

Antimicrobial activity of essential oil from *Oliveria decumbens* and its synergy with vancomycin against *Staphylococcus aureus*

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S u m m a r y

Oliveria decumbens Vent (*Umbelliferae*) is a shrub commonly found in the South-East of Iran. Its aerial section is extensively used in herbal medicine. The Disk Diffusion Test and Microbroth Dilution Assay were used to determine the antimicrobial activity of the essential oil from *Oliveria decumbens* Vent against *Staphylococcus aureus*.

To detect synergy, vancomycin was added to Mueller-Hinton agar at sub-inhibitory concentrations and the inhibitory zones were recorded in millimeters. The main components of oil were thymol (22%), carvacrol (22%) and p-cymene (19%). The *O. decumbens* oil exhibited strong antistaphylococcal activity (18.0 ± 0.86). Carvacrol was considerably more effective (29.8 ± 1.5) than thymol (17.2 ± 1.13) and p-cymene (0.0 ± 0.0) against *Staphylococcus aureus*. The oil presented strong synergism with vancomycin (24.9 ± 0.75 vs. 19.3 ± 0.54 , $p < 0.001$). However, further studies are required to evaluate its *in vivo* efficacy.

Key words: *Staphylococcus aureus*, *Oliveria decumbens*, essential oil, drug resistance, thymol, carvacrol, p-cymene, vancomycin, synergy

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INTRODUCTION

Staphylococcus aureus is the second pathogen after *Escherichia coli* encountered in primary and secondary skin infections. It is also colonized in 30% of burn wounds [2]. Outbreaks of methicillin resistant *S. aureus* (MRSA) have created major infection problems for burn units and intensive care units (ICUs) [3]. The resistance of *S. aureus* to other antimicrobial agents has increased in recent years. Consequently, the search for a new prototype of drugs to combat infections with multi-drug resistant isolates of *S. aureus* is necessary. The plant's essential oils may offer preferred alternatives for current drugs.

Oliveria decumbens which is endemic to Iran belongs to the family of *Umbeliferae*. Its local names are "den", "denak" and "moshkorak". It is used in traditional medicine for treating indigestion, diarrhea, abdominal pains and fever [1]. Thus far, the antimicrobial properties of *O. decumbens* essential oil have only been investigated on standard strains of bacteria and fungi [1]. This paper presents the first detailed investigation of anti-staphylococcal properties of *O. decumbens* essential oil and evaluation of the synergism rate of oil with vancomycin against clinical strains of *S. aureus*.

MATERIALS AND METHODS

Oliveria decumbens

The herb was collected in May 2007 from the Kazeron area in the Fars province, southern Iran. The hydro-distillation method was used to extract the essential oil from the aerial segment (100 mg) of *O. decumbens* by Clevenger-type apparatus for 6 h. The essential oil was dried by sodium sulphate. Afterwards, 3.2 ml of essential oil was obtained (3.2% v/w). The main components of the essential were thymol (22%), carvacrol (22%) and p-cymene (19%). Thymol (99.9%), p-cymene (99.6%) and carvacrol (95%) were purchased separately from Roth (Carl Roth KG, Karlsruhe, Germany) and Merck (Darmstadt, Germany), respectively. Vancomycin was purchased from Fluka (Chemie AG, Switzerland).

Staphylococcal isolates

The clinical isolates of *S. aureus* cultured from patients at Tehran University hospitals (n=13) and *S. aureus* ATCC 25923 were used in all experiments.

Screening for the antimicrobial potential of essential oil on *S. aureus*

The isolates were grown in brain heart infusion (BHI) overnight. After the growth, 100 μ l of broth culture from each isolate were suspended in saline solution and its turbidity was adjusted to 0.5 McFarland. The adjusted solution was inoculated on the surface of Mueller Hinton agar plates where disks containing 0.5 μ l of den oil and its main components were then placed on the inoculated Mueller Hinton agar. To impregnate the disk with essential oils and its components, the sterile filter paper disks (6 mm in diameter) were saturated with 10 μ l of DMSO containing 0.5 μ l of Den oil. The same method was used to impregnate the blank disks with thymol, p-cymene and carvacrol, either separately or with different combinations of these components of the essential oil. Disks containing 30 μ g of vancomycin (Oxoid, UK) were also placed. The DMSO was used to dissolve the den oil or its components. The blank disk impregnated with DMSO was used as a control. The plates were incubated at 37°C for 24 h and the diameters of inhibitory zones were measured. The DMSO disks showed no inhibition zone in this study [4]. The assay was performed in duplicate.

