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Original article

The dual impact of Jordanian *Ephedra alte* for inhibiting pepsin and treating microbial infections

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ABSTRACT

Screening of phytochemical *Ephedra alte* crude extract by GC–MS and HPLC analysis indicated the presence of alkaloids, tannins, flavonoids, terpenoids, and phenolic acid in the extract. The total phenolic content of *E. alte* methanol extract was 39.43 mg of Gallic acid eq/g, crude *E. alte* with 56.74, and 2.42 μg Trolox equivalent antioxidant capacity (TEAC)/g of plant extract according to DPPH and FRAP assay, respectively. The antimicrobial activity of *E. alte* against *Staphylococcus aureus, staphylococcus epidermidis, Escherichia coli,* and *Klebsiellaoxytoca* demonstrated a mean zone diameter of inhibition ranging from 0 to 17 mm. The MIC of the extracts ranged from 0.5 to 1.0 mg/mL. *E. alte* extract inhibits pepsin enzyme activity with IC50 values of 213.67 μg/ml. This study revealed that *E. alte* extract has pepsin enzyme inhibitory, antibacterial, antioxidant activities. The current outcomes indicate that *E. alte* might be employed as a natural agent for managing GERD and infectious diseases.

1. Introduction

Pepsin is a gastric enzyme secreted by the chief cells as the precursor pepsinogen, and it is considered the vital digestive enzyme which plays an essential role in peptic ulcer. Moreover, reflux disease is a widespread condition involved in the ulceration of esophageal tissues and known as Gastroesophageal reflux disease (GERD) (Lenham et al., 2019), which usually triggers inflammation and oxidative stress (Kwon et al., 2016). The reflux contains pepsin, HCl pancreatic enzymes, and bile acids. Recent studies showed that pepsin is the responsible element accused of inducing tissue deterioration (Lenham et al., 2019). On the other hand, it was reported that microbial infections stimulate intestinal epithelia to generate ROS, which in turn increases the oxidative stress (Kumar et al., 2007). Therefore, the antioxidant activity of natural compounds that possess an antimicrobial effect is highly recommended in treating infectious diseases. Many medicinal plants contain bioactive compounds exhibiting free radicals scavenging, such as phenolic compounds, vitamins, terpenoids and other endogenous metabolites. These secondary metabolites play an important role in antioxidant activity and significantly suppress pathogen (Al-Mustafa and Al-Thunibat, 2008, Al-Rimawi et al., 2018, Al-Zereini et al., 2018, Karadeniz et al., 2015, Prakash and Gupta, 2009).

Ephedra Pachyclada Boiss is a folk medicinal plant that belongs to the Ephedraceae family. It is known in folk knowledge against gastric disorders in ethnopharmacological inventories of the Kerman tribal in central Iran (Pirbalouti et al., 2013). This effect was tested by using the hydroalcoholic extract of the plant in experimentally healing rat ulcers. The histo-pathological analysis results indicated that the extract accelerates ulcer healing in rats. Such

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finding supports the role of using this plant as a folk medicinal plant by Kerman people (Konar and Singh, 1979, Sharififar et al., 2010). The leaves extract of this plant inhibited *B. subtilis*, *S. aureus* and *B. cereus*, while the flower extracts were reported to be effective against most types of these bacterial strains (Chebouat et al., 2014).

Based on the best of our knowledge, no documented reports were published on the effect of methanolic extract of Jordanian *Ephedra alte* on gastric ulcers. *Ephedra alte* belongs to the plant family *Ephedraceae* (Schaneberg et al., 2003). *Ephedra* plants are classified as *E. MajorHost*, *Ephedra sinica*, *E. gerardiana Wall*, *E. equisetina Bunge*, *E. Distachya*, and *E. intermedia* (Morton, 1978). These plants are known as evolutionarily primitive plants and are found in dry, sandy, or rocky environments (Blumenthal and King, 1995). *Ephedra* species is recognized worldwide for its high ecological, economic, and medicinal value. In Jordan, four *Ephedra L.* species have been reported. These species include *Ephedra foliate Boiss*, *Ephedra alata Decne*, *Ephedra alte*, and *Ephedra transitoria Riedl* (Post, 1932, Taifour and El-Oqlah, 2014).

