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Essential oil from *Oliveria decumbens* accelerates *in vivo* wound healing: a possible mechanism by the involvement of glycogen synthase kinase-3

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Abstract

Background and objective: The development of new products for skin care and wound treatment is continuous, and herbal medicine plays an important role in the treatments. *Oliveria decumbens,* a plant from the family Apiaceae, has been reported to enclose many pharmacological properties. This study aimed to detect the effect of the essential oil of *Oliveria decumbens* as an antibacterial and wound healing agent.

Methods: The antibacterial properties of *Oliveria decumbens* were studied by the disk diffusion method. In addition, the excision model of wound healing was applied to rats, and the AutoDock method was used to study the mechanism of action.

Results: The essential oil extracted from *Oliveria decumbens* showed antibacterial activities against gram-positive and gram-negative bacteria, and the wound healing effect was comparable to the standard skin cream. Thymol was predicted to be the strongest binder of GSK-3 protein active site forming the most stable complex with hydrogen bond and hydrophobic interaction. The second-best binders were P-Cymene, Limonene, Gamma-Terpinene, and Carvacrol.

Conclusion: The observed data backed the original claim of antibacterial and wound healing properties of *Oliveria decumbens* extracted essential oil.

Keywords: *Oliveria decumbens;* Wound healing; Disk diffusion; AutoDock Vina method.

Introduction

The skin has the ability to heal or repair lost or damaged tissues by rejuvenating the tissues and forming a collagenous scar. This progression is termed "wound healing."[1] The process includes four phases named coagulation, inflammation, proliferation, and remodeling phases, in which a selection of growth factors and cytokines are produced.[2]

Among the important cytokines in the wound healing process are interleukin (IL-1β) and tumor necrosis factor (TNFα).[3] TNF-α is mainly produced by activated monocytes, macrophages, natural killer (NK) cells, and T lymphocytes.[4] it has pleiotropic effects on normal and malignant cells[5] and exerts host-damaging effects in tumor cachexia, in sepsis, as well as in autoimmune diseases.[6] Interleukin family of cytokines is known to be produced by almost all species of activated immune cells, including B cells, granulocytes, mast cells, dendritic cells, macrophages, and T cells. Their principal actions are primarily considered anti-inflammatory, inhibitory, or self-regulating.[7]

Geographically, *Oliveria decumbens* is a shrub commonly found in southwest Anatolia, Iran, Syria, and Iran.[8] The common name for the Kurdish people is "Giamamza." The essential oil was documented to enclose a large amount of oxygenated monoterpene constituents such as thymol, carvacrol, g-terpinene, and ρ-cymene.[9-12] Many studies have shown that the extracted oil of *Oliveria decumbens* exhibited strong antimicrobial

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and antifungal activities against filamentous fungi and yeast[13] and the antibacterial activity on the growth of some clinical and standard bacterial strains that cause infection such as (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Streptococcus pyogenes*) has been documented,[14] while the efficiency of herbal formulation containing *Oliveria decumbens* essential oils was evaluated against methicillin resistant *S. aureus* (MRSA) related skin wound infection in experimental mice model.[15] Den oil also has been extracted from *Oliveria decumbens* aerial part, which was used as an antiseptic agent and known as a broad spectrum antimicrobial agent due to its strong antibacterial activity.[16]

Moreover, the essential oil of *Oliveria decumbens* was tested for its antimicrobial activity against six bacterial strains and two fungi. The oil exhibited a large antimicrobial activity against the tested Gram positive and Gram negative bacteria and fungal strains.[9] Also, another team worked on *Oliveria decumbens* as a valuable medicinal plant. They investigated the antibacterial activities and phytochemical analysis of the essential oil on the growth of some clinical and standard strains causing infection (*Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyogenes*, and *Staphylococcus epidermidis*).[17] Another research investigated the antimicrobial activity of *Oliveria decumbens* on well-known fungi (*Candida albicans*) by using the disk diffusion agar method.

The essential oil was reported to enclose antibacterial and antifungal effects. With increasing essential oil concentration, the inhibitory zone increased.[18] The current study aimed to test the extracted oil from *Oliveria decumbens* as an antibacterial efficacy and wound healing activity using the experimental rat model and then to test its mechanism of action computationally by using the AutoDock Vina method.

