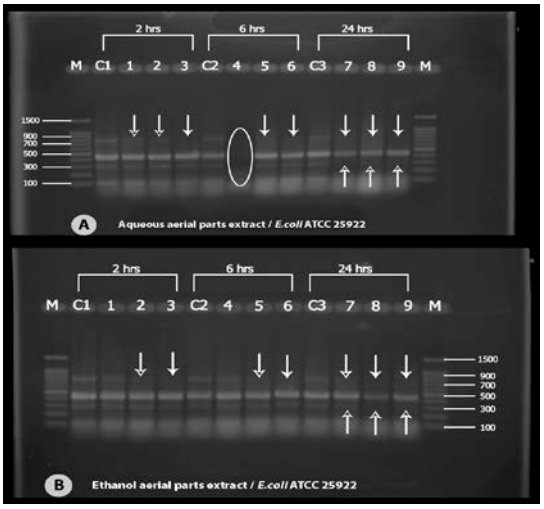

Figure 1. ERIC-PCR profile of *E. coli* strain untreated and treated with different aerial parts extract concentrations. A: aqueous aerial parts extract, B: ethanolic aerial parts extract of *E. foeminea* at different time intervals. Lanes C1, C2 and C3 are untreated (negative controls); lanes 1, 4 and 7 treated with 3.5 mg/ml; Lanes 2, 5 and 8 treated with 1.75 mg/ml; Lanes 3, 6 and 9 treated with 0.875 mg/ml of plant extract.

4. Discussion

In the present study broth microdilution method was used to examine the potential antimicrobial activity of both aqueous and ethanolic aerial parts extracts of *E. foeminea* against *E. coli*. The results confirmed that both aqueous and ethanolic aerial parts extracts of *E. foeminea* exhibited antibacterial activity against *E. coli* strain. Antimicrobial activity of some *Ephedra* species has been reported using different types of extracts (Al-Khalil *et al*., 1998; Feresin *et al*., 2001; Cottiglia *et al*., 2005; Parsaeimehr *et al*., 2010; Rustaiyan *et al*., 2011; Dehkordi *et al*., 2015; Dosari *et al*., 2016). According to previously conducted studies, phenolic compounds are the active ingredients of *Ephedra* plant (Dehkordi *et al*., 2015; Dosari *et al*., 2016).

In this study, the potential genotoxic effect of the aqueous and ethanolic aerial parts extracts of *E. foeminea* against *E. coli* was examined using ERIC-PCR technique. Reviewing the scientific literature showed that this study is the first of its kind that studied the genetoxicity of *E. foeminea* extracts on prokaryotes using ERICPCR technique. Besides, many plants were previously tested to detect their genotoxicity potential by different techniques (Basaran *et al*., 1996; Lalrotluanga *et al*., 2011; El-Tarras *et al*., 2013; Hajar and Gumgumjee, 2014; Ciğerci et al., 2016; Abu-Hijleh *et al*., 2018). ERIC-PCR profiles showed significant differences between the treated and untreated *E. coli* strain used in this study. The changes in the treated *E. coli* strain with both aqueous and ethanolic aerial parts extracts included the disappearance of certain bands as well as the change in the band intensity in comparison with untreated control. The changes in the profile of the treated *E. coli* strain in comparison with the untreated control samples could be explained due to the effect of the genotoxic molecules that were present in the plant extracts. These molecules can induce different

changes such as breakdown in DNA strands, point mutations and/or rearrangements in chromosomes. These changes in the DNA might have a potential effect on the primer binding sites and/or inter-priming distances (Abu-Hijleh *et al*., 2018). DNA sequencing or probing and other techniques can help in understanding the proposed mechanisms that lead to such differences in ERIC-PCR profiles (Lalrotluanga et al., 2011). Ma-huang is a traditional Chinese medicinal preparation derived from *Ephedra sinica* Stapf and other *Ephedra* species that are used to treat different diseases. Studies on cytotoxity of the ma-huang extracts showed that, cytotoxicity of all ma-huang extracts could not be totally accounted for by their ephedrine contents, suggesting the presence of other toxins in the extracts which may modify its pharmacological and toxicological activities (Lee *et al*., 2000).

5. Conclusion

The results of this study showed that aqueous and ethanolic aerial parts extracts of *E. foeminea* possess genotoxic and mutagenic potential against *E. coli*. In addition, the results also point out the capability of using *E. foeminea* to treat infections caused by *E. coli*. More studies are recommended to reveal the exact molecules that are responsible for *E. foeminea* genotoxicity as well as the mechanisms responsible for that genotoxicity.

Competing Interests

Authors have declared that no competing interests exist.

References

Abu-Hijleh A, Adwan G and Abdat W. 2018. Biochemical and molecular evaluation of the plant *Ecballium elaterium* extract effects on *Escherichia coli*. *J Adv Biol Biotechnol*., **9** (**2**): 1-11.

Adwan G, Adwan K, Jarrar N, Salama Y and Barakat A. 2013. Prevalence of *seg*, *seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates. *Br Microbiol Res J.*, **3**(**2**):139-149.

Al-Khalil S, Alkofahi A, El-Eisawi D and Al-Shibib A. 1998. Transtorine, a new quinoline alkaloid from Ephedra transitoria. *J Nat Prod*., **61**(**2**):262-263.

Al-Rimawi F, Abu-Lafi S, Abbadi J, Alamarneh AAA, Sawahreh RA and Odeh I. 2017. Analysis of phenolic and flavonoids of wild *Ephedra alata* plant extracts by LC/PDA and LC/MS and their antioxidant activity. *Afr J Tradit Complement Altern Med*., **14**(**2**): 130-141.