Ephedra species have been used in folk medicine to treat asthma, nasal congestion, cough, and dietary supplements for weight loss and athletics. *Ephedra* species contain Ephedrine- alkaloids (ephedrine and pseudoephedrine) as a significant active gradient (Huang et al., 2020). *Ephedra* herb has anti-inflammatory (Hikino et al., 1980), analgesic, anti-influenza (Mantani et al., 1999), and anti-metastatic effects (Hyuga et al,. 2011). In *Ephedra,* the phytochemical content varies with plant parts (Liu et al., 1993), plant varieties and species (Cui et al., 1991), seasons of harvest, and geographical origins (Zhang et al., 1989). In addition that, *Ephedra* contains other components that might improve its toxicological and pharmacological activities, such as phytoconstituents. Most of the important bioactive compounds are extracted from plants (Ziani et al., 2019). Many of the available drugs have been derived directly or indirectly from medicinal plants. These compounds and their bioactive properties such as antimicrobial and antioxidant *in vitro,* have been studied *in vitro* (Nakatani, 2000, Saad et al., 2008, Silvia Martins et al., 2013, Yanishlieva et al., 2006). The bioactive compounds that have antioxidant and antimicrobial activity are mainly due to their capacity to chelate metals, redox ability, and reactivity as quenching species of mono oxygen (Djeridane et al., 2006, Ghanem and El-Magly, 2008, Krishnaiah et al., 2011). In addition to these properties, medicinal plants were used as a traditional method for many years to deal with health disorders and prohibit diseases. The irrational use of antibiotics led to the development of several bacterial strains with multi-drug resistance. It also makes an immense clinical problem in treating infectious diseases caused by *Escherichia coli* and *Klebsiella pneumonia* (Ahmad and Beg, 2001, Manges et al., 2006, Webster et al., 2011) and others. Therefore, there is a need to search for the novel antimicrobial drug. The binding of bioactive molecules with protein will change their bioactivity (Blois, 1958).

Until now, there is no biological activity study of the Jordanian *Ephedra* plant and this fact motivated us to study and get more insight into the antibacterial, antioxidant and pepsin activity of this plant. Observing the biological activity of *Ephedra* on human health will give a good picture of their combination and interaction with pepsin (Monogioudi et al., 2011). Hence, this work investigates the inhibitory effect of methanol extract of *E. alte* for pepsin enzyme and its potential as an antimicrobial agent. In addition, we aimed to evaluate the bioactivity and using GC–MS, and HPLC- PDA to analyze the chemical compositions of *E. alte* as a Jordanian medicinal plant used as a folk medicine in treating various diseases.

2. Materials and methods

2.1. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchromane-2- carboxilic acid (Trolox) were purchased from Merck (Germany). Pepsin, bovine albumin, and 2, 4, 6 - tri (2-pyridyl) 2, s-trizine (TPTZ) were obtained from Sigma-Aldrich Chemical Company (USA). All other chemicals and solvents used in this study were of analytical grades.

2.2. Plant

E. alte was collected from a local region in Al-Tafila, Jordan, in the spring of 2017. It belongs to the *Ephedraceae* family with *the Ephedra L.* genus (Taifour and El-Oqlah, 2014). The aerial part of the *E. alte* plant was identified by Prof. Al-Qur'an S., Biological Sciences Department, Mu'tah University, Jordan.

2.3. Plant processing and extraction

A blender grounded the dried plant materials into a fine powder and 25 g were soaked in 250 ml methanol solution and kept in the shaker at 150 rpm, in a dark place for three days at room temperature according to Cichewicz and Thorpe (1996) (Cichewicz and Thorpe, 1996). The mixtures were then filtrated using a Buchner funnel under a vacuum. The filtrate was centrifuged at 3000 rpm for 10 min. The extracts were concentrated in rotary evaporator under reduced pressure at 55 °C. The extracts were left in open vials in the fume hood for three days at room temperature. A resulting extract was stored in the refrigerator at 4 °C in a container until use.

2.4. Determination of yield extract (%)

The % yield (w/w) from the dried extract was calculated as follows:

Yield (%) = (W1 * 100)/W2, where W1 is the weight of the extract after evaporation of solvent, and W2 is the weight of the plant powder (Díaz Dellavalle et al., 2011).

2.5. Antibacterial activities

Antibacterial activities of *E. alte* extract were determined by agar diffusion test according to the standard methods followed in Clinical and Laboratory Standards Institute and as described by Bauer et al. (1996). A bacterial test was performed on four bacteria strains. Bacterial strains were *Staphylococcus aureus* ATCC43300, *Staphylococcus epidermidis* ATCC12228, *Escherichia coli* ATCC25922, and *Klebsiellaoxytoca* SLK089. Different concentrations (1 and 2 mg/disc) of tested extract were applied on a 6-mm blank filter disk that placed on the top of Muller Hinton Agar plates seeded with bacterial test strains at a cell density of 106 cell/ml. The test was performed in triplicate. Streptomycin (10 μg) and Tetracycline (30 μg) discs were used as positive controls and 10% DMSO was used as a negative control. Growth inhibitory activity was calculated by measuring the diameter of the clear zone around the disc using a ruler.

2.6. Determination of minimum inhibitory concentration (MIC)

The MIC was determined according to Patel et al., (2011) (Author: Mitesh H. Patel, 2011). 2 × 106 cell/ml was inoculated into tubes of 5 ml Muller-Hinton broth containing a serial dilutions

of plant extract (0, 62.5, 125, 250, 500, 1000, 2000 μg/ml). Cultures were incubated at 37 °C for 24 hrs.

