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Research Article

Chemical Composition and Antibacterial Properties of *Chenopodium botrys* L. Essential Oil

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ABSTRACT
Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in treatment of antibiotic-resistant bacteria. As we know, there is no documented proof on antibacterial effects of *Chenopodium botrys L.* essential oil in west of Iran. Gas chromatography mass spectrometry was done to determine chemical composion. As a screen test to discover antibacterial properties of the essential oil, agar disk and agar well diffusion methods were employed. Macrobroth tube test was performed to specify MIC. The findings show that the most substance found in *C. botrys* essential oil was α-eudesmol. Also, the results indicated that *C. botrys* essential oil prevented *Escherchia coli O157:H7* and *Staphylococcus aureus* from the growing in concentrations 0.007 g/ml. Thus, the present research demonstrates the antibacterial effects of the medical plant on Gram-negative and Gram-positive pathogenic bacteria, suggesting to use as antibacterial supplements in the developing countries towards the development of new therapeutic agent.

Keywords: *Chenopodium botrys L*; Essential oil; Chemical composition; Antibacterial effect.

INTRODUCTION

Bacterial infections are responsible for many deaths each year. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant[1]. The spread of drug resistant pathogens is one of the most serious threats to successful therapy of microbial diseases. Down the ages plants have evoked interest as sources of innate products. They have been screened for their potential uses as alternative remedies for the treatment of several infectious diseases[2]. Some medicinal plants used in traditional Iranian medicine are efficient in treating diverse ailments caused by bacterial and oxidative stress[3]. There is growing concern in correlating phytochemical constituents of plant with its pharmacological activity. In herbal medicines, raw plant essential oils in the form of infusion, decoction, tincture or herbal essential oil are traditionally consumed by the population for the treatment of diseases including infectious diseases. Plant-derived products have a major variety of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds. The antibacterial properties of essential oils have been identified for many years, and their rudiment have found applications as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food maintenance, etc. There are reports of the active principles of essential oils from different plants with antifungal or antibacterial effect. Herbal essential oils have antimicrobial activity on a wide number of bacteria, and most of these compounds have phenolic groups in their structure. The original benefit of natural factors is that they do not increase the "antibiotic resistance", an event usually encountered with the long-term use of synthetic antibiotics; because they have a significant role in the defense system of the plant to microbial diseases due to their intrinsic anti-oxidative and anti-microbial properties[4]. The compounds of plant essential oils contain numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic antithrombotic and vasodilatory activities[5]. The genus *Chenopodium* (Family: *Chenopodiaceae*) includes differents of weedy

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herbs (more than 200 species) native to much of Europe, Asia, and both North and South America[6]. *C. botrys* has been found in Kermanshah, Azerbaijan, Hamedan, Khorasan, Mazandaran, Sistan & Bluchestan and Tehran provinces of Iran. In Iranian herbal medicine, *C. botrys* is used as expectorant, antibacterial, anticonvulsant, and tonic[7]. The herb contains flavonoids, alkaloids and different terpenoids. *C. botrys* of various origins produced 0.08-2% essential oil[8-13]. The essential oil varied in amount and composition. The significant components of *C. botrys* oil from Kermanshah were α-eudesmol, epi- α-muurolol and cubenol, respectively. The aim of this study was to screen the in vitro antibacterial activity of the plant essential oil against some bacteria including *E. coli O157:H7, S. aureus, and B. subtilis.*

MATERIAL AND METHODS

Plant sample collection

In the empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

Essential oil extraction

Essential oil from fresh, clean, weighed aerial part *C. botrys* fruits extracted by hydro-steam distillation using the Clevenger apparatus were collected and stored in sterile vials. Briefly, 100 to 150 g of plant was introduced in the distillation flask (1L), which was connected to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oil were released from the plant material and evaporated into hot steam. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system in order to condense the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was filtered through anhydrous Na_2SO_4 to dry the yielded essential oil. Afterward, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the essential oil were done using dimethyl sulfoxide (DMSO).

Gas chromatography mass spectrometry (GC/MS)

C. botrys essential oil was analysed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 220°C and 250°C, respectively. One microliter of each solution in hexane was injected and analyzed with the column held initially at 60°C for 2 min and then increased by 3°C/min up to 300°C. Helium was employed as carrier gas (1 ml/min). The relative amount of individual components of the total essential oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds and mass spectra.