The synergy of den oil with vancomycin

Initially the minimal inhibitory concentration (MIC) of vancomycin against all isolates (n=14) was determined using micro-broth dilution assay [5]. The MIC 90% for this glycopeptide was determined and one fourth of its value was used in synergy with essential oil [6]. Vancomycin was added to Mueller Hinton agar plates at sub-inhibitory concentrations (0.125 μ g). A plate of Mueller Hinton agar without vancomycin was used as a control. The turbidity of bacterial suspension was adjusted to 0.5 McFarland before inoculating onto the plates. The blank disks impregnated with 0.5, 1, 2, 3 μ l of the essential oil were put into the plates containing Mueller Hinton agar plus vancomycin. The diameters (mm) of the inhibitory zones were recorded after incubation at 37°C/18 h.

STATISTICAL ANALYSIS

The diameters of inhibitory zones were subjected to the one-sample Kolmogorov-Smirnov test to check their distribution throughout the population. The me-

ans obtained for antistaphylococcal activity of the essential oil and its components were analyzed by One-Way ANOVA. In case of any differences between the means, the Scheffé post hoc tests were performed. The two-sample t-test was used to compare the average inhibition zones for the MHA medium without vancomycin and the MHA medium with vancomycin ($0.125 \mu\text{g/ml}$) obtained in the synergy test.

RESULTS AND DISCUSSION

In comparison to vancomycin, the antimicrobial activity of *O. decumbens* oil was high ($p < 0.01$, see tab. 1, fig. 1A, 1B, 1C).

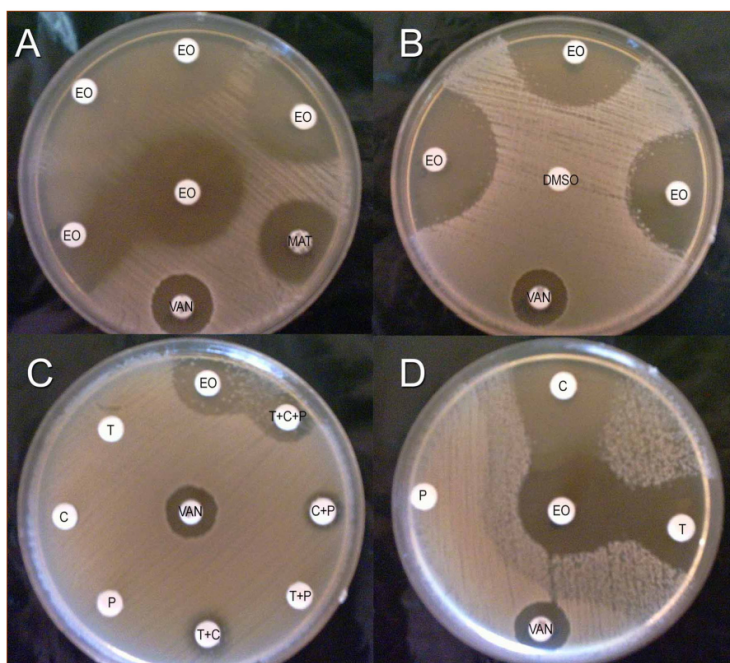


Figure 1.

