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Antibacterial Activities of *Ephedra sinica* Herb Extract on Standard and Clinical Strains of *Pseudomonas aeruginosa*

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ABSTRACT

Background: *Ephedra sinica* Stapf (Ephedraceae) is one of the most important plants in traditional medicine and medicinally important as the botanical origin of crude drugs. The main active constituents of *Ephedra sinica* are the unique and taxonomically restricted adrenergic agonist's phenylpropylamino alkaloids, also known as ephedrine alkaloids: norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine, methylephedrine and methylpseudoephedrine.

Mthods: In this study, ethanolic and hydroalcoholic herb extract of *Ephedra sinica* was assayed against on standard and clinical *Pseudomonas aeruginosa* bacteria activities and then the MIC and MBC were assayed.

Results: The results were shown that the lowest MIC of ethanolic herb extract of *Ephedra sinica* on clinical and standard *Pseudomonas aeruginosa* bacteria respectively was 25 and 12.5 µg/ml but the lowest MIC of the hydroalcoholic herb extract were 25 and 25 µg/ml respectively. The lowest MBC of ethanolic herb extract on clinical and standard strains of *Pseudomonas aeruginosa*were 50 and 25 µg/ml, respectively. But, the lowest MBC of the hydroalcoholic herb extract against clinical and standard strains was 25 and 25 µg/ml.

Conclusion: Based on these results, suggest to antibacterial activity have to use hydroalcoholic herb extract that the toxicities of purified extracts of these plants need to study and hopefully improved traditional medicines will be developed.

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Introduction

Ephedra sinica Stapf (Fam. Ephedraceae) is taxonomically classified as gymnosperms that is one of the most important plants in traditional medicine and medicinally important as the botanical origin of crude drugs. It's used as a diuretic, antipyretic, diaphoretic and as bioresources that contain pharmacologically active chemicals (1, 2). It is famous for containing six alkaloids of the ephedrine series [(1R, 2S)-norephedrine, (1S,2S)-norpseudoephedrine, (1R,2S)-ephedrine, (1S,2S)-pseudoephedrine, (1R,2S)-N-methylephedrine and (1S,2S)-N-methylpseudoephedrine](3). It has an official monograph in some Pharmacopoeias in the world, where it is standardized against the major alkaloids, ephedrine, and pseudoephedrine (4). The final stages of ephedrine and pseudoephedrine biosynthesis in genus members of *Ephedra* involve N-methylation of norephedrine and norpseudoephedrine, respectively (5).

Because of ephedrine and pseudoephedrine, *Ephedra sinica* has been used to treat cold, cough, asthma, edema, and urine-negative embolism from ancient times (6). the effects of ephedrine and pseudoephedrine on the blood pressure and heart rate of resting healthy volunteers were compared (7) and related that Ephedrine 60 or 90 mg was required to raise the diastolic blood pressure above 90 mmHg, whereas 210 or 240 mg pseudoephedrine were required to produce the same effect and then they related that both isomers produced some bronchodilation, but the effect of pseudoephedrine was less than half that of ephedrine but in another research (8) related that Pseudoephedrine was a weaker constrictor than ephedrine for the common carotid, external iliac and superior mesenteric arteries. Unlike ephedrine, pseudoephedrine caused vasodilatation in the area supplied by the vertebral artery. The stimulation and depression of myocardial contraction for both isomers were approximately equal.

Therefore, with all the above mentioned actions, the main emphasis is conventionally given to its alkaloidal content, despite the fact that these substances represent only about 0.7–0.8% of the whole plant (9, 10). Recent advances emphasize that it's proven the clinical effects of these Secondary metabolisms on the respiratory, central nervous and cardiovascular systems (11). However, many species of *Ephedra* have also been shown to contain significant amounts of oligomeric flavonoids (12).