2.7. Estimation of total phenols

The total phenolic content was measured according to Singleton and Rossi (1965) (Singleton and Rossi, 1965). Briefly, a reaction mixture of 0.2 ml of crude extract (5 mg/ml), 1 ml of diluted Folin-Ciocalteu'sreagent and 0.8 ml NaHCO3 (7.5%) was incubated for 45 min at 45 °C. The Gallic acid (GA) compound was applied as a standard and total phenol was calculated in terms of gallic acid equivalents (mg of GA/g of extract).

2.8. Phytochemical screening

E. alte extract was screened for the presence of alkaloids, flavonoids, saponins, terpenoid as described by Kokate CK (2014). Tannins were tested as shown by Sofowara (1993), glycosides and sterols were assayed according to Trease & Evans (1989).

2.9. 2, 2-Diphenyl-1-picrylhydrazyl -DPPH- radical scavenging method

The total antioxidant activity of *E. alte* extract was assayed using DPPH, as described by Tepeet al. (2005). Aliquots of various concentrations of the extract (0–2000 μg/ml) were added to 1 ml of 0.004% methanol solution of DPPH. Samples were incubated for 30 min at room temperature; after that absorbance was measured at 517 nm. All determinations were done in triplicate. The inhibition of free radical scavenging activity was expressed by the following equation:

$$Inhibition\ (\%) = 100\ \times (Abs1 - - Abs2)/Abs1.$$

where, Abs1 is the absorbance of the negative control which is a solution of 100 μl methanol 95% and Abs2 is the absorbance of the sample or the positive control. Extract concentration providing 50% inhibition (IC50) was determined from a graph plotting percentage inhibition against extract concentration. Trolox was used as a standard, in the concentration range of 0–100 μg to construct a calibration curve and DPPH radical- scavenging activities were expressed as mg Trolox® equivalents per Gram of plant extract (Chung et al., 2002). Trolox® (final concentration 0–1 μg/mL) was used as a standard for the construction of the calibration curve.

2.10. Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out according to the method described by Benzie and Strain (1996). The FRAP assay is based on the ability of antioxidants to decrease ferric ions to Fe^{+2} ions. The FRAP reagent was freshly prepared by mixing 10 mM TPTZ (1 ml) and 20 mM ferric chloride (1 ml) in 0.25 M acetate buffer (10 ml, pH 3.6). Plant extract sample (50 μl) was added to 3 ml of the FRAP reagent (The final concentration of the plant extract in the solution was 100 μg/ml). The tests were carried out in triplicate. The absorbance was measured at 593 nm after 8 min incubation at room temperature. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as mg Trolox® equivalents per g of plant extract (final concentration 0–1 μg/mL was used as a standard for the construction of the calibration curve).

2.11. Thin-layer chromatography (TLC)

TLC was carried out with the crude extract on aluminum plates impregnated with silica gel 60 (20 × 20 cm) using toluene: acetone: formic acid (70:30:1) as mobile phase. The resulting bands were detected with UV light lamps. Silica gel 60 (20 × 20 cm) plates are sheets of glasses coated with a thin layer of a solid adsorbent (silica). A small amount of the crude extract is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent - toluene: acetone: formic acid (70:30:1) in a developing chamber. When the solvent has reached the top of the plate, the plate is removed, dried, and the separated components of the mixture are visualized by using 254–366 nm UV lamp.

2.12. Estimation of pepsin activity

The modification of Kalra et al. (2001) method was used to estimate the pepsin activity by methanolic *E. alte* extract. In this experiment, two test tubes were used and placed in an ice bath. The first test tube containing: 0.3 g of pepsin was added to 1 ml of bovine albumin (0.5% w/v in 0.01 N HCl, pH 2), the second test tube containing: 0.5 g of bovine albumin (0.5% w/v in 0.01 N HCl, pH 2) as a background control tube. The test tubes were kept in a water bath at 37 °C for 20 min. After that, 2 ml of 10% trichloroacetic acid was added to each test tube to stop hydrolysis. The denaturation step took place by heating all tubes in boiling water for 5 min. The precipitate in each tube was uptake by centrifugation (5000 × g for 10 min). A 1 ml of the supernatant ofeach tube was mixed with 0.4 ml of 2.5 N NaOH and 0.1 ml of Folin- Ciocalteu reagent and the volume was added to 10 ml with distilled water. The absorbance of each tube was measured at 700 nm. All the experiments were done in triplicate. The same procedure was repeated using different crude concentrations (0, 10, 20, 50, 100, 200, 400 μg/ml) to calculate the IC50 pepsin. The percent inhibition of pepsin by methanolic *E.* extract is calculated as:

$$Inhibition\ (\%) = [(A_{Negative\ control} - - A_{Sample})/A_{Negative\ control}] \times 100\%$$

where A is absorbance and the result is recorded as IC50.

