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Original Research

Antibacterial Activity of Aerial Part Extracts and Fractions of *Ajuga chamaecistus* ssp. *tomentella*

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Abstract

The genus *Ajuga* [Kamafitos] has been used as wound healing in traditional Persian medicine. Recent studies have shown that some species of this plant has antibacterial effects. Methanol 80% and aqueous extract and partition fractions of n-hexane, diethyl ether, and n-butanolic from a methanolic extract of *Ajuga chamaecistus* ssp. *tomentella* were evaluated for antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* using cup-plate method. Furthermore, (minimum inhibitory concentration) MIC was determined using micro plate method. Gentamicin and vancomycin were used as positive control and methanol 5% was used as negative control. Zone of inhibition (mm) and the MIC (µg/ml) were measured in three times. Based on the results, all extracts and fractions showed antibacterial effect *against S. aureus, B. subtilis, E. coli* and *P. aeruginosa*, among them the diethyl ether fraction exhibited the most antibacterial effect against *S. aureus* (zone of inhibition =18mm) and *B. subtilis* (zone of inhibition =16.5mm). Also, n-butanolic fraction showed the most antibacterial effect against *E. coli* (zone of inhibition =16mm) and methanolic extract presented the most antibacterial effect against B. subtilis (zone of inhibition =15mm). S. aureus was the most sensitive (MIC > 333µg/ml) and *P. aeruginosa* was the most resistant (MIC > 4000µg/ml) bacteria to the extracts. The above results revealed that most of the extracts and fractions from this plant possess antibacterial activity against micro-organisms specially *S. aureus*. According to traditional use of *Ajuga* species as wound healing medicinal plant, it can be used for treatment of various skin infections and possibly reduction of healing time.

Keywords: *Ajuga chamaecistus* subsp *tomentella*, Antibacterial, Cup-plate method, MIC

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Introduction

Plants often are unique sources of new drugs playing an important role in treatment of human diseases. Infectious diseases are one of the most important problems faced by people all over the world. Because of the adverse side effects of synthetic drugs and the emergence of antibiotic resistance bacteria, the new natural compounds with a broad spectrum of activity against bacterial species are required [1]. *Ajuga* was mentioned as "*kamafitos*"or" *khamanitos"* in traditional Persian medicine with warm and dry nature. This medicinal plant is an antiblockage [*Mofattih*], diuretic [*Modirr*] and emmenagogue agent. Also, it has cleansing [*Monaqqi*] and detergent [Jali] property for internal organs in addition to purgative for condensed phlegm materials in the body. "*Kamafitos"* has beneficial effect in treatment of edema, jaundice, joint pains, and sciatica. Topically, it has been used for wound healing, and breast hardness [2, 3]. Five species of the genus *Ajuga* are found in Iran such as *A. austro- iranica, A. chamaecistus, A. comata, A. orientalis* and *A. chamaepitys*. *Ajuga chamaecistus* contains five subspecies in Iran, including *A. chamaecistus* ssp. *tomentella* [4]. Several biological activities have been reported from some species of *Ajuga* such as treatment of joint disease [5], anti-inflammatory [6], hypoglycemic [7], antimalarial [8], antioxidant, and antimicrobial effects [9].

The main components of essential oil from the aerial parts of *Ajuga chamaecistus* ssp. *tomentella* was reported as thymol, *exo*-fenchol, β-pinene, 1-octen-3-ol, α-terpineol, 2-hexanol, α-thujene, and α-pinene [10]. Recently, ten natural compounds including 20-hydroxyecdysone, cyasterone, ajugalactone, makisterone A, and 24-dehydroprecyasterone (phytoecdysteroids), 8-acetylharpagide (iridoid), *cis*- and *trans*-melilotoside, lavandulifolioside, leonoside B, and martynoside (phenylethanoid glycosides), have been isolated from diethyl ether and n-butanol fractions of *Ajuga chamaecistus* ssp. *tomentella*. Cytotoxicity evaluation of this plant confirmed nontoxic effects of total extract and isolated compounds in normal and cancer cell lines [11, 12]. Moreover, analgesic property of total water, hexane and diethyl ether fractions of *A. chamaecistus* ssp. *tomentella* has been described, that supports traditional use of *Ajuga* genus for joints pains and other inflammatory diseases [13]. Most recently, hexane fraction of *A. chamaecistus* ssp. *tomentella* showed larvicidal activity against malaria vector *Anopheles stephensi* and the main phytoecdysteroid compound, ajugalide-E, separated from this fraction [14].

The aim of this study was to evaluate anti-bacterial effects of aqueous and methanolic extract and some fractions from aerial parts of *Ajuga chamaecistus* ssp. *tomentella* against four bacteria such as Gram positive strains (*Staphylococcus aureus* and *Basilus sabtilus*) and Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*).