Methods

Plant extraction

Flowering aerial parts of *Oliveria decumbens* were collected from Darbandy Kandenawa and were used in the study. The plant materials were cleaned from any unwanted substances. The hydro-distillation method was used to extract the essential oils by Clevenger-type apparatus for 6 hours at Pharmacognosy lab, College of Pharmacy, Hawler Medical University. The obtained pale yellow essential oil with a strong aroma was collected and dried over anhydrous magnesium sulfate and stored in amber glass at 4 °C before using.

Antibacterial effect

Kirby – Bauer assay was tested for antibacterial activity.19 Different concentrations of the essential oil were impregnated in blank discs (200-25 mg/ml) using dimethyl sulfoxide (DMSO) as the vehicle. The bacteria *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25922) were cultured on Mueller Hinton Agar (MHA) plates at 37°C, and the growing cultures were adjusted to 0.5 McFarland. For examining the antibacterial activity, the diameters of the inhibition zones around the discs were measured after 24 hours. Ceftazidime/clavulanic acid 30/10 mcg/ disc was used as the control of the study. The whole assays were repeated three times, and the mean values were set.

Wound healing method

An excision model of wound healing was performed. Male Wistar rats weighing (200-250 grams) were divided into three groups, each of 10 rats. Rats were housed separately and got free excess to diet and water. The skin was shaved by electrical shaver after anesthetizing the animals with a light dose of both ketamine and xylazine. Then the area was disinfected with 70% alcohol, and a uniform wound (2.00cm) in diameter was excised from the nape of the dorsal neck of each rat.

All treatments were applied twice a day topically, whereas 0.2 mL of the vehicle (10% Tween 20) was applied to Group 1

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rats. Group 2 rats were topically dressed with 0.2mL of *Oliveria decumbens* extracted essential oil, and Group 3 rats were dressed with the reference drug (MEBO cream 0.25% w/w).

The wound closure area of each animal was assessed by tracing thewound on days 0, 5, and 10 after surgery, and the wound closure rate was expressed as the percentage of wound are acompared with that on a postoperative day following the method of Zahra et al.[20]

Computational methods

Preparation of Glycogen Synthase Kinase 3 protein and studied compounds

The crystal structure for Glycogen Synthase Kinase 3 (GSK-3) (PDB ID: 1Q5K) was obtained from Protein Data Bank (PDB) (http://www.rcsb.org).[21] Discovery Studio 4.1 (http://accerys.com)[22] and Molecular Graphics Laboratory (MGL) Tools 1.5.6 (http://mgltools.scripps.edu)[23] used to prepare GSK-3and saved as (.pdbqt) file for molecular docking.

A total of 12 compounds as *Oliveria decumbens* (aerial parts) essential oil (Table 1), were investigated in our study.

The 3D structures of all available compounds (Figure 1) were constructed using UCSF Chimera Ver. 1.10.1 program (http://www.cgl.ucsf.edu/chimera/).[24]

Open Babel graphical user interface (GUI) (http://openbabel.org/)[25] used to convert the structures to (.pdbqt) file format, which is necessary for AutoDockVina.[26]

AutoDock Vina

After defining the active site of GSK-3 protein using Discovery Studio 4.1 (http://accerys.com).[22] The line cube was established using Molecular Graphics Laboratory (MGL) Tools 1.5.6 (http://mgltools.scripps.edu).[23] The coordinates of the grid box defined in a configuration file were the dimensions of a cube was 20 Å, covering the active site with a grid point spacing of 1.0 Å, and center grid boxes of 23.598, 22.348 and 10.968 in X, Y and Z dimensions, respectively. which is required to supply AutoDock Vina 1.1.2 via the command line. AutoDock Vina was run on the Windows 8.1 operating system with 4 CPUs and 12 GB of RAM. All available compounds docked ten times to the active site of GSK-3 protein.