2.13. HPLC system and chromatographic conditions

HPLC is a Waters Alliance instrument (e2695 separations module), it is equipped with 2998 Photo diode Array detector (PDA). Data acquisition and the control were carried out by Empower 3 chromatography data bank (Waters, Germany). HPLC analytical experiments of methanolic extracts of the three aerial samples were run by using ODS column of Waters (XBridge, 4.6 ID × 150 mm, 5 μm) in addition to guard column of Xbridge ODS, 20 mm × 4.6 mm ID, 5 μm. A mixture of acidic water (0.5% acetic acid) (Solvent A) was used as mobile phase and the acetonitrile (solvent B) ran in a linear mode of gradient. In addition, solvent A descended from 100% to 70% in 40 min. After that, 40% in 20 min then 10% in 2 min; it stayed there for 6 min and after that back to the basic conditions in 2 min. The HPLC system was calibrated for 7 min by using the initial acidic water as a mobile phase (solvent A) before adding the next sample of extract. The crude sample was filtered using a 0.45 μm of PTFE filter. The wavelength range of PDA was from 210 to 500 nm. The applied flow rate was 1 ml/min. The injection volume of sample was 20 μl and the temperature of column was set at 25 °C.

2.14. GC–MS analysis

The GC was Agilent technology type 6890 GC equipped with split-splitless injector and HP- 5MS capillary column coated with a film of 5% phenylmethylpolysiloxane (30 m × 0.25 mm, 0.25 μm of film thickness). This agilent 6890 GC is equipped with a mass spectrometer type 5973C Inert MSD (Mass Spec, Mass Spectrometer, Mass Selective Detector, MS, GC–MS). The column oven temperature was programmed as follows: start temperature at

60 °C, increased to 300 °C with a ramp of 15 °C/min, the temperature was held at 300 °C for 7 min until elution was complete. After 15 s the split valves were opened for 3 min to purge the injector. All injections (1 μl) were made with a 10 μl syringe. Helium gas (purity of 99.999%) was used as the carrier gas at a flow rate of 1.0 ml/min (Krafczyk and Glomb, 2008).

Method: 1 g of powdered *E. alte* was soaked in 10 ml of methanol with continuous shaking at room temperature for 4 days. The filtrate was centrifuged at 4500 rpm for 5 min. Then, 3.0 ml of the supernatant was transferred to a test tube and evaporated at 25 °C. The residue was reconstituted with 100 μl of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) derivatization solution and injected in GC/MS.

3. Statistical analysis

All data analyses were expressed by the Microsoft excel 2010. Results were reported as mean ± standard deviation (SD).

4. Results

4.1. Extraction yield and free radical scavenging activity of E. alte

The extraction yield (%) of *E. alte* methanolic extract was 11.6% (w/w) and is shown in Table 1. To explore the antioxidant potential of the *E. alte*, the extract was analyzed for its capacity to scavenge oxidative radicals. A Trolox standard curve was established using eight concentrations of Trolox standard solution (0–100 μg/ml). The *E. alte* DPPH radical scavenging potential and FRAP were assessed in contrast to the positive control (Trolox) and expressed as TEAC [mg Trolox/g of plant extract]. The TEAC for the *E. alte* extract was found to be 56.74 μg/ml and IC50 was 13.2 mg/ml for the *Ephedra* plant by using DPPH. Similarly, with respect to FRAP radical scavenging activity, *Ephedra* extract had 2.42 TEAC (μg Trolox/g of plant extract) (Table 1).

4.2. Antimicrobial activity of E. alte

The antibacterial activity of *E. alte* might be attributed to the presence of metabolic natural products such as toxins or broad-spectrum antimicrobial compounds that can act against both Gram-positive and Gram-negative bacteria (Al-Rimawi et al., 2017). The results of antibacterial activity of methanolic extract of *E. alte* against various human pathogens are listed in the Table 2. The MIC of methanolic extract that inhibited Escherichia coli and Klebsiella oxytoca was 1000 μg/ml; however, the MIC inhibits *Staphylococcus epidermidis* and *Staphylococcus aureus* were 500 μg/ml (Table 2).

4.3. Pepsin percentage of inhibition

Methanol extract of E. alte inhibited pepsin with IC_{50} value of 213.7 μg/ml, as shown in table 1. The kinetic results of the enzyme by the Line weaver-Burk plot of 1/velocity versus 1/[albumin as substrate] in the presence or absence of *E. alte* extract showed that both V_{max} and K_m changed with increasing concentration. The K_m was changed from 18.38 to 23.1 and V_{max} was changed from 292.7 to 190.5. This behavior indicates that *E. alte* extract inhibits the enzyme in a mixed- noncompetitive manner (Fig. 1).

4.4. Preliminary phytochemical screening (qualitative analysis)

The phytochemical analysis conducted on *E. alte* extract revealed the presence of tannins, terpenoids, flavonoids and alkaloids as shown in Table 3.