Source of microorganisms

Tow bacterial species namely *E. coli O157:H7* (ATCC No. 25922) and *S. aureus* (ATCC No. 25923) were procured from Veterinary school of Tehran University as lyophilized. Each bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10^8 cfu/ml using Muller Hinton broth.

Culture media

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

Evaluation of antimicrobial activities

Agar disk diffusion and agar well diffusion were used as screen tests to evaluate antibacterial property of essential oil of *C. botrys* based on standard protocol. The solution of the essential oil was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk in order. After a period of 24 hours' incubation, the diameters of growth inhibition zones around the disks were measured. DMSO was used as negative control whereas kanamycin and cephalexin were used as positive controls in case of *E. coli* and *S. aureus*, respectively. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In the case, after incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter[14].

Statistical Analysis

Antibacterial effect was determined by One-way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.05$.

RESULTS

Chemical composition

The most substances found in *C. botrys* essential oil was α-eudesmol. In contrast, β-cubebene was the least constituents discovered in *C. botrys* essential oil. Composition of these plants using Gas chromatography mass spectrometry method can be perceived in table 1.

Agar disk diffusion test

In case of *C. botrys*, the widest zone was formed due to 0.031 g/ml of the essential oil in *E. coli* culture (equivalent to positive control: kanamycin) and it was no halo in 0.002 g/ml and less for all bacteria. No inhibition zone was observed due to DMSO. Growth inhibition zones due to different dilutions are listed in table 2.

Agar well diffusion test

In regard to *C. botrys* essential oil, the widest zone was seen in 0.031 g/ml, due to *E. coli* (16 mm) and it was no halo in 0.002 g/ml and less for all bacteria. It was no growth inhibition in 0.002 g/ml and less for all bacteria. The data are discoverable in table 3.

MIC determination

Toward *C. botrys* essential oil, MIC was 0.007 g/ml for all bacteria (Table 4).

MBC ascertaining

0.007 g/ml was the MBC for all bacteria in *C. botrys* essential oil (Table 5).

DISCUSSION

In spite of the current interest in drug discovery by molecular modelling, combinatorial chemistry and other synthetic chemistry methods, plant-derived compounds are still substantiating to be an important source of medicines for human being. Because of their safety and low cost as well as their impact on a wide number of microbes[15], medicinal plants may have the potency to treat bacterial resistance to different types of antibiotics. The antimicrobial effects of aromatic oils extracted from a vast number of plants have been appraised and reviewed, and the mechanisms that enable the natural components of herbs and spices to resist microbes have been discussed[16-18]. The results show that these mechanisms vary greatly depending on the components of the plant[19,20]. Since the antibacterial effectiveness of medicinal plants destabilized significantly depending on the phytochemical characteristics of plan families and subfamilies, it is not surprising to note the difference in this efficacy even when using samples taken from the similar plant, but from two various regions[21]. Chenopodium botrys is native to Asia (India, China, and Iran), Europe and adventive in much of North America. The plant has been used traditionally for medicinal purposes; generally, these therapeutic uses and health benefits of C. botrys are largely based on scientific substantiation, making it a good candidate to gather documentations, including the phytochemical content, in vitro experiments, animal models and human studies available in the scientific studies[6,7]. In this study, Presence of α-eudesmol, epi- α-muurolol, cubenol, germacrene D-4- ol, elemol, bis (2-ethyl hexyl)-phethalates, 8-cadinene, phethalates, A-chenopodiol, germacrenen D, γ-elemene, hinesol, α-eudesmol acetate, γ-eudesmol acetate, β-elemene, carotol, B-chenopodiol, botrydiol, viridyflorol, α-copaene- 11 –ol, guaiol acetate, β-myrcene, β-gurjunene, α-cadinene, γ-eudesmol, juniper camphor, β-funebrene, cubenene, β-caryophyllene, β-cubebene were identified in the composition of the obtained *C. botrys* essential oil using mass gas-chromatograph. The most substances found in *C. botrys* essential oil was α-eudesmol (15.5%), respectively. In contrast, β-cubebene (0.2 %) was the least constituent discovered in *C. botrys* essential oil. As the tables showed, *C. botrys* essential oil have prevented the growth of *E. coli*, *S. aureus* and *B. subtilis* and destroyed them. Also, by increasing the concentration of *C. botrys* essential oil, the inhibition zone increased ($p \leq 0.001$). The results determined that in tested bacteria, there was a significant difference ($p \leq 0.001$) in terms of sensitivity to *C. botrys* essential oil. In other words, the most sensitivity was observed in *E. coli*. Also, the results indicated that *C. botrys* essential oil with 0.007 g/ml concentration has prevented *E. coli*, *S. aureus* and *B. subtilis* from the growth. There are similarities between this result and the resembling studies. Maksimović et al extracted essential oil from aerial parts of *C. botrys* collected (Southern Serbia) indicated considerable bactericidal and fungicidal effect to selected strains of microorganisms, including *Aspergillus niger*, *Candida albicans*, *Sarcina lutea*, *Klebsiella pneumoniae*, *Salmonella enteridis* and *Shigella flexneri*[22]. El-Sayed et al showed that the essential oil of *C. botrys* growing in Saudi Arabia have antimicrobial effect[11]. Tzakou et al presented essential oil from aerial parts of *C. botrys* growing in Greece have antimicrobial

