مجله دنیای میکروب‌ها
سال نهم شماره چهارم (پیاپی ۲۹) زمستان ۱۳۹۵
صفحات ۳۵۴-۳۴۶

Original Article

بررسی ترکیبات شیمیایی و فعالیت ضد باکتریایی اسانس و عصاره درمنه کوهی جمع آوری شده در ایران

پرنیان سعیدی[۱]، ساغر کتابچی[*۲]، وحید روشن[۳]

[۱] کارشناس ارشد، گروه بیماری شناسی گیاهی و گیاه پزشکی، واحد شیراز، دانشگاه آزاد اسلامی، شیراز، [۲] استادیار، گروه بیماری شناسی گیاهی و گیاه پزشکی، واحد شیراز، دانشگاه آزاد اسلامی، شیراز، [۳] استادیار، گروه منابع طبیعی، مرکز تحقیقات کشاورزی و منابع طبیعی استان فارس، شیراز.

چکیده

سابقه و هدف: فعالیت ضد میکروبی اسانس و عصاره های گیاهی از سال ها قبل، مورد توجه بوده است. مشکلات و کمبود های بسیاری در کنترل بیماری های باکتریایی گیاهی وجود دارد. از لحاظ تاریخچه ای، ترکیبات طبیعی گیاهی از منابع مهم با خاصیت درمانی به شمار می آیند. این مطالعه با هدف بررسی ترکیب شیمیایی و فعالیت های ضد باکتریایی اسانس و عصاره درمنه کوهی (*Artemisia aucheri*) انجام شد.

مواد و روش ها: در این مطالعه مقطعی، ترکیبات اسانس و عصاره اتانلی درمنه کوهی به وسیله دستگاه کروماتوگرافی گازی (GC/MS) و کروماتوگرافی مایع با کارایی بالا (HPLC) شناسایی شدند. فعالیت ضد باکتریایی اسانس و عصاره با روش انتشار دیسک و رقیق سازی متوالی ارزیابی گردید.

یافته ها: اصلی ترین ترکیب اسانس ۱/۸ سینئول (۲۲/٦٥ درصد) و مهمترین ترکیب پلی فنلی عصاره اسید کلروژنیک (۲٦٤/۹ mg/l) بود. در بررسی MIC و MBC اسانس، بیشترین قطر هاله بازدارنده (در محدوده ٦/٤ تا ۸ میلی متر و ٦/٤ تا ۹ میلی متر) علیه باکتری های *باسیلوس سوبتیلیس*، *برنریا نیگریفلوئنس*، *استرپتومایسس اسکبیس*، *رایزوبیوم رادیوباکتر*، *رایزوبیوم ویتیس*، *زانتوموناس آکسونوپودیس* پاتوار *سیتری* و *زانتوموناس آربوری کولا* پاتوار *جوگلندیس* مشخص گردید. همچنین در ا رزیابی MIC و MBC عصاره بیشترین قطر هاله بازدارنده (در محدوده ٦/٤ تا ۸ میلی متر و ٦/٤ تا ۹ میلی متر) علیه *رایزوبیوم رادیوباکتر*، *برنریا نیگریفلوئنس*، *رایزوبیوم ویتیس*، *استرپتومایسس اسکبیس*، *باسیلوس سوبتیلیس*، *زانتوموناس آکسونوپودیس* پاتوار *سیتری*، *زانتوموناس آربوری کولا* پاتوار *جوگلندیس* و *رالستونیا سولاناساروم* مشاهده شد.

نتیجه گیری: نتایج این پژوهش نشان داد که اسانس و نیز عصاره اتانلی درمنه کوهی از فعالیت ضد میکروبی مناسبی علیه باکتری های بیماری زای گیاهی برخوردار می باشند. بنابراین درمنه کوهی می تواند به عنوان یک آفت کش زیستی در نظر گرفته شود.

واژگان کلیدی: عصاره، اسانس، درمنه کوهی، MIC، MBC.