4.5. Fractionation of Ephedra extract by thin-layer chromatography (TLC)

The methanol crude extract of *Ephedra* gave nine fractions using TLC method. These fractions were tested for their antibacterial activity against bacterial strains and only three fractions showed antibacterial activity (Table 4), Fig. 2 shows the fraction on thin-layer chromatography. Fraction 2 gave (10, 11, 9 and 8) mm inhibition zones on bacteria (*S. aureus*, *S. epidermis*, *E. coli* and *K. oxytoca*) respectively. Fraction 8 gave (10, 12, 9 and 10) mm inhibition zones on bacteria (*S. aureus*, *S. epidermis*, *E. coli* and *K . oxytoca*) respectively. Finally, fraction 9 gave (11, 12, 10 and 9) mm inhibition zones on bacteria (*S. aureus*, *S. epidermis*, *E. coli* and *K. oxytoca*), respectively.

4.6. HPLC- PDA profile of the extract

Fig. 3 shows the chromatogram of the crude extract of *E. alte* at 350 nm. The major eluted compounds were detected in the range of 2–28 min. Four minor peaks were detected in the range of 46–68 min indicating nonpolar phytochemicals. The UV–Vis maximum absorptions were in the range of 210–350 nm. The major peaks eluted at 9.2 and 17.2 min share a similar UV–Vis spectrum with two λmax of about 254, 255 and 346, 349 nm respectively.

4.7. GC–MS analysis of the extract

The extracted phytoconstituents of *E. alte* was investigated by GC–MS. Each constituent in the methanolic extract was quantified and identified by comparing mass fragmentation patterns with standards like Wiley 9 library spectral data and NIST.

5. Discussion

Traditionally, *E. alte* medicinal plant has a strong reputation for treating many diseases and is used as an input of different herbal formulas to treat cough, influenza and skin disorder. Because of the rare occurrence of adverse effects and increasing multiple pharmacological effects such as anti-inflammatory, antimicrobial and antioxidant, its clinical application has increased worldwide. The results showed that *Ephedra* extract investigated in this study is richer in phenolic compounds than that of plum fruits (Thaipong et al., 2006).

Ephedra, as one of the plants, contain phenolics, quench and scavenging of free radicals. Thereafter, the obtained standardized extract was evaluated for their potential antibacterial, antioxidant activity as well as pepsin inhibitory activities. Starting with pepsin which contributes in various physiological functions, such as upper airway, oropharynx, hypopharyngeal, peptic ulcer and gastro-esophageal reflux disease where it could cause injury to the esophagus (Yue et al., 2017). Several published works have been investigated the effect of different natural extracts on the pepsin enzyme activity (Rege and Chowdhary, 2014a,b, Kalra et al., 2011, Moradi et al., 2013). In this study, the methanolic extract of *Ephedra* was examined against pepsin activity for the first time, and the experimental results described here evidently showed that the methanolic extract of *Ephedra* significantly inhibits pepsin activity more than other alcoholic plants extracts such as *Phyllanthus amarus* leaves (Abdulla et al., 2010). It was demonstrated that polyphenols exhibit inhibitory effects on pepsin (He et al., 2006).

As the current data revealed that *E. alte* has excellent contents of polyphenolic compounds and tannins compared to other plants (Abdulla et al., 2010). Therefore, the inhibitory effect on pepsin might be attributed to the potential effect of polyphenols content in the methanolic extract of *Ephedra.* The natural compounds have

Table 1
Percentage extraction yield, total phenol and IC50 values of methanolic plant extracts for DPPH scavenging and FRAP assays.

Scientific name	*Yield (%) extract	Total phenol (mg GAE/g plant extract)	**DPPH scavenging activity (IC50) (μg TEAC/ml)	**FRAP activity (IC50) (μg TEAC/ml)	Pepsin % of inhibition (IC50 μg/ml)
E. alte	11.60	39.43	56.74	2.42	213.67

Trolox equivalent/ml of plant extract.
* Percentage extraction yield (%) is expressed as w/w g of dried extract.
** DPPH radical scavenging activity and FRAP activity of extract is expressed as μg.

Table 2
Antibacterial activities of methanolic extracts of *E. alte*.

Bacteria	*Zone of inhibition (mm)			
	500 μg/ml	1000 μg/ml	2000 μg/ml	MIC (μg/ml)
Escherichia coli	05 ± 0.4	09 ± 0.5	14 ± 0.3	1000
Staphylococcus aureus	09 ± 0.4	12 ± 0.6	17 ± 0.5	500
Staphylococcus epidermidis	06 ± 0.5	10 ± 0.4	15 ± 0.4	500
Klebsiellaoxytoca	07 ± 0.5	10 ± 0.5	15 ± 0.4	1000

* Three different experiments measuring the zone of inhibition (mm).