Methods

Plant material

Aerial parts of *Ajuga chamaecistus* Ging. ssp. *tomentella* (Boiss.) Rech. F. were collected from Tehran, Iran, in June 2013 and verified by Prof. G. Amin. A voucher specimen (THE-6697) has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Methanol extraction and fractionation

The air-dried and ground aerial parts of *A. chamaecistus* ssp. *tomentella* (250 g) were extracted with methanol 80% (3 × 0.5 L) at room temperature. The solvent was evaporated on a rotary evaporator and in a vacuum oven to give

a dark brown extract (45 g). The extract (30 g) was suspended in water and partitioned successively by n-hexane, diethyl ether, and n-butanol. Removal of the solvents with a rotary evaporator resulted in the production of n-hexane, diethyl ether, and n-butanol fractions.

Water extraction

Fifty grams of the powdered plant from the aerial parts of *A. chamaecistus* ssp. *tomentella* were extracted with boiling distilled water at 90 temperatures for 20 min. The aqueous extract was dried by using freeze drying process.

Bacterial strains

Gram-positive bacteria comprising *Staphylococcus aureus* (PTCC25923), and *Bacillus subtilis* (PTCC6633), and gram-negative bacteria such as *Escherichia coli* (PTCC25922), and *Pseudomonas aeruginosa* (PTCC25823) were prepared from the Persian type culture collection (PTCC) of Iranian Research Organization for Science and Technology.

Antimicrobial activity assay

The methanol 80% and aqueous extracts and partition fractions of n-hexane, diethyl ether, and n-butanol from the methanol extract of *Ajuga chamaecistus* ssp. *tomentella* were tested against four bacteria using the cup plate method [15]. Mueller-Hinton agar plates were seeded with 100 µlit of culture. Seven cups were made in each petri plate for seven concentrations (250-8000 µg/ml) and 50 µL of different concentration of each extract and fraction were added into each well. Then bacterial plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring zone of inhibition around each well in plate. Each experiment was repeated three times and average values were reported.

Determination of minimum inhibitory concentration (MIC)

Micro dilution method was used to determine MIC[16]. Plant extracts and fractions were dissolved in methanol 5% to achieve concentration of 8000 µg/ml. Micro titer plates filled with a 100 µl of Mueller-Hinton broth. 100 µl of the extracts and fraction with concentration of 16000 µg/ml were added to the first column of wells, and mixed with the broth in the wells to obtain concentration of 8000 µg/ml. 100 µlit of first well were transferred to next well and from next to next until last well to obtain concentrations 8000-250 µg/ml. Finally, bacterial suspensions with 0.5 McFarland standard turbidity were added to each well. The plates were incubated at 37°C for 24 hours. Gentamicin and vancomycin were used as positive control. MIC was defined as the first concentration without visible turbidity comparing to the negative control. This test was repeated three times and the average of values was reported.

Results

The antibacterial effect of the methanol 80% and aqueous extract and partition fractions of n-hexane, diethyl ether, and n-butanol from *Ajuga chamaecistus* ssp. *tomentella* were studied using cup plate method with following strains: *Staphylococcus aureus, Basilus sabtilus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Results showed that all samples possessed antibacterial activity in concentration of 8000 µg/ml. The zone of inhibition by each extract and fraction observed in the nutrient agar media is represented in Table 1. The diethyl ether fraction was the most active against *Staphylococcus aureus* and *Basilus subtilus* (diameter of zone of inhibition = 18.5 and 16.5 mm, respectively), the n-butanol fraction was significantly active against *Escherichia coli*, (diameter of zone of inhibition = 16 mm), and the methanol extract was very active against *Pseudomonas aeruginosa*. (diameter of zone of inhibition = 15 mm). *S.*

aureus was the most sensitive (MIC 333 µg/ml) and *P. aeruginosa* was the most resistant (MIC 4000 µg/ml) to the extracts. (Table 2, Figure1)

Table1. Antibacterial activities of different extract and fractions (8000 µg/ml) from aerial parts of *A. chamaecistus* subsp. *tomentella* in terms of zone of inhibition (mm).

Sample	zone of inhibition(mm)			
	S. a	*B. s*	*E. c*	*P. a*
Met. Ext.	17±0.5	16±0.5	15±0.5	15±0.5
Aq. Ext.	17.5±0.5	15.5±0.5	13±0.5	11±0.5
Hex. Fr.	18±0	15±0	15.5±0.5	12±0
De. Fr.	18.5±0.5	16.5±0	14±0.5	11±0.5
n-But. Fr.	17.5±0.5	15±0.5	16±0.5	15±0.5

Met. Ext.: methanol extract, Aq. Ext.: aqueous extract, Hex. Fr.: hexane fraction, De. Fr.: Diethyl ether fraction, n-But.Fr.: n-butanolic fraction. *S. a*: *Staphylococcus aureus*; *B. s*: *Bacillus subtillis*; *E. c*: *Escherichia coli*; *P. a* : *Pseudomonas aeruginosa*.

