Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Antibacterial Activity of Essential Oils and Plant Extracts of Artemisia (Artemisia annua L.) In Vitro

Alireza Massiha,[*1] Mohammadreza Majid Khoshkholgh-Pahlaviani, [2] Khosro Issazadeh, [1] Sirus Bidarigh, [3] Saeed Zarrabi[4]

1. Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Lahijan, Iran
2. Department of Biotechnology, Faculty of Basic Sciences, Islamic Azad University, Lahijan, Iran
3. Department of Agriculture, Faculty of Natural Resource, Islamic Azad University, Lahijan, Iran
4. Department of Chemistry, Faculty of Basic Sciences, Islamic Azad University, Lahijan, Iran

Article information

Article history:
Received: 20 July 2011
Accepted: 6 May 2012
Available online: 29 Oct 2012
ZJRMS 2013; 15(6): 14-18

Keywords:
Artemisia annua
Plant extract
Anti-bacterial
Minimum inhibitory Concentration

*Corresponding author at:
Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Lahijan, Iran.
E-mail:
amirmassiha@yahoo.com

Abstract

***Background*:** Many of the plants used to treat certain diseases, because they have showed antimicrobial activity. In this case, many studies have conducted on antimicrobial and antioxidant activity of *Artemisia annua*.

***Materials and Methods*:** The purpose of this study is to determine the antibacterial effects of aqueous, chloroform, methanol and ethanol extracts of *A. annua* against eight bacterial species. Antimicrobial activity, minimum inhibitory concentration and minimum bactericidal activity of the essential oil and extract was performed by agar disc diffusion and microdilution broth methods.

***Results*:** The obtained results showed antibacterial activity of the organic and chloroformic extracts of *Artemisia annua* against the tested microorganisms. Presence of tannins, saponins, alkaloids, amino acids, phenolic compounds, quinines and terpenoids were identified in the composition of the obtained extract using mass gas-chromatograph. The best result for the minimum inhibitory Concentration and minimum bactericidal concentration was reported for the 32 mg/ml of chloroformic extract.

***Conclusion*:** The results indicate the fact that the extracts and essential oils of the plants can be useful as medicinal or preservatives composition.

Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.

Introduction

Studies conducted in the world show that the essential oils of many plants have the ability to inhibit the growth of microorganisms. Therefore, medicinal plants have found many applications as antimicrobial agents [1]. From the chemical perspective; essential oils have mainly composed of poly propanoeids , aromatic compounds and sesquiterpenes. Herbal essential oils have antimicrobial activity on a large number of bacteria; and most of these compounds have phenolic groups in their structure. In fact they are considered due to their large amounts of aromatic compounds, because they have an important role in the defense system of the plant against microbial diseases due to their intrinsic anti-oxidative and anti-microbial properties.

Secondary metabolites that are produced as inactive precursors stored in the plant tissues include phenolic compounds, flavonols and flavonoids, glycosides, alkaloids and poly acetylenes. Recently, these compounds have been considered due to their inhibitory and bactericidal properties to kill pathogenic microorganisms [2]. Excessive usages of antibiotics often causes increasing bacterial resistance to these drugs. On the other hand, the uncontrolled use of antibiotics is often associated with the adverse effects in human; therefore, the use of medicinal plants with the active pharmaceutical and nutritional compounds can be effective on the improvement of treatment methods and probably as a substitute for the classic therapies [3]. According to the World Health Organization, using medicinal plants in developed countries has led 80 percent of the population of these countries to the traditional medicine.

Artemisia with the scientific name of *Asteraceae annua* L. which belongs to the Asteraceae family is one the main genera of the herbs which contains a wide number of species mostly found in the countries of northern temperate regions, where the rainfall varies from zero to 50 cm [4].

There are 34 species of annual and perennial herbaceous plants of this genus in Iran that spread throughout this country [5]. Various studies have shown that the annua species has been traditionally used to treat bacterial and parasitic infections [6]. Today their role in regulating plant growth and coping with the cancer has also been proved [7]. When assessing the chemical composition of the essential oil of this herb, it was also found that there are important derivatives, such as alkaloids, flavonoids, tannins and phenols in the aqueous extract of the leaves of this herb.