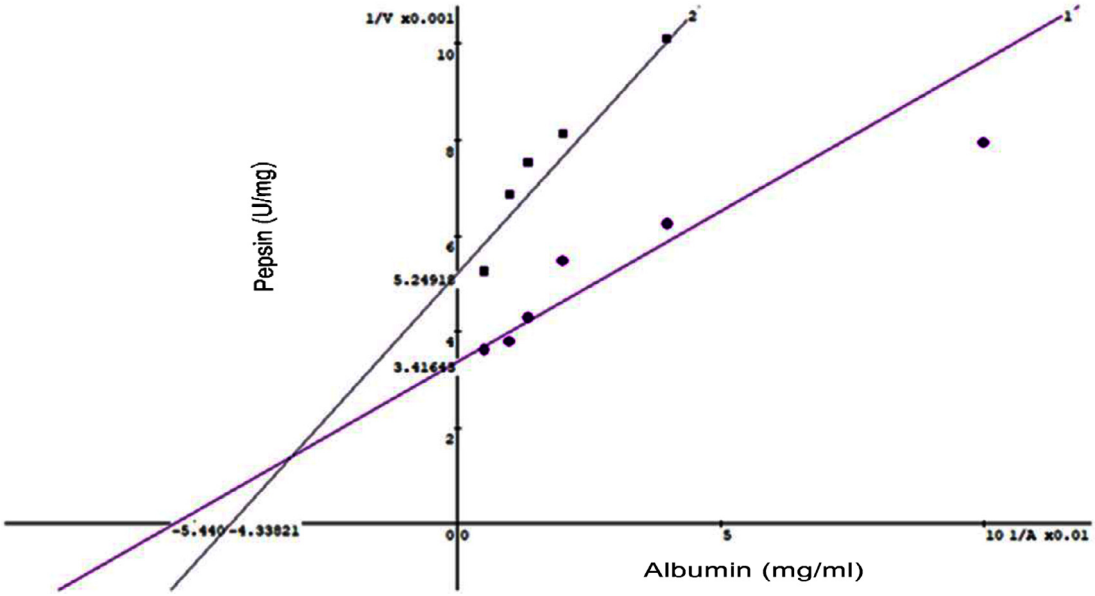

Fig. 1. Line weaver–Burk plots for inhibition of pepsin in the *E. alte* extract.

Table 3
Preliminary phytochemical screening of *E. alte* extract.

Chemical compounds	Results
Tannins	+
Terpenoids	+
Flavonoids	+
Sterols	–
Alkaloids	+

+: compound detected, –: compound not detected.

been proved to possess cytoprotective properties (Gonzales et al., 2000) and have antiulcer potential and putative HIV-protease inhibitor (Rege and Chowdhary, 2014a,b) due to their antioxidants activity. Moreover, these inhibitory effects on pepsin could be also related to the flavonoids, according to earlier research that reported the inhibitory effect of flavonoid compounds on pepsin utilizing molecular docking and spectroscopic methods (Zeng et al., 2015). Furthermore, α-Tocopherol which was detected in the methanolic extract of *Ephedra* was previously found to possess an inhibitory effect on pepsin (Rao and Vijayakumar, 2008).

Ephedraceae family plants are characterized by alkaloids of the ephedrine series. The antibacterial activities of these plants were reported in several articles based on the origin of plants (Al-Snafi, 2017, Yousif et al., 2012). In Jordan, six crude extracts of *E. alata* (butanol, ethanol, dichloromethane extract of flowers and leaves) were tested against several Gram+ and Gram−. The results proved antimicrobial activities of *E. alata* due to the sites and number of —OH groups of the *Ephedra* phenolic compounds that are thought to be related to the toxicity against bacteria.

The methanol was used in this study to extract most of the plant materials which might be work as biological active compounds. *E. alte* showed antibacterial activity against both Gram+ and Gram− bacteria. The antibacterial activities of methanol extract of this plant were used against two positive bacteria: *S. aureus* and *S. epidermidis*, and two gram - bacteria: *E. coli* and *K. oxytoca*. The results were observed using well diffusion method by measuring the

Table 4
Antibacterial activity of *E. alte* extract fractions.

Bands number	Diameters of inhibition zones (in mm) Bacteria			
	S. aureus	*S. epidermis*	*E. coli*	*K. oxytoca*
Band 1	na	na	na	na
Band 2	10	11	9	8
Band 3	na	na	na	na
Band 4	na	na	na	na
Band 5	na	na	na	na
Band 6	na	na	na	na
Band 7	na	na	na	na
Band 8	10	12	9	10
Band 9	11	12	10	9

na: no activity.

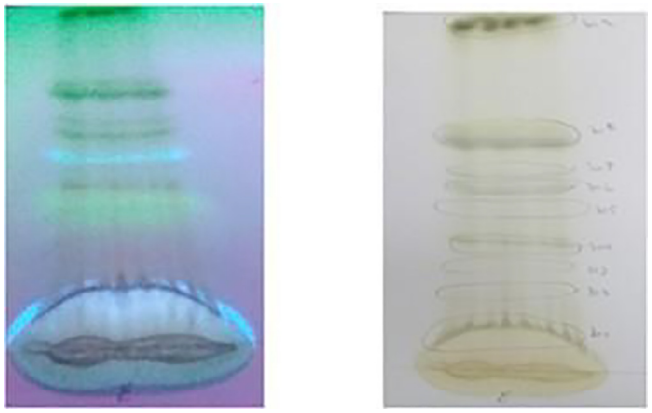

Fig. 2. Fractionation of *Ephedra* extract by TLC.

diameter of the growth inhibition zone. The inhibitory zone increased in a dose-dependent manner. The results of the inhibition zone of test organisms were in complete accordance but they showed different values of MICs for the plant extract. MIC values of methanolic extract were the same against *S. aureus* and *S. epidermidis*, i.e. the growth of these microorganisms was inhibited at a low concentration of the extract (500 µg/ml), while *E. coli* and *K. oxytoca* were inhibited at higher concentration (1000 µg/ml) as shown in Table 2.