- A: antistaphylococcal activity of (0.5, 1, 2, 3, 4 μl) of essential oil of *O. decumbens* (EO) compared with methicillin (MAT) and vancomycin (VAN);
- B: antistaphylococcal activity of different concentrations of EO, VAN and Dimethylsulfoxide (DMSO) as a solvent;
- C: the effect of 0.5 μl of essential oil of *O. decumbens* and main components (is equal to quantity components in 0.5 μl of EO), T* – thymol, C* – carvacrol, P* – p-cymene;
- D: the effect of 0.5 μl of essential oil of *O. decumbens* and 0.5 μl of main components (T1 – thymol, C1 – carvacrol, P1 – p-cymene).

The inhibitory zone (17–23 mm) obtained for 0.5 μl of den oil was larger than that of vancomycin. The antistaphylococcal activity was enhanced when the con-

centration of the essential oil was increased on the disks ($p < 0.001$). There was no inhibition zone around the disk containing DMSO (tab. 1, fig. 1B). The antistaphylococcal effects of the main components ($0.5 \mu\text{l}$) of den oil purchased from the reliable suppliers (Roth and Merck) were determined and compared with $0.5 \mu\text{l}$ of essential oil (tab. 2). p-cymene (P^1 , $0.5 \mu\text{l}$) showed no antimicrobial activity in comparison with $0.5 \mu\text{l}$ of its oil. In contrast, both thymol (T^1 , $0.5 \mu\text{l}$) and carvacrol (C^1 , $0.5 \mu\text{l}$) showed antistaphylococcal activity. Antistaphylococcal activity of carvacrol was stronger than thymol when they were used at similar concentrations ($p < 0.01$, see tab. 2, fig. 1D). In contrast to commercially obtained thymol (T^* ; equal to 22% of $0.5 \mu\text{l}$ of essential oil) and p-cymene (P^* ; 19% of $0.5 \mu\text{l}$ of essential oil) both of which did not inhibit the growth of *S. aureus*, carvacrol (C^*) produced an inhibition zone around the disk under similar conditions. However, thymol and p-cymene either separately or together increased the inhibitory zone of carvacrol (fig. 1C). Thymol and p-cymene did not show an inhibition zone individually or when combined. Thymol/carvacrol, p-cymene/carvacrol, carvacrol/p-cymene and thymol/carvacrol/p-cymene had synergy effects compared to thymol, carvacrol and p-cymene alone. The inhibition zone of the three main components of the essential oil (63% of its contents) is smaller than that of the essential oil itself. It appears that the other components in the essential oil may also have anti-staphylococcal activity. A high concentration of aromatic compounds, particularly phenol rich oil, seems to account for the strong antibacterial activity.

Table 1.

The inhibitory zone (mm) obtained for vancomycin and essential oil from *Oliveria decumbens* against *S. aureus* by disk diffusion method

statistics	vancomycin (30 μg)	essential oil from <i>Oliveria decumbens</i> on disks				DMSO
		0.5 μl	1 μl	2 μl	3 μl	
mean \pm SE	16.0 \pm 0.10	19.3 \pm 0.54	26.0 \pm 0.62	32.4 \pm 0.53	37.4 \pm 0.60	0.0 \pm 0.0
min.-max.	15.0–17.0	17.0–23.0	22.0–30.0	30.0–37.0	34.0–43.0	–

DMSO – dimethyl sulfoxide as a solvent

The inhibitory zone around the disk of essential oils increased when they were used in the medium containing vancomycin (tab. 3). The essential oil enhances the antimicrobial activity of vancomycin. The emerging of resistance to vancomycin necessitates the search for alternatives and efficient compounds to treat the infections. Topical anti-microbial therapy is the most important method of wound care to prevent staphylococcal infection. The physical nature of essential oils (low molecular weight combined with pronounced lipophilic properties) allows them to penetrate membranes quicker than other substances. The essential oils penetrate tissue roughly 100 times faster than water and 1000 times faster than salts [7]. Therefore, the results of this study appear promising and may enhance the natural product uses, showing the potential of plant oils in the treatment of skin infections caused by *S. aureus*. Further studies should be carried out regarding antimicrobial activity of oil in topical agent formula in animal models.

Table 2.