Table 1 List of *Oliveriadecumbens* essential oil compounds

#	Essential oil	References
1	Alpha-pinene	(Ecormier et al 2003)[27], (Fierascu et al, 2018)[28]
2	Beta-pinene	(Ecormier et al 2003)[27], (Fierascu et al, 2018)[28]
3	Beta-Myrcene	(Lüddeke and Harder, 2011)[29]
4	p-Cymene	(Fierascu et al, 2018)[28]
5	Limonene	(Fierascu et al, 2018)[28]
6	Gamma-Terpinene	(Santos et al, 2011)[30]
7	4-Terpineol	(Grillo et al, 2018)[31]
8	Thymol	(Ramos et al, 2012)[32], (Fierascu et al, 2018)[28]
9	Carvacrol	(Ramos et al, 2012)[32], (Fierascu et al, 2018)[28]
10	Myristicin	(Shulgin, 1966)[33]
11	Elemicin	(Kobets et al, 2016)[34]
12	Nonadecane	(Hayase et al, 2020)[35]

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Statistical Analysis

All data were expressed as Mean ± SEM. The statistical analysis was achieved using one-way ANOVA, Post Hoc Bonferroni multiple comparisons in the IBM statistical package for the social sciences (SPSS, version 23) program. A *P* value ≤0.05 was considered statistically significant.

Results

Pale yellow (with strong aroma) essential oil of *Oliveria decumbens* was extracted and used in the experiment. The results revealed that *Oliveria decumbens* essential oil had positive activity against Gram-positive and Gram-negative bacteria. The highest plant dose (200 µg/mL) gave results almost the same as the positive antibiotic control ceftazidime/clavulanic acid, as shown in Table 2.

Table 2 Antibacterial activity of the essential oil of *Oliveria decumbens*

Concentration (µg/ml)	*S. aureus*	*E. coli*
OD200	>5	4
OD100	4	3
OD50	3.5	2.5
OD25	R	R
CAC 30/10	5	5
DMSO	R	R

OD: *Oliveria decumbens,* CAC: Ceftazidime/Clavulanic acid, R: Resistant.