This study showed that Gram− bacteria had less susceptibility to the plant extract than Gram+ bacteria. It has been previously shown that Gram− bacteria are commonly more resistant to common antibiotics than Gram+ ones (Cos et al., 2006). Moreover, Gram+ bacteria were found to have more susceptibility as compared to Gram− bacterial species. This is in agreement with earlier studies which attributed this to the differences in chemical composition and structure of cell wall of both types of microorganisms.

(Tarawneh et al., 2010, Yaghoubi et al., 2007). According to the table (4), fractions 2, 8 and 9 from plant *Ephedra* extract exhibited antibacterial activity via bacterial strain (*S. aureus*, *S. epidermidis*, *E. coli* and *K. oxytoca*).

Many researchers investigate the antioxidant and bioactivity potentials of plant extracts that are attributed to the presence of different classes of secondary metabolites such as polyphenols, alkaloids, flavonoids, quinines, lignins, terpenoids, carotenoids, vitamins and many others (Mishra et al., 2010; Wong and Kitts, 2006). Furthermore, some flavonoids exhibit antibacterial activity against both Gram+ and Gram− species by disrupting the

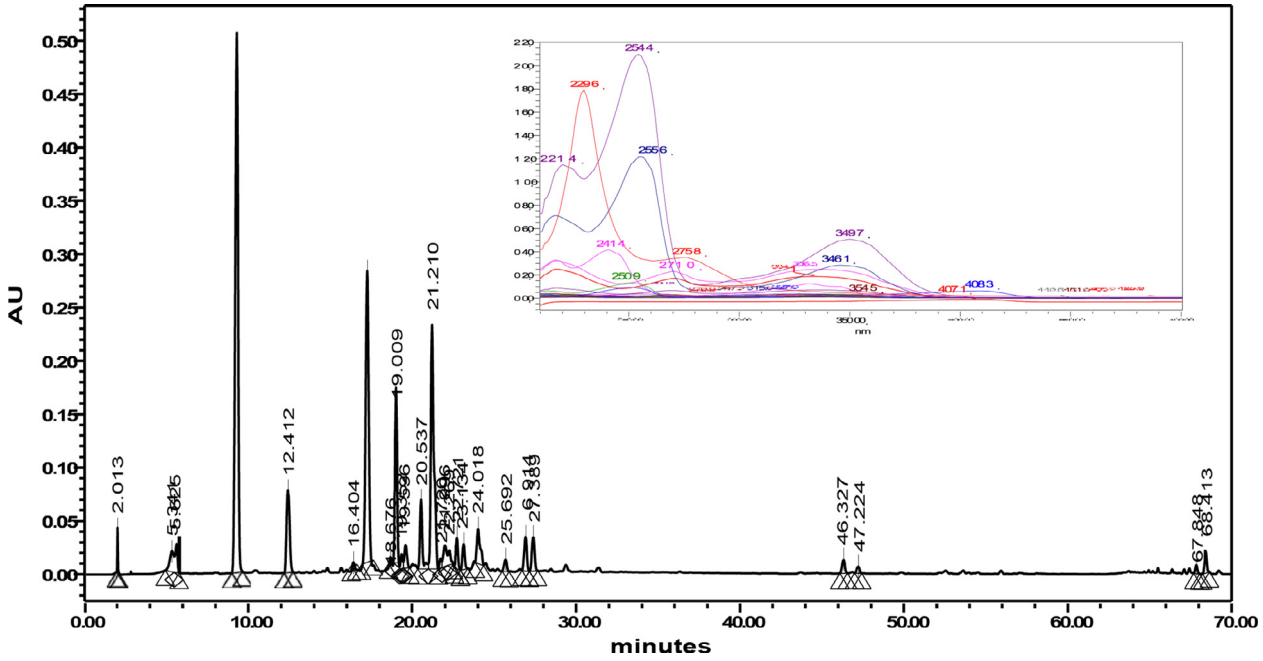

Fig. 3. Typical HPLC-PDA chromatogram and overlaid UV–Vis spectra of crude extract of *E. alte* at 350 nm.

replication of bacterial DNA (Manderfeld et al., 1997), so it can explain the potential activity of methanolic extract of *Ephedra in* inhibiting the growth of both Gram− and Gram+ bacteria.