Many health benefits of foods and medicinal plants have been attributed to proanthocyanidins (13-15), and some of their pharmacological activity, such as hypotensive and vasorelaxant effects (16, 17), enhancement of the airflow obstruction, airway hyper-responsiveness and the airway microenvironment in asthma (18), and the inhibition of inflammation and remodeling in murine models of a chronic inflammatory disease like asthma (19), are responsible for the aforementioned activities of *Ephedra sinica*, especially its respiratory and cardiovascular effects. In some researches showed that *Ephedra* spp, also display additional pharmacological activities that are not attributed to alkaloids, including antioxidant (20), antiviral (21), anti-inflammatory (22, 23), antimicrobial (3, 24), immunosuppressive (25, 26), anti-invasive, antiangiogenic, antitumor (27), and cytotoxic (28) properties and primarily their antioxidant activity. Tannins, mainly proanthocyanidins, were proved by colorimetric reactions to occur in large amounts in the stems of many species of *Ephedra* (e.g., Eurasian *Ephedra*: *E. intermedia*, E. przewalskii, *E. alata, E. distachya* and *E. fragilis*; North American species of *Ephedra*: *E. californica*, *E. fasciculata*, *E. nevadensis*, *E. torreyana*, *E. trifurca* and *E. viridis*) (29). The dimeric proanthocyanidins of *Ephedra sinica* exhibit cytotoxic activity against the cancer cell lines SGC-7901, HepG2, and HeLa (28). In addition, the administration of proanthocyanidin oligomers of *Ephedra sinica* induces a decrease of the uremic toxin parameters in rats (30).

Very recently plant extract of marigold flower (31), *Ziziphora tenuior* (32), *Spirogyra varians* (33), *Solanum tricobatum* (34), *Melia dubia* (35), *Erythrina indica* (36), beet root (37), mangosteen (38), *Ocimum tenuiflorum* (39), olive (40), leaf extract of *Acalypha indica* with high antibacterial activities (41) and of *Sesuvium portulacastrum* also reported in literature with nanoparticle size ranging from 5 to 20 nm (42) are brimming in literature as a source for the synthesis of silver nanosilver particles as an alternative to the conventional methods.

A single herb may have several pharmacological actions, but only at sufficient concentrations. Synergy among components of different medicinal plants will enhance anti-bacterial activities and can be an effective strategy for fighting drug-resistant microorganisms (43). This plant was used as a folk medicine for the treatments of various ailments in Persian tribes. Ethnobotanical data (local name, mode of preparation, medicinal uses) were collected through interviews and discussions with tribal practitioners in their local language.

The aim of this research was assayed of antibacterial activities of ethanolic and hydroalcoholic herb extract of *Ephedra sinica* against the standard and clinical *Pseudomonas aeruginosa* bacteria activities.

Materials and Methods

Plant Materials and Preparation of Herb extract

The herb of *Ephedra sinica* was collected in 2017 from dahane-sanklider village, sabzvar region of Khorasan-Razavi province of Iran and identified by the Department of Plant Biology, University of Zabol.

The *Ephedra sinica* herbs were washed several times with deionized water and dried at room temperature. 10g of the herbs was homogenized in 100 ml of ethanolic and hydroalcoholic (30 cc double distilled water and 70 cc ethanol) and stirred and kept on a rotator shaker at 190–220 rpm for 24h at room temperature in an airtight container till further use with the help of electric grinder, glass dish and filtered (44). After centrifugation at 10,000 rpm for 15 mins, the supernatant was collected and stored at 4 °C.

MIC and MBC of Plant Extracts

The antibacterial activity of *Ephedra sinica* herb extract (ethanolic and hydroalcoholic) was assessed against *Pseudomonas aeruginosa* (ATCC 27853) bacteria.

In this research, some Antibiotics such as Amikacin (AN), Amoxycillin/Clavulanic Acid (AMC), Cefazolin (CZ), Gentamycin (Gm), Ampicillin (Am) and Azithromycin (AZM) were used for positive control.

We used a broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to Yu et al. (45). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of ethanolic and hydroalcoholic herb extract of *Ephedra sinica* were prepared in a 96-well microtiter plate ranged from 3.1 to 100 ppm. To each well, 10 µL of indicator solution (prepared by dissolving a 10-mg extract in 2 mL of DMSO) and 10 µL of Mueller Hinton Broth were added. Finally, 10 µL of bacterial suspension (106 CFU/mL) was added to each well to achieve a concentration of 10^4 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37 °C for 18-24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

After MIC determination of the *Ephedra sinica* herb extract, the MBC was considered the lowest concentration of the *Ephedra sinica* herb extract which prevented growth and kills >99.9% of the initial bacterial population where no visible growth of the bacteria was observed on the Mueller Hinton plates. All tubes were incubated for a total of 72 h, and progressive changes in visual turbidity were noted after 48 and 72 h (46).

Results

Antibacterial activity of herb extract of Ephedra sinica

The results of the study of the antibiotic resistance pattern of standard and clinical strains of *Pseudomonas aeruginosa* to all antibiotics showed that the strains were resistant to all antibiotics and the resistance percentages are presented in Table 1.