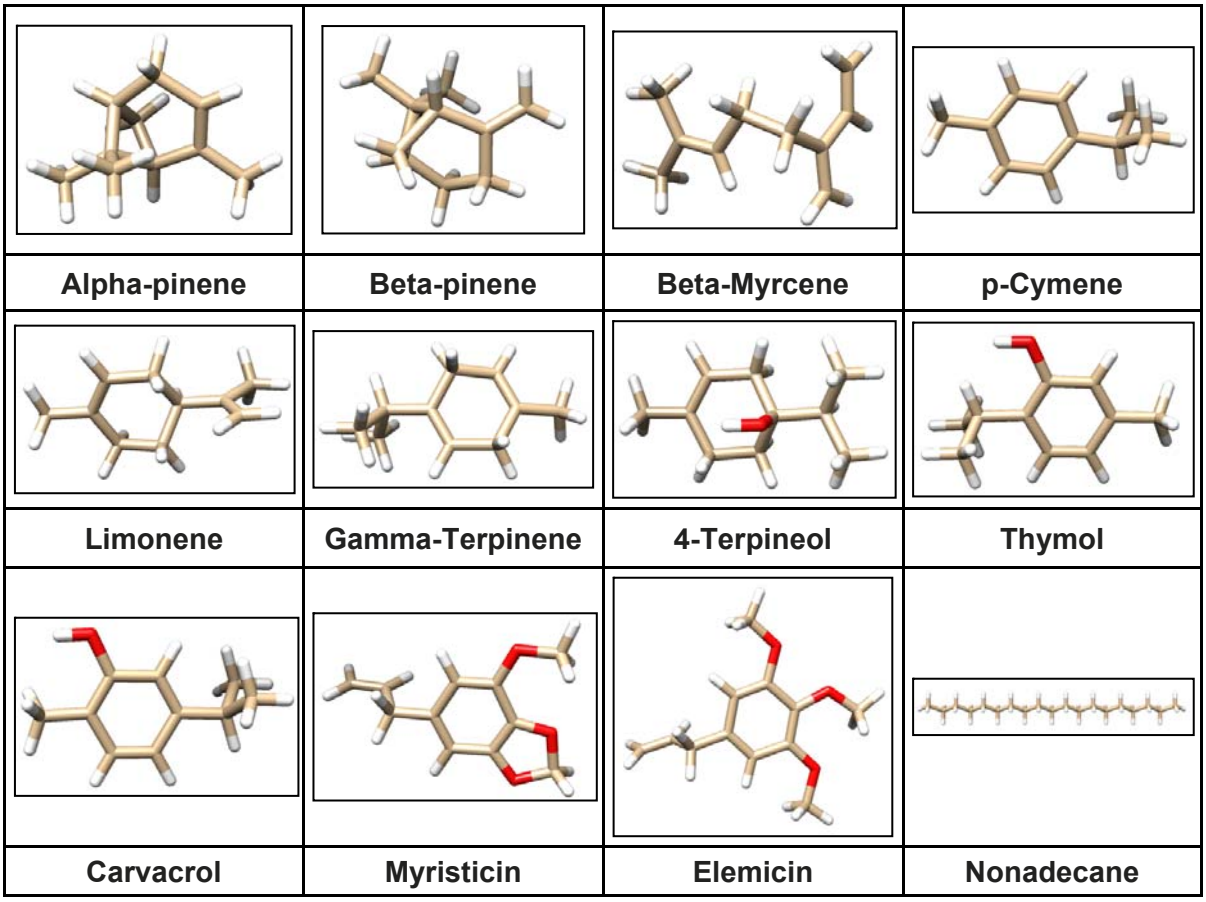

Figure 1 3D structures of studied *Oliveria decumbens* essential oil compounds

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In the wound healing experiment, on day 5 post surgery, a decrease in the skin wound surface was observed (wound closure = 42%), while the MEBO control group rats showed more decrease in wound closure (43%). However, regarding the vehicle control group (10% Tween 20), the changes in the skin surface area were much less (14%), as shown in Figure 2 and Table 3.

On day 10 post surgery, the wound closure was much better (64%, 55%, and 14%) for MEBO control, *Oliveria decumbens,* and the vehicle treated groups, respectively.

Table 3 Wound healing measurements of experimental rats after exposure to different treatments

GROUPS	DAY 0	DAY 5		DAY 10	
	wound area	wound area	Wound closure %	wound area	Wound closure %
10% Tween 20	330±0.4	300±1.5	9	285±1.5	14
MEBO	332±2.4	190±2.1*	43	120±2.1*	64
Oliveria decumbens	344±1.7	201±4.3*	42	154±1.8*	55
P (ANOVA)	1	0.03		0.004	

Data expressed as Mean±SEM,(*) Significance was set when *P* <0.05.

Figure 2 Macroscopic appearance of excision wound healing area on rat skin at day 10 after surgery within different groups of treatments (O) *Oliveria decumbens* group, (T) 10% Tween 20 group, (M) MEBO ointment group

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Docking studies predicted the type and potency of the most important requirements of these interactions, in addition to the suitable orientation and conformation of compounds that fitted to the GSK-3 binding site. Therefore, optimal AutoDock Vina scores were used as standards to recognize the best conformation among the 12 studied compounds. All compounds were well docked into the binding site of GSK-3 protein, ten runs were performed by AutoDock Vina software for every single compound, and the average docking scores (kcal/mol) were calculated.

According to the result obtained (Table 4), out of the 12 studied compounds, thymol was predicted to be the strongest binder of GSK-3 protein active site forming the most stable complex (Figures 3) with hydrogen bond and hydrophobic interaction with the score or binding affinity of -6.4 kcal/mol. The second-best binders were P-Cymene, Limonene, Gamma-Terpinene, and Carvacrol, with docking scores -6.2 kcal/mol.

Table 4 Average of docking scores (kcal/mol) for essential oil docked to GSK-3 Protein

#	Compounds	Docking Score (kcal/mol)	#	Compounds	Docking Score (kcal/mol)
1	Alpha_pinene	-4.4	7	4_Terpineol	-5.7
2	Beta_pinene	-5.0	8	Thymol	**-6.4**
3	Beta_Myrcene	-5.3	9	Carvacrol	-6.2
4	P_Cymene	-6.2	10	Myristicin	-6.0
5	Limonene	-6.2	11	Elemicin	-5.6
6	Gamma_Terpinene	-6.2	12	Nonadecane	-5.2