Oxidative stress occurs when the production of oxidants or reactive oxygen species (ROS) exceeds local antioxidant capacity and it is thought to be associated with the pathogenesis of many diseases including diabetes mellitus (Evans et al., 2002). Antioxidants are molecules that decelerate or quench free radical reactions and hence, delay or prevent cellular damage (Ning et al., 2012). Medicinal plants are rich sources of secondary metabolites that act as natural antioxidants such as phenolic compounds (cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins), ascorbic acid and carotenoids (Foti et al., 1996, Hollman, 2001). There are many kinds of antioxidant assays that can be used to evaluate the antioxidant activity of medicinal plant extracts. Most of these methods depend on either measuring the potential of the plant to reduce oxidants such as FRAP assay or to scavenge free radicals such as DPPH (Al-Fatimi et al., 2007, Atta ur et al., 2002). The *Ephedra* plant has an antioxidant activity band on the FRAP and DPPH assay (Ahmadi et al., 2011). The % of inhibition of DPPH at different concentrations of crude extract was found to be dose-dependent. The result of methanol crude extract yield of *Ephedra* was 11.6%.

The result proved that the plant extract has high antioxidant properties due to the high total phenols and other phytochemicals. Such phenolic compounds were reported by many studies to be potent antioxidants and radical scavenging agents (Kähkönen et al., 1999). The antioxidants compounds are capable of donating a single electron or H atom for reduction. In this study, the compounds present in the methanolic plant extract capable of scavenging DPPH radicals and were also able to reduce ferric ions.

In addition, the ferric ion reducing ability of antioxidants correlates with the results from other methods used to estimate antioxidant capacity (Pulido et al., 2000).

Furthermore, the lower TEACFRAP value of the plant could be due to the presence of compounds reactive towards DPPH but are not capable of reducing Fe (III)-TPTZ into the blue Fe(II)-TPTZ complex, not all reducing agents are antioxidants. Moreover, the polarity of solvent plays a crucial role in increasing the solubility of antioxidant compounds from plant extract (Silva et al., 2007).

The HPLC-PDA results of *E. alata* showed many phytochemicals that belongs to phenolics, flavonoid glycosides and flavonoids (Al-Rimawi et al., 2017). PDA was used to control wavelengths from 210 to 350 nm. All the prominent peaks shared maximum wavelengths near to 348.5–352.1 nm. These types of compounds are very close to isomeric flavonoid glycosides (Al-Rimawi et al., 2017). The resulted GC–MS chromatogram showed that the methanolic extract of *E. alte* contains twenty-five peaks with retention times between 5.55 and 24.67 (presented in Table 5). The twenty-three components representing 93.50% of the total composition were identified, while two peaks representing 6.50% of total composition were unknown, Table 5. The analysis showed that the target extract is mainly composed of aromatics and oxygenated hydrocarbons. The major identified compounds were α-D-Glucopyranose (30.82%), Phenobarbital (16.85%), 3-OH-Dodecenedioic Acid (10.13%), 3-OH-Tetradecenedioic Acid (8.33%), Melibiose (5.40%), D-Glucose (5.39%), L-Ascorbic acid (3.53%), 1,3- Propanediol (2.51%), Cyanuric acid (2.13%), Malic Acid (1.81%), Undecenedioic acid (1.73%), Glucuronic Acid (1.52%), and Ethylamine (1.03%).

Table 5
List of chemical components of *E. alte.*

Components	Retention time	Composition percentage %
Ethylamine	5.55	1.03
Cyanuric acid	6.06	2.13
1,3-Propanediol	9.49	2.51
Androstan-3-one	11.45	0.03
Malic Acid	11.45	1.81
N-Butylglycine	12.79	0.02
Unknown	13.94	1.65
Unknown	14.01	4.85
3-OH-Dodecenedioic Acid	14.13	10.13
L-Ascrobic Acid	14.13	3.53
3-OH-Tetradecenedioic Acid	14.63	8.33
Undecenedioic acid	14.91	1.73
Palmitic Acid	15.45	0.66
Glucuronic Acid	15.45	1.52
Silane	16.26	0.13
Isobutyric	16.51	0.53
Hexadecanoic acid	18.58	0.33
Phenobarbital	19.10	16.85
α-D-Glucopyranose	19.11	30.82
2,6,10,14,18,22-Tetracosahexaene	19.89	0.03
D-Glucose	20.25	5.39
Melibiose	20.25	5.40
Maltose	20.88	0.39
α-Tocopherol	22.26	0.04
β-Sitosterol	24.67	0.16
Total identified components	93.50%	
Unknown components	6.50%	

6. Conclusions

In the present study, *E. alte* plants were screened for their potential antioxidant activity, pepsin inhibitory and antibacterial activities. Based upon the results, it could be concluded that *E. alte* exhibited a considerable effect on the tested bioactivities, which highlights and supports their potential use as natural antipeptic agents in the treatment of GERD, Peptic ulcer with strong antioxidant and antibacterial effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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