This herbal species can be used for coping with bacterial infections in human and plants [8]. Studies have shown that ethanol and chloroformic extracts of this plant has been able to prevent the growth of *E. coli* and *Bacillus subtilis*. Chloroformic extract of this plant has shown a significant inhibitory effect on the growth of Mucor fungi. The antifungal effect of the essential oil has been further emphasized in various studies compared to its antimicrobial activity [7].

In examining the chemical composition of the essential oil of this herb using Gas chromatography mass spectrometry (GC/MS), the presence of camphor (48%), 1, 8 cineole (9.39%), camphene (6.98%) and spathulenol (4.89%) in this herb were specified. The aim of this study was to determine the primary biological activity using the agar disk diffusion method and minimum levels of antibacterial activity of the essential oil and extracts obtained from the leaves of the annua species against standard strains and bacterial clinical isolates and environmental isolates of Bacillus species.

Materials and Methods

Sample Collection and Preparation of Different Herbal Essential Oils: In this empirical-experimental study, leaves of the *Artemisia annua* L. was collected from Lahijan flower and plant research station in September, during the flowering (blooming) stage. Then, they were washed with water and dried at the room temperature after being approved by a botanist expert. In order to prepare the essential oil, 10 ml of the distilled water was added to the 25 g powder and was placed on the shaker for 24 hours. This solution was dried after passing through the 0.22 µm membrane filter and the resulting powder was then dissolved in distilled water after weighing to obtain final concentrations of 5, 10, 25, 50 and 100 mg/ml of this solution.

Collection of Plant Materials and the Essential Oil Preparation: 400 grams of fresh leaves of annua species were dried in the air after washing and were exposed to the distilled water using Clevenger apparatus for 4 hours. Distilled essential oil was dried using anhydrous sodium sulfate and were stored at 4ºC in dark bottles.

Preparation of the Test Microorganisms: In this study, microbial strains were provided as lyophilised from Bacteria and fungi collection center of Scientific and Industrial Research Organization of Iran. Bacterial strain was opened in sterile conditions according to the manufacturer's instruction. The basic culture medium was prepared in tryptone soy broth and tryptone soy agar medium. A storage culture medium was prepared from the resulting culture and was used in the subsequent stages. A part of the culture was inoculated to the nutrient agar slope medium and was incubated at 37ºC for 24 hours at the room temperature. The colonies of medium surface were washed with normal saline and bacterial suspension was diluted with normal saline to make the level of absorption at a wavelength of 53 nm to be equal to the 0.5 McFarland.

Evaluation of the Antimicrobial Activity of the Crude Extract: Antimicrobial activity of the extracts was measured twice using agar dilution tests and based on recommendations by the National Committee for Clinical Laboratory Standards (NCCLS).

In summary, 15 ml of agar Mueller - Hinton medium was mixed with different concentrations of the essential oil of Artemisia and 10^4 CFU/ml of each sample of bacteria was inoculated to it. Plates were incubated for 24 hours at 37ºC. Minimum inhibitory concentration (MIC) was determined as the lowest concentration of each extract that prevents bacterial growth in the culture medium. During the next step, fluid diluting technique was used to determine the minimum bactericidal concentrations of the extracts of Artemisia. To perform this experiment; equal volumes of each bacterial suspension including 10^5 CFU/ml were inoculated to the Mueller - Hinton broth medium containing various concentrations of the extract of Artemisia.

Then mediums were incubated for 24 hours at 37ºC. 100 µl of each liquid culture were subsequently cultured on Mueller - Hinton agar medium and again were incubated at 37ºC for 24 hours. The minimum bactericidal concentration (MBC) was determined as the lowest concentration of the extract of Artemisia that completely killed the bacteria and no growth was observed.