دریافت مقاله: اسفند ماه ۹٤ پذیرش برای چاپ: اردیبهشت ماه ۹۵

*) آدرس برای مکاتبه: شیراز، دانشگاه آزاد اسلامی، گروه بیماری شناسی گیاهی و گیاه پزشکی.
تلفن: ۰۹۱۷۷۱۵۲۰۸۰ پست الکترونیک: ketabchi@iaushiraz.ac.ir

Journal of Microbial World
Volume 9, No. 4, January 2017

Original Article

Assessment of chemical compositions and antibacterial activity of the extract and essential oil of *Artemisia aucheri* collected from Iran

Parnian Saeidi[1], Saghar Ketabchi[2], Vahid Rowshan[3]

[1]MS.c., Department of plant pathology and plant protection, Shiraz branch, Islamic Azad University, Shiraz, Iran.
[2]Assistant Professor, Department of plant pathology and plant protection, Shiraz branch, Islamic Azad University, Shiraz, Iran.
[3]Assistant Professor, Department of Natural Resources, Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran.

Abstract

Background & Objectives: The antimicrobial activity of plant oils and extracts has been recognized for many years. There are many difficulties and deficiencies to control plant photogenic bacteria. Historically, natural plant products have been considered as great sources with therapeutic properties. This study was aimed to evaluate the chemical compositions and antibacterial activity of extract and essential oil of *Artemisia aucheri* collected from Iran.

Materials & Methods: In this sectional study, the compositions of essential oil and ethanolic extract of *Artemisia aucheri* were determined by GC/MS and HPLC assays. Antibacterial activity of the essential oil and extract was evaluated using micro broth dilution and disc diffusion methods.

Results: The main component of the oil was, 1.8 Cineol (22.65%), and the main polyphenolic compounds of the extract was chloregenic acid (264.9 mg/l). Evaluating MIC and MBC values of the essential oil, the maximum inhibition zone diameter (in the range of 6.4-8 mm, and 6.4-9 mm) was measured against *Bacillus subtilis*, *Brenneria nigrifluens, Streptomyces scabies, Rhizobium radiobacter, Rhizobium vitis, Xanthomonas axonopodis* pv.*citri,* and *Xanthomonas arboricola pv. juglandis*. Furthermore, evaluating MIC and MBC values of the extract the maximum inhibition zone diameter (in the range of 6.4-8 and 6.4-9 mm) was observe against *Rhizobium Radiobacter*, *Brenneria nigrifluens, Rhizobium vitis, Streptomyces scabies, Bacillus subtilis, Xanthomonas axonopodis* pv.*citri, Xanthomonas arboricora* pv. *Juglandis,* and *Ralstonia solanacearum*.

Conclusion: In general, both essential oil and ethanolic extract showed desirable antimicrobial activity against plant photogenic bacteria. Therefore, *Artemisia aucheri* can be considered as a biopesticide.

Keywords: Extract, Essential oil, *Artemisia aucheri,* MIC, MBC.
Received: April 2015 Accepted: June 2015

Introduction

Plants are abundant natural sources of effective antibiotics, chemicals that kill pathogenic cells or inhibit their division, and are classified into antibacterial, antifungal, antiviral, and antineoplastic types according to their target (1). Herbs and their essential oils

Correspondence to: Saghar Ketabchi
Tel: +98 9177152080
E-mail: ketabchi@iaushiraz.ac.ir

have been known to have varying degrees of antimicrobial activity. Using plants essential oils and extracts may also be effective for managing plant pathogenic bacteria. The *Asteraceae* family are well known for their antimicrobial activity. There are many reports on their bioactivities, including antimicrobial activity of the extract and essential oil of different species of the *Artemisia* genus (2).

Artemisia santonicam and *Artemisia vulgaris* show antimicrobial activity against *S. aureus*, *Streptococcus epidermidis*, and *Enterococcus faecalis* (3).

The *Artemisia* genus has 34 annual and perennial species that have been distributed in tropical and subtropical regions of Iran. *Artemisia aucheri* is an aromatic and indigenous plant from *Asteraceae* family that is distributed in Iran. In this investigation, we have evaluated the chemical composition and antibacterial activity of the essential oil and extract of *A. aucheri* collected from Iran (4).