Amakura Y, Yoshimura M, Yamakami S, Yoshida T, Wakana D, Hyuga M, Hyuga S, Hanawa T and Goda Y.2013. Characterization of phenolic constituents from ephedra herb extract. *Molecules*, **18**(**5**):5326-5334.

Andrews, J. M. 2006. BSAC standardized disc susceptibility testing method (version 5). *J Antimicrob Chemother*., **58**(**3**): 511-529.

Atienzar FA, Venier P, Jha AN and Depledge MH. 2002. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutation Research/Genetic Toxicol and Environ Mutagen*., **521**(**1-2**): 151-163.

Basaran AA, Yu TW, Plewa MJ and Anderson D. 1996. An investigation of some Turkish herbal medicines in *Salmonella typhimurium* and in the COMET assay in human lymphocytes. *Teratogen, Carcinogen, and Mutagen*., **16**(**2**):125-138.

Caveney S, Charlet DA, Freitag H, Maier-Stolte M and Starratt AN. 2001. New observations on the secondary chemistry of world Ephedra (Ephedraceae). *Am J Bot*., **88**(**7**):1199-1208.

Ciğerci IH., Cenkci S, Kargıoğlu M and Konuk M. 2016. Genotoxicity of *Thermopsis turcica* on *Allium cepa L.* roots revealed by alkaline comet and random amplified polymorphic DNA assays. *Cytotechnol*., **68**(**4**):829-838.

Clinical and Laboratory Standards Institute (CLSI). 2017. **Performance Standards for Antimicrobial Susceptibility Testing**. 27th ed. CLSI supplement. M100S. Wayne, PA, USA.

Cottiglia F, Bonsignore L, Casu L, Deidda D, Pompei R, Casu M and Floris C. 2005. Phenolic constituents from *Ephedra nebrodensis*. *Nat Prod Res*., **19**(**2**):117-23.

Danin A. 2018. Flora of Israel on line. http://flora.huji.ac.il/browse asp. Accessed (Dec. 10, 2018).

Dehkordi NV, Kachouie MA, Pirbalouti AG, Malekpoor F and Rabei M. 2015.Total phenolic content, antioxidant and antibacterial activities of the extract of *Ephedra procera* fisch. et mey. *Acta Pol Pharm*., **72**(**2**):341-345.

Dosari AS, Amin Norouzi A, Moghadam MT and Satarzadeh N. 2016. Antimicrobial activity *of Ephedra pachyclada* methanol extract on some enteric gram negative bacteria which causes nosocomial infections by agar dilution method. *Zahedan J Res Med Sci.*, **18**(**11**):1-4.

El-Tarras AA, Hassan MM and El-Awady MA. 2013. Evaluation of the genetic effects of the *in vitro* antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning electron microscope. *Afr J Biotechnol*., **12**(**21**):3171-3180.

Eberhardt MV, Lee CY and Liu RH 2000. Antioxidant activity of fresh apples. *Nature* **405**:903–904.

Feresin GE, Tapia A, López SN and Zacchino SA. 2001. Antimicrobial activity of plants used in traditional medicine of San Juan province, Argentine. *J Ethnopharmacol.*, **78**(**1**):103-107.

Hajar AS and Gumgumjee NM. 2014. Antimicrobial activities and evaluation of genetic effects of *Moringa peregrina* (forsk) fiori using molecular techniques. *Inter J Plant and Animal Environ Sci.*, **4**(**1**):65-72.

Ibragic S and Sofić E. 2015. Chemical composition of various *Ephedra* species. *Bosn J Basic Med Sci.*, **15**(**3**): 21-27.

Lalrotluanga, Kumar NS and Gurusubramanian G. 2011. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage in mosquito larvae treated with plant extracts. *Science Vision*, **11**(**3**): 155-158.

Lee MK, Cheng BW, Che CT and Hsieh DP. 2000. Cyto-toxicity assessment of Ma-huang (*Ephedra*) under different conditions of preparation. *Toxicol Sci*., **56**(**2**):424-430.

Mendelovich M, Shoshan M, Fridlender M, Mazuz M, Namder D, Nallathambi R, Selvaraj G, Kumari P, Ion A, Wininger S, Nasser A, Samara M, Sharvit Y, Kapulnik Y, Dudai N and Koltai H. 2017. Effect of *Ephedra foeminea* active compounds on cell viability and actin structures in cancer cell lines. *J Med Plants Res.*, **11**(**43**): 690-702.

O'Dowd NA, McCauley G, Wilson JAN, Parnell TAK and Kavanaugh D. 1998. *In vitro* culture, micropropagation and the production of ephedrine and other alkaloids. *In*: **Biotechnology in Agriculture and Forestry**; Bajaj YPS. (Ed.), p. 41, Springer, Berlin.

Parsaeimehr A, Sargsyan E and Javidnia K. 2010. A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of *Ephedra*. *Molecules*, **15**(**3**): 1668-1678.

Pirbalouti AG, Azizi S, Amirmohammadi M and Craker L. 2013. Healing effect of hydro-alcoholic extract of *Ephedra pachyclada* Boiss. in experimentally gastric ulcer in rat. *Acta Pol Pharm*., **70** (**6**):1003-1009.

Rustaiyan A, Javidnia K, Farjam MH, Aboee-Mehrizi F and Ezzatzadeh E. 2011. Antimicrobial and antioxidant activity of the *Ephedra sarcocarpa* growing in Iran. *J Med Plants Res.,* **5** (**17**):4251-4255.