Phytochemical Analysis of the Extract: In this study, Heywood [8] method was used to identify the active components of the herb with little changes in the method. 0 mm = Negative, (Weak) +1=1-4 mm, (Average) +2 =5-10 mm , (Strong) +3=11-15 mm, (Very strong) +4= ≥16 mm

Results

Results of antimicrobial activity of aqueous and alcoholic essential oil of Artemisia on bacterial species are shown in tables 1 and 2.

Artemisia extract phytochemical evaluation revealed the presence of alkaloids, flavonoids, phenols, quinines and terpenoids (Table 3).

Table 1. Antimicrobial activity of the organic extracts of Artemisia (*Artemisia annua*) using the method of disk diffusion in the agar (inhibitory zone diameter in millimeter)

Microorganism / Type of extract	S. aureus	E. coli	S. aureus	B. cereus	Bacillus sp	E. faecalis	UPEC	P. aeruginosa
Chloroform	7	10	12	10	10	7	12	10
Methanol	10	14	10	8	10	8	12	12
Ethanol	8	16	11	8	12	10	11	10
Solvent control (DMSO 10%)								
Positive control(P-S-C)	12	13	14	13	15	14	16	13

Table 2. Antibacterial activity of the aqueous extract of Artemisia (A.annua) (Inhibitory zone diameter in m/m)

Microorganism Extract concentration	S. aureus	E. coli	S. aureus	B. cereus	Bacillus sp	E. faecalis	UPEC	P.aeruginosa
5mg/ml	0	9	0	0	0	0	10	0
10mg/ml	10	13	10	10	14	13	14	9
25mg/ml	14	17	18	17	18	28	18	13
50mg/ml	18	19	28	33	28	33	28	18
Control negative(H_2O)	0	0	0	0	0	0	0	0
Penicillin	0	0	0	5	5	5	0	0
Streptomycin	33	14	28	18	28	18	14	28
Chloramphenicol	18	28	14	14	33	14	18	17

Table 3. Phytochemical composition of the organic solvent extracts of Artemisia (*A.annua*)

Organic solvents Compounds	Chloroform	Ethanol	Methanol
Alkaloids	+	++	++
Amino acids	-	+	+
Carbohydrates	-	+	+
Flavonoids	+	++	++
Glycosides	-	+	+
Tannins	-	++	+
Phenol	+	++	++
Phlobatannins	-	+	-
Quinines	+	+	+
Saponin	-	++	+
Terpenoids	+	++	++
Volatile oils	-	-	+

Table 4. Measurement of antimicrobial activity of the essential oil of Artemisia (*A.annua)*

Antimicrobial activity	Essential oil of Artemisia		Streptomycin	
Microorganism	MIC (g/l)	MBC (g/l)	MIC (µg/disc)	MBC (µg/disc)
S. aureus	0.031	0.031	0.24	0.24
B. cereus	0.053	0.055	0.98	0.98
E. faecalis	0.026	0.031	3.91	3.91
P.aeruginosa	0.025	0.053	1.95	1.95
S. aureus	0.033	0.073	62.50	62.50
E. coli	0.017	0.024	0.24	0.24
Bacillus sp.	0.026	0.053	1.95	1.95
UPEC	0.026	0.031	0.24	0.24

Table 5. Identified composition of Artemisia essential oil (Artemisia annua) using Gas chromatography mass spectrometry method

Component name	Retention index	Percent
1, 8 cineole	4.30	11.40
Ethyl-heptanoate	5.29	0.83
Linalool	5.24	8.01
Ethyl benzoate	6.48	1.17
4 terpineol	6.60	1.07
α terpinol	6.82	0.62
camphor	9.39	1.92
α Pinene	9.41	3.67
Eugenol	9.55	1.09
Camphene	9.86	0.21
Spathulenol	12.98	4.97
Gamma-Dodecalactone	14.35	2.36
Methyl Palmitate	17.48	0.91
Palmitic Acid	17.93	2.56
Ethyl Palmitate	18.31	1.20
Methyl Oleate	19.55	2.86
Stearic Acid	20.24	0.78
Ethyl Stearate	20.59	1.96
Tricosane	21.68	1.29
Tetracosane	22.69	0.83
Di Octyl Adipate	22.73	1.16
Geranyl acetate	22.86	0.98
Pentacosane	23.68	4.85
Di-(2-ethylhexyl) Phthalate	24.25	1.38
Triphenyl Phosphinsulphid (Ph3P=S)	25.25	1.57