This study has been carried out for the first time to determine antibacterial activity of *A. aucheri* essential oil and methanolic extract against plant pathogenic bacteria.

Materials and Methods

Plant material

Aerial parts of *A. aucheri* in pre flowering stage from Sarvestan area, Fars Province, Iran were collected during February 2013. The harvested plants were dried at room temperature (25 °C) for 2 weeks. Taxonomic identification of plant was confirmed by Dr. Rowshan. The plant voucher specimen number in the Fars Research Center Agriculture and Natural Resource is P920275.

Essential oil extraction

Essential oil was obtained from dried aerial parts (100 g) of *A. aucheri* that was subjected to hydro-distillation using a clevenger-type apparatus for three hours. Then the oil was dried by anhydrous sodium sulfate. The isolated oil was stored in tightly closed vials at 4 °C for future analysis (5).

Essential oil analysis

Essential oil was analyzed by Hewlett–Packard GC/MS (model 6890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl polysiloxan (30 m×0.25 mm×0.25 µm film thickness) with helium as the carrier gas and a split ratio of 1:20 (6).

Preparation of ethanolic extracts

The finely dry powdered aerial parts (20 gr) was transferred to dark colored flask and mixed with 100 ml ethanol and stored at room temperature. The extracts were filtered through a Buchner funnel, with Whatman number 1 filter paper. The extract was concentrated under reduced pressure of 22-26 mmHg at 45 °C, and the residue obtained. All residues were kept in tightly stopper bottle and kept in refrigerator at 4 °C for future analysis (7).

Extract analysis

It was carried out by chromatographic system HPLC (model Agilent Technologies), equipped with a XDB-C_{18} capillary column (4.6mm×150 mm, 5µm film thickness) at ambient temperature. The mobile phase

included methanol: water with 1% formic acid: (10:90). The sample was run for 15min, and detection was done at 230 and 280 nm by UV detector. All chromatographic data were recorded and processed using Chemstation software (8).

Bacterial species

The bacterial strains used in this study were included *Xanthomonas axonopodis* pv. *citri, Xanthomonas arboricora* pv. *juglandis, Xanthomonas campestris, Brenneria nigrifluens, Rhizobium radiobacter, Pseudomonas florescence, Pantoea agglomerans, Ralstonia solanacearum. Rhizobium vitis, Pseudomonas syringae* pv. *syringae, Erwinia amylovora, Bacillus subtilis*, and *Streptomyces scabies*. All the strains were isolated from diseased samples, and identified by Dr. Ketabchi, faculty of Islamic Azad University, Shiraz, Iran.

Antibacterial activity, MIC and MBC inhibition zone

In vitro antibacterial activity of the essential oil and extract as well as inhibition zone (IZ) of minimum inhibition concentrations (MIC) and minimum bactericidal concentration (MBC) of *A. aucheri* were evaluated by disc diffusion method. The bacterial suspensions were prepared with a concentration of 10^8 CFU/ml (OD_{600}= 0.1).

The essential oil or ethanolic extract were dissolved in dimethylsulfoxide (DMSO), and diluted in a two-fold manner to make the concentrations of 10000, 5000, 2500, 1250, 625 µg/discs. The suspensions were then spread on the plate containing nutrient agar as slim layer. Then sterile discs (6 mm in diameter) were impregnated with different concentrations of essential oil or ethanolic extract, and placed on the surface of the test plate. Negative control discs were DMSO. The diameters of the growth inhibition zones around each disc were measured following incubation at 27°C for three days (9).

Determination of MIC

Minimum inhibition concentrations (MIC) of *A. aucheri* essential oil and extract against the tested bacterial strains were determined using micro-broth dilution method. Briefly, serial two-fold dilutions of *A. aucheri* (10% w/v) were prepared in 96-well micro-titer plate containing nutrient broth (Merck, Darmstadt, Germany). Bacterial suspensions were adjusted to the concentration of 10^8 CFU/ml (OD_{600}= 0.1).

