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Original Article

The Chemical Composition and Antimicrobial Activity of Essential Oils of *Vaccinium Arctostaphylos* L.

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ABSTRACT

Objective: The growing resistance of different bacteria to antibiotic chemical drugs have become the global issue. This investigation is the first study of the antimicrobial activity of the essential oil of flowering aerial parts of *Vaccinium arctostaphylos*. The current research was conducted to evaluate the antibacterial effects of essential oils and hydroalcoholic extract extracted from aerial parts of *Vaccinium arctostaphylos*, native species. **Methods:** The chemical composition of the essential oils extracted by hydrodistillation from the flowering aerial parts of *Vaccinium arctostaphylos* L. was assessed by the GC and GC/MS analysis. Twentynine different compounds, constituting 86.61% of the oil, were recorded, among which the major compounds were α-Pinene (15.5%), Linalool (11.7%), Sandaracopimaradiene (5.9%) and Safranal (8.8%). The *invitro* antibacterial activity of the extracted essential oil was evaluated against some Gram positive and negative bacteria. **Results:** According to the obtained results from the current research, the essential oil of *Vaccinium arctostaphylos* showed the antibacterial activities against most of the bacteria tested, including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Streptococcus pneumonia*, contrasted to *Staphylococcus aureus.* In contrast to the essential oil, the hydroalcoholic extract of *Vaccinium arctostaphylos* did not display the antibacterial effects against the mentioned microorganisms.

1.INTRODUCTION

Nowadays, the growing resistance of different microorganisms to antibiotic drugs produced by the various pharmacological industries have become the global issue (Teimouri, 2012). Therefore, the medicinal plants as a valuable source of natural products for improving human health should be studied to identify their phytochemical characteristics and determine their potential for applying in food and pharmacology related industries (Nascimento *et al*., 2000; Rathee *et al*., 2012**)**. The studies of aromatic and medicinal plants, especially native Traditional medicine, is regarded as a suitable way of finding plants rich in the sufficient essential oils (Mohammed and Al-Bayati, 2009). The essential oils display various renges of pharmacological activities, especially antibacterial, antifungal, antiviral and antioxidant (Nezhadali *et al*., 2014). The qualitative and quantitative properties of essential oil vary between species and considerably is affected by the different environmental factors. Terpenoids are the structurally diverse class of plant secondary metabolites (Cheng *et al*., 2007; Van Schie *et al*., 2007; Schmidt *et al*., 2010) which play a critical role in plant interaction with surrounding environment (Oraghi Ardebili *et al*., 2013). The genus *Vaccinium* (Ericaceae) consist of about 200 species which

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is found in northern forests of Iran between 1600-1800 m above sea level, among which *Vaccinium arctostaphylos* L. (*V. arctostaphylos)* is a native to Iran and rich in essential oil applied in alternative medicine (Nickavar *et al*., 2002; Feshani *et al*., 2011). Vaccinium has many benefits for health, such as hypoglycemic effects, spreading out the arteries, antibacterial effects on the urinary tract infections, avoid stroke, heart and brain (Feshani *et al*., 2011). *Vaccinium arctostaphylos* fruit has been introduced as a potent antidiabetic agent with antihyperglycemic, antioxidant and triglyceride lowering roles (Feshani *et al*., 2011).

The presence of various chemicals, including Delphinidin 3-O-b-glucoside, Ptunidin 3-O-b-glucoside, Malvidin 3-O-b-glucoside flavonol glycosides (Nickavar and Amin, 2004; Mzhavanadze, 1971a), Coumarins (Mzhavanadze *et al*., 1971b) in the leaves as well as the phenolic acids and their derivatives (Mzhavanadze *et al*., 1971b) in the essential oil of *V. arctostaphylos,* extracted from different organs, have been recorded in severa researches. Furthermore, Nickavar *et al*. (2002) has assayed the essential oil of flowering aerial parts. The present study is the first study of the antimicrobial activity of the essential oil extracted from the flowering parts of *Vaccinium arctostaphylos.*

2. MATERIALS AND METHODS

2.1. Plant material

The flowering aerial parts of *Vaccinium arctostaphylos* were collected from the forest region of Talesh in the north of Iran in June 2013.Voucher specimens were deposited in the Herbarium of Faculty of Science Research Kerman University Kerman, Iran (no. 7521 KHR).

2.2. Oil isolation

The air dried flowering aerial parts (200 g) were crushed and hydrodistilled for 4 h in a Clevenger apparatus. The distillate was extracted with petroleum benzene 40–60 °C. The organic layer was separated, dried over anhydrous sodium sulphate and concentrated to 0.5 ml under reduced pressure. The concentrated extract had a strong odor and yellow color.

2.3. GC/MS analysis

GC analysis was performed using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out on fused silica capillary DB-1 column (25 m × 0.25 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 and 300°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 1.1 $mlmin^{-1}$; the oven temperature program was 60 to 250°C at the rate of $4°min^{-1}$ and finally held isothermally for 15 min; the split ratio was 1:50. GC-MS analysis was carried out by use of Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-1column (60 m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200 and 250°C, respectively. Mass range was from 35 to 456 amu. The oven temperature program was the same given earlier for the GC. The constituents of the essential oils were identified by the calculation of their retention indices under temperature programmed conditions for n-alkanes (C6 to C24) and the oil on the DB-1 column under the same chromatographic conditions. The identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by the comparison of their retention indices with authentic compounds or with those reported in his literature (Adams, 2001; Shibamoto, 1987). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

2.4. Preparation of hydroalcoholic extract

The hydroalcoholic extract from the air dried flowering aerial parts were prepared using the maceration method.