Antibacterial activity potentials of Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic and hydroalcoholic herb extract of *Ephedra sinica* against *Pseudomonas aeruginosa* bacteria were evaluated and then the results showed that the ethanolic and hydroalcoholic herb extract of *Ephedra sinica* (table 2, 3 and 4) have inhibiting activity on bacteria in tested concentrations.

The lowest MIC of ethanolic herb extract of *Ephedra sinica* on clinical *Pseudomonas aeruginosa* strains was 25 µg/ml but on standard *Pseudomonas aeruginosa* bacteria was 12.5 µg/ml and also the lowest MIC of the hydroalcoholic herb extract of *Ephedra sinica* on clinical *Pseudomonas aeruginosa* bacteria was 25 µg/ml but on standard *Pseudomonas aeruginosa* bacteria was 25 µg/ml (Table 2 and 3).

The lowest MBC of ethanolic herb extract of *Ephedra sinica* on clinical *Pseudomonas aeruginosa* bacteria was 50 µg/ml but on standard *Pseudomonas aeruginosa* bacteria was 25 µg/ml and also the lowest MBC of the hydroalcoholic herb extract of *Ephedra sinica* on clinical *Pseudomonas aeruginosa* bacteria was 25 µg/ml but on standard *Pseudomonas aeruginosa* bacteria was 25 µg/ml (Table 2 and 4).

Discussion

The results of the current study showed that the lowest MIC and MBC of the ethanolic herb extract of *Ephedra sinica* was 12.5 and 25 mg/mL respectively but the lowest MIC and MBC of hydroalcoholic herb extract of *Ephedra sinica* was 25 and 50 mg/mL.

It is related (47) that the lowest MIC values were 6.25 mg/mL, 12.5 mg/mL for *S. aureus* and *B. subtilis* respectively, at 24 h and 48 h of incubation period. The MBC value for *S. aureus*, *B. subtilis* and *B. cereus* showed no different, within 24 h and 48 h. The MIC and MBC of glycyrrhetinic acid against *P. aeruginosa* were found to be 160 µg/mL and 420 µg/mL, respectively (48) and also in anither research (49) that the extract of *Sphareranthu hirtus* was the most active against multi-drug resistant *Pseudomonas aeruginosa* and enterohemorrhagic *E. coli* 0157 EHEC. It is related that the MIC and MBC values of 44.2 and 51.2; 40.7 and 52.1; 35.2 and 37.9; 40.2 and 49.8; 25.4 and 32.5; 32.9 and 40.0; 40.1 and 42.0 were recorded for ethanolic extract and 40.4 and 44.0; 30.7 and 52.1; 31.4 and 37.4; 36.2 and 38.6; 25.2 and 30.9; 22.5 and 30.0; 30.1 and 32.0 are values for water extract of *A. arabica* against Methicilin- resistant *S. aureus*, *B. subtilus*, enterohemorrhagic *E. coli* 0157 EHEC, *P. vulgaris*, multi-drug resistant *P. aeruginosa*, *Salmonella. typhi*, and *Klebsiella. pneumoniae*, respectively.

In a research, related that The MIC of the essential oils (of *Acorus calamus*, *Allium sativum*, *Mucuna pruriens*, and *Sesamum indicum* L) against bacteria were ranged from 11.3 to 617 µg/ml but in this research, The lowest MIC and MBC of ethanolic herb extract of *Ephedra sinica* on clinical *Pseudomonas aeruginosa* bacteria was 25 and 50 µg/ml, respectively (50). But, on standard strains of *Pseudomonas aeruginosa* 12.5 and

Table 1. Percent resistance of clinical strains of *Pseudomonas aeruginosa* to the antibiotics.

	AN	CZ	AMC	Gm	Am	AZM
S	37.5	12.5	50	25	25	25
R	37.5	75	50	62.5	62.5	62.5
I	25	12.5	0.0	12.5	12.5	12.5

Table 2. Antimicrobial activity of ethanolic and hydroalcoholic herb extract of *Ephedra sinica* on standard and clinical *Pseudomonas aeruginosa* strains.

Bacterial strain	ethanolic herb extract	hydroalcoholic herb extract
	MIC(µg/ml)/ MBC(µg/ml)	MIC(µg/ml)/ MBC(µg/ml)
1	50/100	50/100
2	25/50	25/50
3	25/50	25/50
4	50/100	25/50
5	25/50	25/50
6	50/100	25/50
7		50/100
8	100/100	25/50
9	50/100	25/50
standard	12.5/25	25/50

Table 3. Standard and Clinical Growth of *Pseudomonas aeruginosa* strains at different concentrations of ethanolic extract

Bacterial strain	Different concentrations of the ethanolic herb extract of *Ephedra sinica* (µg/ml)					
	3.1	6.25	12.5	25	50	100
1	++	++	++	++	+	-
2	++	++	++	+	-	-
3	++	++	++	+	-	-
4	++	++	++	++	+	-
5	++	++	++	+	-	-
6	++	++	++	++	+	-
7	++	++	++	++	++	+
8	++	++	++	++	++	+
9	++	++	++	++	+	-
standard	++	++	+	-	-	-

Table 4. Standard and Clinical Growth of *Pseudomonas aeruginosa* strains at different concentrations of hydroalcoholic extract.