A constant amount of bacterial suspensions was added to all wells and the plate was incubated at 28°C for 24-48 hour. Micro-titer plates containing medium and bacteria, without any essential oil and extract were considered as controls. Each well was examined for bacterial growth by comparing each well to the control. The MIC was defined as the lowest concentration in which no visible growth of the organisms was observed (10).

Determination of MBC

Bacterial suspension was added to the plate. Culturing was performed in NA medium to determine minimum bactericidal concentration (MBC) of wells without turbidity. Then the medium was incubated at proper temperature for each bacterium . Finishing incubation time,

Table 1. Essential oil components of *Artemisia aucheri* analysis by (GC/MS).

Compound	RI	Percentage in oil	Compound	RI	Percentage in oil
Geranyl acetate	1383	1.13%	1,8-Cineole	1031	22.05%
Methyl eugenol	1404	0.15%	Davanone	1587	13.82%
(E)-Caryophyllene	1418	0.46%	Camphor	1148	9.92%
Germacrene D	1479	0.44%	(Z)-Jasmone	1397	7.4%
Geranyl acetate	1383	1.13%	Chrysanthenone	1126	4.11%
Davana ether	1491	0.13%	Tricyclene	922	0.11%
Bicyclogermacrene	1495	0.93%	α-Thujene	925	0.2%
Artedouglasia oxide C	1522	1.26%	α-Pinene	934	3.6%
Artedouglasia oxide A	1534	1.79%	Camphene	948	1.99%
Spathulenol	1577	1.16%	Sabinene	973	3.34%
γ-Terpinene	1016	0.53%	ß-Pinene	977	2.04%
p-Cymene	1029	1.37%	Bornyl acetate	1284	0.25%
dehydro-1,8-Cineole	990	0.44%	Thymol	1293	0.07%
Benzene acetaldehyde	1043	0.03%	Carvacrol	1303	0.55%
α-Terpinene	1057	0.98%	§-Elemene	1335	0.18%
cis-Sabinene hydrate	1066	1.15%	α-Terpinyl acetate	1348	1.18%
Terpinolene	1087	0.27%	Unknown	1270	0.63%
Trans-Sabinene hydrate	1099	1.04%	Verbenone	1209	0.19%
trans-Pinocarveol	1143	2.38%	trans-Carveol	1218	0.14%
Pinocarvone	1163	2.32%	(E)-Ocimenone	1239	0.61%
α-Terpineol	1166	1.98%	Carvone	1243	0.36%
Terpinene-4-ol	1178	2.41%	Geraniol	1253	0.28%
α-Thujenal	1184	0.16%	Myrtenal	1197	1.56%
α-Terpineol	1192	2.61%			

the lowest essential oil and ethanolic extract concentration in which 99.9% of bacteria showed no growth, was considered as bactericidal concentration (10).

Statistical analysis

All tests were performed in four replicates. Analysis of variance (ANOVA) and mean comparison with Duncan's multiple range test ($P \leq 0/01$) was carried out in SPSS software (version 18, SPSS Inc., Chicago, IL, USA).

Results

The chemical composition of *A. aucheri* essential oil is given in Table 1. 46 components were identified in plant essential oil representing 99.60% of its total weight. The main components of the oil included 1.8 cineol (22.65%), davanone (13.82%), camphor (9.92%), Z jasmine (7.4%), and crysanthenone (4.11%). HPLC analysis of total phenol content is presented in Table 2.

Gallic acid (30.979 mg/l), catechin (139.177 mg/l), and chloregenic acid (264.938 mg/l) were detected as the first report in this plant (Fig. 1, 2). The inhibition zone diameters of MIC and MBC for bacterial strains that were

Table 2. The type and amount of polyphenols in *A. aucheri* extract.