2.5. Antibacterial activity

2.5.1. Bacterial strains

The essential oils and hydroalcholic extract were tested against bacteria, including Gram negative (*Escherichia coli* ATCC 25832, *Pseudomonas aeruginosa* ATCC 25752), Gram positive strains (*Staphylococcus aureus* ATCC 28523 and *Enterococcus faecalis* ATCC 27311) as well as two other clinically isolated strains *Klebsiella pneumoniae* and *Streptococcus pneumoniae*.

2.6. Disc diffusion method

The agar disc diffusion method was used as a preliminary assay for testing the antibacterial effect of essential oil extracted from the flowering aerial parts of *Vaccinium arctostaphylos* (NCCLS, 2001). A suspension of the tested bacteria, $2×10^6$cfu/ml, was spread on the solid media plates. Filter paper discs of 6 mm in diameter were individually impregnated with 20 ml of the essential oil and then placed on the previously inoculated agar plates. The petri dishes were kept at 4^oC for 2 h and then incubated at 37^oC for 24 h. The diameters of inhibition zones were measured and expressed in millimeters. Levofloxacin was used as a positive control.

2.7. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and the

minimum bactericidal concentration (MBC) (NCCLS, 2001). All tests were performed in Muellere Hinton Broth (MHB). The essential oil was dissolved in 10% dimethylsulphoxide (DMSO). A serial doubling dilution of oil was prepared in a 96 well micro-titer plate over the range of 0.05-100 $mgml^{-1}$. Overnight broth cultures of each strain were prepared, the final concentration in each positive well was adjusted to 2×10^6 $cfuml^{-1}$ and the plates were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the essential oil at which the bacteria do not show any visible growth. To determine MBC, broth was taken from each well and inoculated in Muellere Hinton agar for 24 h at 37°C. The MBC is defined as the lowest concentration of the essential oil at which inoculated bacteria was, totally killed. Levofloxacin and 10% DMSO solution served as positive and negative controls, respectively.

3. RESULTS AND DISCUSSION

The yield of essential oil extracted using the hydrodistillation method from the flowering aerial organs of *V. arctostaphylos* L. was 0.2%. As it was presented in the table 1, GC analysis revealed that there were 29 different constituents. The presence of 26 compounds in flowering shoots, have been recorded by Nickavar *et al.* (2002), among which the compounds, including Safranal, Vitispirane, α-Terpineol, Carvacrol and Linalool were equal with our findings. The differences found between these studies may mainly attribute to their different samples and habitats of plants. It has been well understood that the productions of the secondary metabolites are considerably influenced by the varieties of ecological characteristics. According to the obtained results given in Table 1, α-Pinene (15.5%) , Linalool (11.7%), Sandaracopimaradiene (5.9%) and Safranal (8.8%) were determined as the major constituents of essential oil of flowering shoots of *Vaccinium arctostaphylos* harvested from Talesh forest.

The evaluation of the *invitro* antibacterial activities of the essential oil extracted from the flowering shoots of *V. arctostaphylos* against the examined bacteria reflected that these extracts showed the moderate antibacterial activities against most of the bacteria tested, including *E. coli, E. faecalis, K. pneumoniae and S. pneumonia*, in contrast to *S. aureus*. This investigation was the first study in the antimicrobial activity of the essential oil of flowering aerial parts of *Vaccinium arctostaphylos* L. The antimicrobial properties of the oil has been attributed to the higher levels of compounds such as Sandaracopimaradiene, a diterpene, which have displayed considerably antimicrobial activity against some examined bacteria and fungi (Van Puyveldevan *et al*., 1986). In conclusion, according to the obtained results from the current research it could be concluded that the essential oils extracted from flowering shoot of *Vaccinium arctostaphylos* harvested from Talesh region, located in Gilan Province in Iran, is rich in α-Pinene, Linalool, Sandaracopimaradiene and Safranal, thereby having considerable antibacterial effects against some bacteria.

Table1.

the composition of the essential oil of *Vaccinium arctostaphylos* L.

NO	Components	RI
1	α-Thujene	921
2	α-Pinene	938
3	Camphene	955
4	β-Pinene	987
5	Myrcene	993
6	α-Phellandrene	1004
7	α-Terpinene	1010
8	para-Cymene	1028
9	safranal	1030
10	1,8-Cineole	1038
11	trans-Ocimene	1048
12	γ-Terpinene	1055
13	Terpinolene	1077
14	Vitispirane	1099
15	Borneol	1122
16	α-Terpineol	1138
17	Linalool	1219
18	Carvacrol	1256
19	Eugenol	1298
20	α-Copaene	1366
21	β-Bourbonene	1392
22	Sandaracopimaradiene	1439
23	β-Gurjunene	1441
24	α-Humulene	1474
25	β-Bisabolene	1494
26	γ-Cadinene	1530
27	δ-Cadinene	1539
28	Spathulenol	1597
29	B-Citroniol	1611

Table 2.

The antimicrobial activity of the essential oil of *Vaccinium arctostaphylos* L.

Microorganisms	DD[a]	MIC[b]	MBC[b]	Levofloxacin		
				DD[c]	MIC[d]	MBC[d]
Escherichia coli ATCC 25832	28	0.34	0.34	32	0.59	0.59
*Staphylococcus aureus*ATCC28523	14	24	24	29	0.2	0.58
Pseudomonas aeruginosa ATCC 25752	15	0.37	0.49	19	2.33	9.66
Enterococcus faecalis ATCC27311	19	1.46	2.99	15	1.33	1.33
Klebsiella pneumoniae	16	0.44	0.74	10	4.58	4.58
Streptococcus pneumoniae	11	5.96	11.9	16	1.33	18.99

[a]Tested at a concentration of 20ml/disc. [b]Values given asmg/ml. [c]Tested at a concentration of 5mg/disc. [d]Values given asmg/ml

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