Table 1: Identified main composition of the *C. botrys* essential oil using Gas chromatography mass spectrometry method.

Compound	Percent
α-eudesmol	15.5
epi- α-muurolol	11.3
cubenol	10.5
germacrene D-4- ol	7.5
elemol	6.9
bis (2-ethyl hexyl)- phethalates	6.3
8-cadinene	5.3
phethalates	4.9
A-chenopodiol	3.9
germacrenen D	3.9
-elemeneγ	3.1
hinesol	2.7
α-eudesmol acetate	2.6
γ-eudesmol acetate	1.9
β-elemene	1.8
carotol	1.6
B-chenopodiol	1.2
botrydiol	1.1
viridyflorol	1.1
α-copaene- 11 -ol	0.9
guaiol acetate	0.7
β-myrcene	0.7
β-gurjunene	0.6
α-cadinene	0.6
γ-eudesmol	0.4
juniper camphor	0.4
β-funebrene	0.3
cubenene	0.3
β-caryophyllene	0.2
β-cubebene	0.1
TOTAL	98.3

Table 2: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *C. botrys* essential oil.

Dilution(g/ml)		Inhibition zone in disk diffusion (mm)	
Microorganism	*E. Coli*	*S. aureus*	*B. Subtilis*
Positive control	22	16	22
1/32 (0.031)	22	17	14
1/64 (0.015)	19	16	9
1/128 (0.007)	14	13	0
1/256 (0.003)	13	0	0
1/512 (0.002)	0	0	0
1/1024 (0.001)	0	0	0
Negative control	0	0	0

Table 3: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *C. botrys* essential oil.

Dilution(g/ml)		Inhibition zone in disk diffusion (mm)	
Microorganism	*E. Coli*	*S. aureus*	*B. Subtilis*
1/32 (0.031)	16	14	13
1/64 (0.015)	13	9	9
1/128 (0.007)	9	0	8
1/256 (0.003)	8	0	0
1/512 (0.002)	0	0	0
1/1024 (0.001)	0	0	0
Negative control	0	0	0

Table 4: MIC for the *C. botrys* essential oil

Microorganism	*E. Coli*	*S. aureus*	*B. Subtilis*
MIC (*C. botrys* essential oil)	1/128 (0.007)	1/128 (0.007)	1/128 (0.007)

Table 5: MBC for the *C. botrys* essential oil

Microorganism	*E. Coli*	*S. aureus*	*B. Subtilis*
MBC (*C. botrys* essential oil)	1/128 (0.007)	1/128 (0.007)	1/128 (0.007)

activity[23]. Kokanova-Nedialkova et al and Lyubenova et al exhibited *C. botrys* essential oil have considerable antibacterial effect to *Bacillus cereus*; Also showed that the residual water solution a good effect versus *Salmonella heidelberg* and *Bacillus cereus*[24,25]. Mahboubi et al collected the essential oil of *C. botrys* from suburb of Kashan (Iran) and indicated strong antimicrobial activity to *Staphylococcus saprophyticus* followed by *Klebsiella pneumoniae*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Listeria monocytogenes* and *Salmonella typhimurium*; The essential oil had inappreciative effect on *Candida albicans* and showed inhibitory effect from it on *Aspergillus* species[26]. Our results support the use of the plant in traditional medicine and suggest that *C. botrys* essential oil possess compounds with good antibacterial properties. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Additional *in vivo* studies and clinical trials would be needed to justify. Also, further evaluation is necessary on potential of it as an antibacterial agent in topical or oral applications.

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