Phenolic compound	Amount(mg/l)
Caffeic acid	-
Catechin	139.17
Chloregenic acid	264.93
Gallic acid	30.97
Quercetin	-

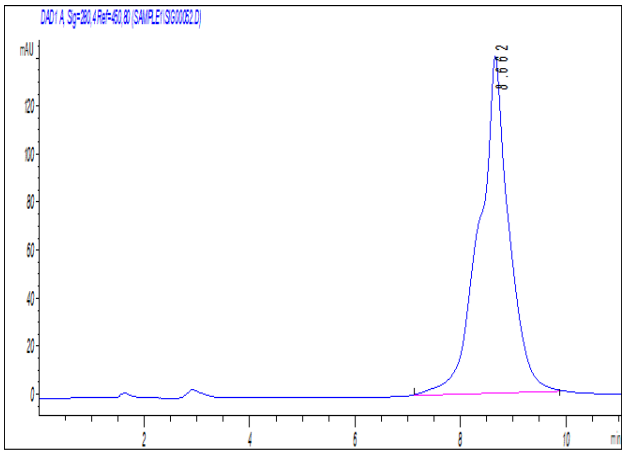

Fig 1. HPLC chromatogram of catechin at 280 nm.

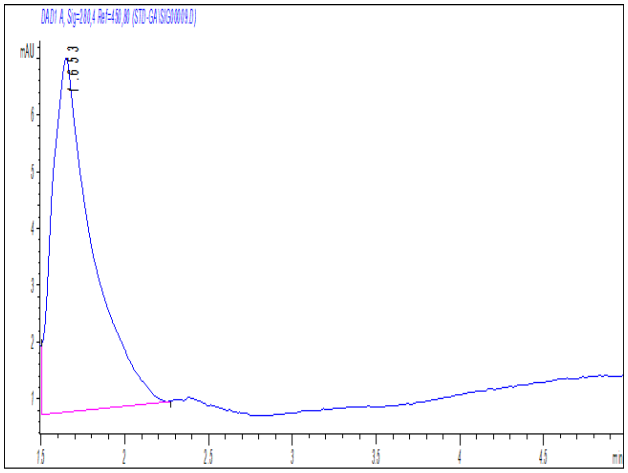

Fig 2. HPLC chromatogram of gallic acid at 280 nm.

sensitive to the plant essential oil was in the range of 6.4-8.0 mm and 6.4-9.0 mm, respectively.

The maximum inhibition zone diameters of MIC and MBC values of plant essential oil was detected for *Bacillus subtilis*, *Breneria nigrifluens, Strepomyces scabies, Rhizobium radiobacter, Rhizobium vitis, Xanthomonas axonopodis* pv. *citri* and *Xanthomonas juglandis* (Table 3).

The inhibition zone diameters of MIC and MBC for bacterial strains that were sensitive to the plant extract was in the range of 6.4-8.0 mm and 6.4-9.0 mm, respectively.

The maximum inhibition zone diameters of MIC and MBC was detected for *Rhizobium radiobacter*, *Brenneria nigrifluens, Rhizobium vitis, Streptomyces scabies, Bacillus subtilis, Xanthomonas axonopodis* pv. *citri, Xanthomonas juglandis* and *Ralstonia solanacearum* (Table 3).

The lowest inhibitory consistency of MIC and MBC values of *A. aucheri* essential oil and

Table 3. Inhibition zone diameter for MIC and MBC values of essential oil and extract of *Artemisia aucheri.*

Bacteria	Essential oil		Extract	
	IZ for MIC (mm)	IZ for MBC (mm)	IZ for MIC (mm)	IZ for MBC (mm)
Pseudomonas florescence	6.4 j	6.4 g	6.4 j	6.4 g
Pseudomonas Syringae* pv. *syringae	6.4 j	6.4 g	6.4 j	6.6 g
Erwinia amylovora	6.6 ij	7f e	6.6 ji	7 ef
Pantoae agglomerans	6.4 j	6.4 g	6.4 j	6.6 g
Xanthomonas juglandis	7 ghi	7.16 f	7 ghi	7.5 f
Ralstonia solanacearum	6.6 ij	7 fgh	7 fgh	7.33 de
Xanthomonas axonopodis* pv. *citri	7.16 hgf	7.5 de	7.33 efg	7.83 cd
Xanthomonas campestris	6.6 ij	7 f	6.8 hij	7.16 ef
Rhizobium vitis	7.66 def	8 c	7.83 bcd	8.66 c
Rhizobium radiobacter	7.66 cde	8.16 c	8.16 ab	9 ab
Brenneria nigrifluens	7.83 bcd	8.16 c	8 abc	8.83 ab
Bacillus subtilis	8.33 a	9.16 a	7.5 def	8 c
Streptomyces scabies	7.66 cde	8.16 c	7.83 bcd	8.16 b

Note: Significant at p≤ 0.01.