The anti-staphylococcal activity of essential oil of *Oliveria decumbens* in comparison with its main components (diameter in mm)

statistics		mean±SE	min.–max.
group			
EO ¹ [0.5 µl]		18.0±0.86	12.0–25.0
thymol	T ¹	17.2±1.13	12.0–26.0
	T [*]	0.0±0.0	–
carvacrol	C ¹	29.8±1.5	23.0–45.0
	C [*]	8.6±0.43	7.0–12.0
p-cymene	P ¹	0.0±0.0	–
	P [*]	0.0±0.0	–
different combination of components	T+C [*]	11.6±0.64	9.0–17.0
	T+P [*]	0.0 ± 0.0	–
	C+P [*]	10.9 ± .88	7.0 – 20.0
	T+C+P [*]	14.6 ± .87	10.0 – 21.0

EO – essential oil, T – thymol, C – carvacrol, P – p-cymene, NZ – no inhibition zone, ¹ – 0.5 µl of component, * – its equivalent concentration in 0.5 µl of essential oil

Table 3.

Antistaphylococcal activity of essential oil of *O. decumbens* in combination with vancomycin against clinical isolates of *S. aureus*

statistics		mean±SE	min.–max.
group			
MHA* medium without vancomycin	0.5	19.3±0.54	17.0–23.0
	1	26.0±0.62	22.0–30.0
	2	32.4±0.53	30.0–37.0
	3	37.4±0.60	34.0–43.0
MHA medium with vancomycin [0.125 µg/ml]	0.5	24.9±0.75	19.0–30.0
	1	31.5±0.80	27.0–38.0
	2	37.5±0.80	32.0–42.0
	3	42.7±0.74	40.0–48.0

*MHA: Muller-Hinton agar

CONCLUSION

1. The main components of the essential oil were thymol (22%), carvacrol (22%) and p-cymene (19%).

2. The essential oil from *O. decumbens*, in particular phenol rich oil, exhibited strong antistaphylococcal activity. A high concentration of aromatic compounds, particularly phenol rich oil, seems to account for strong antibacterial activity. Carvacrol was considerably more effective than thymol and p-cymene against *Staphylococcus aureus*.

3. The essential oil enhances the antimicrobial activity of vancomycin. The essential oil presented strong synergism with vancomycin ($p < 0.001$)

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PRZECIWBAKTERYJNE DZIAŁANIE OLEJKU ETERYCZNEGO *OLIVERIA DECUMBENS* NA *STAPHYLOCOCCUS AUREUS* I JEGO PODOBIEŃSTWO DO WANKOMYCYN

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Streszczenie

Oliveria decumbens Vent (*Umbelliferae*) jest krzewem często występującym w południowo-wschodnim Iranie. Jego części nadziemne są szeroko wykorzystywane w ziołarstwie. W celu określenia działania przeciwbakteryjnego olejku eterycznego *Oliveria decumbens* Vent wykorzystano metodę krążków bibułowych i rozcieńczeń seryjnych w podłożu płynnym.

Dla porównania, na pożywce Mueller-Hintona umieszczono wankomycynę w stężeniu bliskim hamującemu. Średnicę stref zahamowania wzrostu zmierzono w milimetrach. Głównymi składnikami olejku były tymol (22%), karwakrol (22%) i p-cymen (19%). Olejek z *O. decumbens* charakteryzował się silnym działaniem przeciwbakteryjnym (18.0 ± 0.86). Działanie karwakrolu na *Staphylococcus aureus* było stosunkowo bardziej efektywne (29.8 ± 1.5) niż tymolu (17.2 ± 1.13) i p-cymenu (0.0 ± 0.0). Działanie olejku było bardzo zbliżone do działania wankomycyny (24.9 ± 0.75 vs 19.3 ± 0.54 , $p < 0.001$). Jednakże w celu oceny działania olejku *in vivo* należałoby przeprowadzić dalsze badania.

Słowa kluczowe: *Staphylococcus aureus*, *Oliveria decumbens*, olejki eteryczne, p-cymen, wankomycyna, podobieństwo