Discussion

Screening for new antimicrobial agents from plant sources is increasing worldwide. Several studies have reported the antimicrobial effect of *Ajuga* species and their chemical compounds. In a previous study, antibacterial effect of essential oil from aerial parts of *Ajuga chamaecistus* ssp. *tomentella*, against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* were investigated and it was found that essential oil of the plant did not show antibacterial effect up to a dose of 25 ml [10]. However, other species of the genus *Ajuga* suggested the antibacterial effects. Extracted essential oils from *A. iva* showed antibacterial effects, specifically in gram positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* [17]. In addition, essential oil obtained from *A. bracteosa* exhibited antimicrobial activity with MIC-values 0.33 mg/ml against *S. aureus* [18]. Also, Rahman et al (2013) presented the antibacterial effect of the crude extract of *Ajuga parviflora* against *Citrobacter* and *Pseudomonas aurogenosa* with a maximum zone of inhibition of 12 mm [19].

Antimicrobial effects of the plants related to the presence of various compounds such as quinones, phenols, alkaloids, flavonoids, terpenoids, essential oil, tannins, lignans, glucosinolates and some other secondary metabolites. Plants extracts and the phytochemicals might be able to exert their antibacterial activities by targeting the physiological factors of the bacteria that include cell membrane permeability, efflux effect, and biofilm development [1].

Neo-Clerodane diterpenoids (lupulins A, B and D) isolated from *A. lupulima* exhibited antibacterial activity against *Pseudomonas aeruginose* and *Escherichia coli* [20]. In our earlier study, some phytoecdysteroids and phenolic compounds as well as 8-acetylharpagide were isolated from diethyl ether and n-butanol fraction of *A. chamaecistus ssp. tomentella* [11, 12]. According to the report of Chaari et al. (2000), phytoecdysteroids from *A. pseudovia* and also 8-o-actetylharpagide from *A. iva* can possess antibacterial activity. While Mamadalieva et al. (2013) showed that the isolated ecdysteroids from *A. turkestanica* were weak antimicrobial compounds. Nevertheless, the chloroform extract of *A. turkestanica* showed antimicrobial activity against multi resistant strains such as *Staphylococcus aureus* MRSA ATCC 1000/93 and *Streptococcus pyogenes* ATCC 12344 [20, 22]. Additionally, two isolated compounds 14, 15-dihydroajugapitin and 8-o-acetylharpagide from the aerial parts of *Ajuga bracteosa* showed antibacterial activity against *Escherichia coli* with zone of inhibitions of 25.0 ± 1.4 mm and 22.6 ± 0.9 mm respectively and the MIC value of compound 1 and 2 were between 500 and 1000 µg/ml [22].

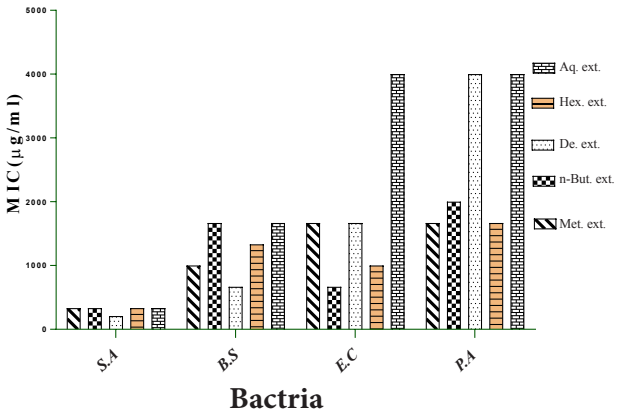

Figure1. MIC (µg/ml) of aqueous and methanol extract, and some fractions from *A. chamaecistus* subsp. *tomentella* against *S. aureus*, *B subtilis, E.coli* and *P.aeruginosa*

Table 2. Minimum Inhibitory Concentration (MIC) of aqueous and methanol extract, some fractions from *A. chamaecistus* subsp. *tomentella*, Gentamicine, and Vancomycine.

Sample	MIC (µg/ml)			
	S. a	*B. s*	*E. c*	*P. a*
Met. Ext.	333.3 ± 8.117	1000 ± 0	1666.6±471.4	1666.6±471.4
Aq. Ext.	333.3±117.8	1666.6±471.4	4000±0	4000±0
Hex. Fr.	333.3±117.8	1333.3±471.4	1000±0	1666.6±471.4
De. Fr.	208.3±58.9	666.6±235.7	1666.6±471.4	4000±0
N-But. Fr.	333.3±117.8	1666.6±471.4	666.6±235.7	2000±0
Gen.	–	–	5±1.4	8±2.8
Van.	10.4±2.9	16.6±5.9	–	–

Met. Ext.: methanol extract, Aq. Ext.: aqueous extract, Hex. Fr.: hexane fraction, De. Fr.: Diethyl ether fraction, n-But.Fr.: n-butanolic fraction. Gen.:Gentamicine, Van.: Vancomycine, *S. a*: *Staphylococcus aureus*; *B. s*: *Bacillus subtillis*; *E. c*: *Escherichia coli*; *P. a* : *Pseudomonas aeruginosa*.

Conclusion

Study on traditional medicinal plants introduced in different countries, can lead to find new drugs. The above results showed that this plant extracts and fractions have antibacterial activity against bacteria specially *S. aureus*. Based on the results, diethyl ether fraction that consists of phytoecdysteroids and 8-acetylharpagide can be considered as antibacterial extract against *S. aureus* and *B. subtilis* that can confirm the traditional use of these plants for treatment of various skin infections as well as wound healing.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

None.

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