Note: in each column, mean with the same letters are not significantly different at 1% level of Duncant test.

Table 4. MIC and MBC values of essential oil and extract of *A. aucheri.*

Bacteria	Essential oil		Extract	
	MIC	MBC	MIC	MBC
Pseudominas flurescens	5000a	10000a	2500b	5000b
Pseudomonas syringae* pv. *syringae	5000a	10000a	2500b	5000b
Erwinia amylovora	5000a	10000a	5000a	10000a
Pantoa agglomeranse	5000a	10000a	2500b	5000b
Xanthomonas juglandis	2500b	5000b	2500b	5000b
Ralstonia solanacearum	2500b	5000b	2500b	5000b
Xanthomonas axonopodis* pv. *citri	2500b	5000b	1250c	2500c
Xanthomonas campestris	2500b	5000b	2500b	5000b
Rhizibium vitis	1250c	2500c	1250c	2500c
Rhizobium radiobacter	625d	1250d	625d	1250d
Brenneria nigrifluens	625d	1250d	625d	1250d
Bacillus subtilis	625d	1250d	625d	1250d
Streptomyces scabies	625d	1250d	625d	1250d

Not: Significant at p≤0/01.

Note: in each column, mean with the same letters are not significantly different at 1% level of Duncan test.

methanolic extract was observed on *Brenneria nigrifluence, Rhizobium radiobacter, Bacillus subtillis, Streptomyces scabies campestris* (with the MIC value of 625 µl and MBC value of 1250 µl) (Table 4).

Discussion

This is the first report on antibacterial activity of essential oil and methanolic extract of *A. aucheri* against plant pathogenic bacteria. In general this plant showed good activity against these selected bacteria. In this study, coumarin 0.051053 mg/g, ellagic acid 4.468689 mg/g, and hesperetin 0.642698 mg/g were reported as the components of total phenol content in this plant for the first time.

The main components of the essential oil included 1.8 cineol (22.65%), davanone (13.82%), camphor (9.92%), Z jasmine (7.4%), and crysanthenone (4.11%).

The composition of *A. aucheri* essential oil has been examined previously by other researchers. Sefidkon et al reported verbenole (21.5%), camphor (21%), and 1.8 cineol (8.3%) as the main components of *Artemisia aucheri* essential oil (11).

Mohammadpoor et al reported camphor (45.5%), and 1.8 cineole (14.3%) as the main components of this plant essential oil (12).

Such differences in the essential oil compositions and the percentage can be attributed to the differences in geographical region, variety, plant age, and the method of drying and extraction of the oil (13). Previous reports showed good antibacterial effect of *A. aucheri* against some human bacteria.

Essential oil of *A. aucheri* has antibacterial activity against a variety of bacterial strains including *Escherichia coli*, *Staphylococcus aureus, Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (14, 15).

In another study (4) the essential oil of *A. aucheri* showed high antibacterial activity against both gram-positive and gram-negative bacteria. This shows the close relationship between the chemical structures of the most abundant compounds of essential oil, and extract, and their antimicrobial activity.

In this study, antibacterial activity of *A. aucheri* can be contributed to major compounds such as 1.8 cineol and catechin in essential oil as well as extract.

These compounds are known to have antibacterial and antifungal activities (16, 17).

Conclusion

In conclusion, our results suggests that *A. aucheri* essential oil and extract may be a source of antibacterial activity against plant bacterial pathogens. This study confirms that the essential oil and extract of *A. aucheri* has high antibacterial activities against 13 microorganisms.

Though both essential oil and extract of plants can be used in treatment of plant diseases, but applying extract instead of essential oil is more straightforward and cost effective, and can be a proper substitute for agriculture toxins.

Acknowledgment

Authors are thankful to Islamic Azad University, Shiraz branch, Shiraz, Iran for their support of the investigation.

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