MIC and MBC oil analysis showed that the highest antimicrobial activity belongs to the 32 microgram concentration [of the essential oil] (Table 4). MIC and MBC of the chemical composition of the essential oil using GC/MS apparatus relvealed that there are 25 major compounds in this essential oil which is equal to the 66.73% of the resulting oil. The most abundant compounds in the leaves of Artemisia essential oil are shown in table 5.

Discussion

In this study it was found that methanol and chloroformic essential oils had a good antibacterial activity against gram-positive and gram-negative bacteria. It seems that the activity of the essential oil of this herb is due to the interactions of its components. Also, the ecological impact of various geographical and climatic factors on the essential oil composition of different populations of a species or inter-species cannot be ignored. Therefore, more studies are needed to be done on antimicrobial effect of the essential oil of this species. In this study, it was found that the essential oil inhibits the growth of all bacteria in the used concentrations. While these microorganisms were sensitive to one or more components of the organic essential oil, alcoholic and chloroformic essential oils used in this study showed a significant level of deterrence against clinical isolates.

This study determined that the *A.annua* leaf oil shows better antimicrobial activity against used microorganisms, so that this effect, in comparison with ciprofloxacin, was more significant than other strains except *P.aeruginosa*. It was found that the minimum inhibitory concentration of the obtained essential oil was varied in the range of 0.026–0.33 g/L, while this value was 0.024-0.098 for ciprofloxacin.

Studies have shown that the phenolic compounds play an important role in the antimicrobial properties of plants. These compounds destroy microorganisms through destroying the cell walls and proteins, interfering in the work of membrane enzymes and affecting DNA and RNA replication. Essential oils having different chemical composition apply different mechanisms in order to destroy microorganisms. The most important properties of this group is having hydrophobic properties, so that these substances penetrate cell membranes of bacteria and mitochondria and cause cell dysfunction followed by increased permeability and subsequent removal of ions and other cell contents.

Removal of molecules and cells cause the death of microorganisms. However, the chemical structure of each essential oil has a direct impact on the level of antimicrobial activity. Research has shown that in many cases, compounds such as 1, 8 cineole, carvacrol, thymol and para cymene are the most important components of the herbal essential oils as far as antimicrobial activity is concerned. As the results of this study showed that 1, 8 cineole and linalool are the major components of the essential oil of Artemisia, the antimicrobial activity of this essential oil can be attributed to these compounds.

Mojarrab et al. [9] evaluated the aerial parts of 14 different species for their antioxidant activity. In this study it was found that the methanol extract shows strong antioxidant activity in all plants tested. Maggi et al. [10] who studied the antimicrobial activity of the extracts of the leaves of this species showed that the ethanol extract of aerial parts causes repelling of insects. In a study it was found that there are different major phytochemical components in methanol extracts of aerial parts of different species of Artemisia in Iran and this is due to the presence of active compounds such as tannins, polyphenols, alkaloids, glycosides, flavonoids, steroids and saponins. Evaluation of the chemical composition of the essential oil of this herb showed higher levels of monoterpene. Based on conducted studies, Chinese and Vietnamese seed genotypes have different percentages of active ingredients such as ketone, myrcene, 1, 8 cineole are particularly Gramicidin.

Woerdenbag et al. [11] showed the highest rate of ingredients of the essential oil before the flowering (blooming) stage at Vietnamese genotype which contained 55% monoterpene.

In a study conducted by Hethelyi et al. [12] 170 different types of Hungarian essential oils were evaluated during the flowering stage and it was found that the rate varies between 0.48-0.81%.

A study conducted in India showed that ketone (% 58.8), camphor (15.8%), 1, 8 cineole (2.2%) and Gramacidin-D (2.4%) are the main components of the essential oil of this herb [13]. Friedman et al. [14] showed different antimicrobial activities for the essential oil. A study conducted in Iran by Verdian et al. [15] showed that there are 32 components in the essential oil of *A.annua* and camphor (48%), 1, 8 cineole (9.31%), camphene% (6.98%) and spathulenol (4.89%) were identified as the main components.