Bacterial strain	Hydroalcoholic herb extract of *Ephedra sinica* (µg/ml)					
	3.1	6.25	12.5	25	50	100
1	++	++	++	++	+	-
2	++	++	++	+	-	-
3	++	++	++	+	-	-
4	++	++	++	+	-	-
5	++	++	++	+	-	-
6	++	++	++	++	+	-
7	++	++	++	+	-	-
8	++	++	++	+	-	-
9	++	++	++	+	-	-
standard	++	++	++	+	-	-

25µg/ml was recorded. Also, the lowest MIC and MBC of the hydroalcoholic herb extract of *Ephedra sinica* on clinical and standard *Pseudomonas aeruginosa* strains 25 µg/ml was recorded for both of them.Additionally, we found that hydroalcoholic extract of *Ephedra sinica* was more effective than some medicinal plants from previous researches (48, 49, 50).

The recorded use of alkaloids for medicinal purposes stretches back some 5000 y (51) and this chemical group has contributed the majority of the poisons, neurotoxins, and traditional psychedelics and social drugs [e.g. nicotine, caffeine, ephedrine (from *Ephedra sinica*), cocaine, and opiates] consumed by humans (52). This group also provides the cholinesterase inhibiting treatments routinely prescribed for the cholinergic dysregulation of Alzheimer's disease (AD), such as galantamine, huperzine, physostigmine, and rivastigmine (53).

Antimicrobial and antibiotic resistances are an increasingly serious threat to human health and then it is necessary to overcome it with the help of nature (54). Therefore, there is an increase in the investigation of plants as a source of human infectious diseases management (55, 56). Though there are two potential problems with the use of any silver antibiotic usage. First, it does appear that silver resistance can occur in at least some bacterial strains. It is extremely important to avoid this circumstance since it appears that, at least in the near future, silver ions may soon be the only remaining effective agent for clinical use. There appears to be a tendency to develop other silver compounds that produce low levels of silver ions in the wound environment or have characteristics permitting long term application with fewer dressing changes as a cost-saving measure. Both of these characteristics would appear to favor the development of additional silver-resistant strains (57).

One of the important characteristics of extracts and essential oils of the plant and their phenolic components is their hydrophobicity (58, 59), which disrupts the cytoplasmic membrane, disrupts the proton energy and electrical current, coagulates the cell contents, and also the penetration of these materials into the cell membrane lipids Bacteria and mitochondria and then disrupts their buildings and increases their permeability (60, 61). This reason causes the leakage of ions and other cellular contents. Although the release of a limited amount of these substances is tolerable to the bacterium, it has a biological effect, and the expulsion of large amounts of cellular contents or the release of vital ions and molecules will cause cell death (62).

The pharmaceutical activity of herbal compositions may be attributable to the natural presence of multiple biologically-active

compounds that interact in synergy to create an effect that is greater than that which occurs with a single chemical component (43) of course in this research Phytochemical screening of the extracts revealed the presence of phytocompounds such as alkaloids and tannins which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs (62). These phyto-constituents may be responsible for the *Ephedra sinica* antibacterial (63). Also, its therapeutic effect cannot be attributed to a single chemical constituent or a group of molecules. All the substances in the plant determine the activity of the plant by a synergistic, but sometimes an antagonistic effect. However, generation of the effect requires a substantial amount of a single ingredient, in other words, a higher concentration than normally found in the plant (43).

Conclusion

The results reveal the presence of antibacterial activity of *Ephedra sinica* herb extract against *Pseudomonas aeruginosa* bacteria and then it's obtained that the antibacterial activity of ethanolic herb extract was more than hydroalcoholic herb extract on standard *Pseudomonas aeruginosa* bacteria but on clinical *Pseudomonas aeruginosa* bacteria the hydroalcoholic herb extract was more and at the end it's suggested to antibacterial activity have to use hydroalcoholic herb extract, of course, The toxicities of purified extracts of these plants need to study and hopefully improved traditional medicines will be developed.

Conflict of interest

None declared.

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