Fabien et al. showed that the essential oil of *A.annua* has been able to significantly inhibit the growth of the tested fungi. The reasons for this case are varied and are depended on the species and the growth conditions, different methods of extracting essential oils, etc.

According to Recio et al. [16] oils represent different antimicrobial activity because of having chemical compounds. It seems that the mechanism of antimicrobial activity of the native essential oils still is not clear and requires further studies.

Acknowledgements

Hereby, authors of this paper would like to appreciate the management and staff of Lahijan Flowers and Plants Research Station and also the staff of Chemistry and Microbiology sector of the Lahijan Islamic Azad University, especially Mrs. Asadi and Mrs Ali Nia due to their cooperation in the successful implementation of this research project with the registration number of 87 (conducted by Dr. Alireza Massiha).

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest
The authors declare no conflict of interest.

Funding/Support
Tarbiat Modares University.

References

1. Zargari A. Medicinal plants of Iran. 4th ed. Tehran: Tehran University Press; 1994: 302-306.
2. Das S, Pal S, Mujib A, editors. Biotechnology of medicinal plants recent advances and potential. 1st ed. Hyderabad: UK 992 Press; 1999: 126-139.
3. Amin G. Traditional medicinal plants of Iran. Tehran: Ministry of Health, Treatment and Medical Education Press; 1991: 69.
4. Burt S. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int J Food Microbiol 2004; 94(3): 223-253.
5. Mozaffarian V. Dictionary of Iranian plant names. Theran: Farhang-e-Moaser; 2007: 56-58.
6. Ghahreman A. Flora of Iran. Res Inst Fores Rang 1984; 15: 18-19
7. Fabien J, Masottia V, Marie Bessie J, et al. Antibacterial and antioxidant activities of Artemisia annua essential oil. Fitoterapia 2002; 73(6): 532–535.
8. Heywood VH, Harborn JB, Turner BL, editors. The biology and chemistry of the composited. London: Academic Press; 1997: 868.
9. Mojarab M, Emami SA, Hassanzadeh MK. Antioxidant activities of methanol extracts of different species of Artemisia from Iran. Pharmacol Online 2009; 2: 797-807.
10. Maggi ME, Mangeaud A, Carpinella MC, et al. Laboratory evalution of Artemisia annua L. extract and artemisinin activity against Epilachna paenulata and Spodoptera eridania. J Chem Ecol 2005; 31(7): 1527-36.
11. Woerdenbag HJ, Luers JFJ, Uden W, et al. Production of the new antimalarial drug artemisinin in shoot cultures of Artemisia annua L. Plant Cell Tissue Org Culture 1993; 32(2): 247-257.
12. Hethelyi E, Tetenyi P, Kettenes-van den Bosch JJ, et al. Essential oils of five Tanacetum vulgare genotypes. Phytochem 1981; 20(8): 1847-1850.
13. Gupta PC, Dutta B, Pant D, et al. In vitro antibacterial activity of Artemisia annua Linn. growing in India. Int J Green Pharm 2009; 3(3): 255-258.
14. Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica. J Food Prot 2002; 65(10): 1545-1560.
15. Verdian-rizi MR, Sadat-Ibrahimi E, Hadjiakhoondi A, et al. Chemical composition and antimicrobial activity of Artemisia annua L. essential Oil from Iran. J Med Plants 2008; 7: 59-62.
16. Rios JL, Recio MC. Medicinal plant and antimicrobial activity. J Ethnopharmacol 2005; 100(1-2): 80-84.

Please cite this article as: Massiha A, Khoshkholgh-Pahlaviani MM, Issazadeh K, Bidarigh S, Zarrabi S. Antibacterial activity of essential oils and plant extracts of Artemisia (Artemisia annua L.) in vitro. Zahedan J Res Med Sci (ZJRMS) 2013; 15(6): 14-18.