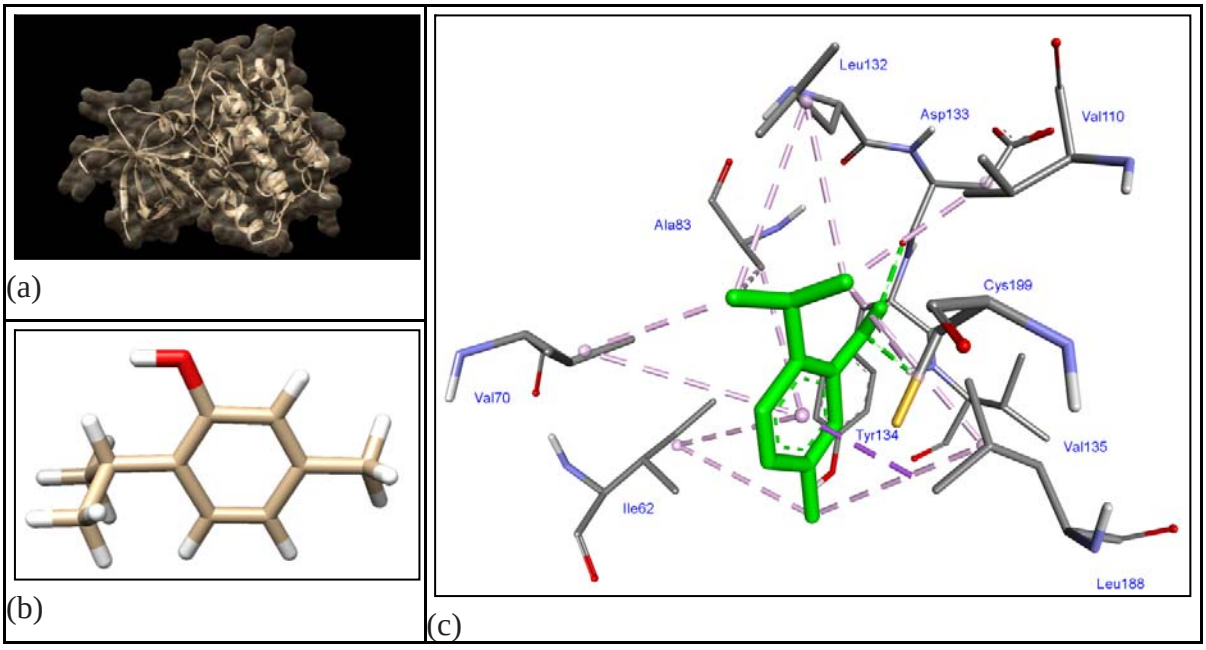

Figure 3 Thymol docked to GSK-3 protein; (a) Showing molecular surface and the compound in the active site (b) 3D structure of the compound (c) Showing interacted residues of protein with compound

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Discussion

The antibacterial effect of *Oliveria decumbens* essential oil was investigated at different concentrations with the inhibition zone diameters compared with antibiotics, as presented in Table 2. The results showed that the *Oliveria decumbens* essential oil was effective against tested gram positive and gram negative bacteria. The antimicrobial activity of *Oliveria decumbens* oil against different microorganisms has been reported by others.[9,17,18]

The antibacterial activity was likely proposed to be due to the presence of phenolic compounds like carvacrol and thymol or possibly their synergistic action.[36-38] Moreover, the results of our study showed that the essential oil extracted from *Oliveria decumbens* had good efficacy in healing wounds comparably to the standard skin cream (MEBO). The effectiveness was suggested to be related to its active constituents.[15,39] The healing capacity of thymol was reported on the 7th day of inducing skin wounds, and a reduction in the acute inflammatory response, thickening of the dermis, and reduction of necrosis areas were observed.[39] Other studies documented the healing power of the plant may be associated with the synergistic effect of various secondary metabolites, including monoterpenes, such as thymol and sesquiterpenes.[40] In addition, the healing effect of a skin dressing containing carvacrol showed that it significantly reduced the surface of a wound lesion, promoting changes in lesion depth and granulation tissue thickness. Subsequently, it was documented that carvacrol was capable of retaining the release of transforming growth factor (TGF-α), tumor necrosis factor-α (TNF-α), and interleukin 1β (IL-1β) during tissue repair.[41] Docking results showed that the highest affinity was observed among essential oil compounds when thymol docked into GSK-3 protein binding site (Figure 2). It formed hydrogen bond interactions with Asp[133], Tyr[134], and Val[135]. Hydrophobic; Alkyl-Alkyl type interaction with Ile[62], Ala[83], Val[70,110], Leu[132,188], and Cys[199]. Also, Pi-Alkyl-Pi-Orbital type interaction with Ile[62], Val[70], and Ala[83] was observed. However, it is hard to know the exact molecular targets of these compounds, but it is possible to suggest the modulation of redox balance, inflammatory cytokines, and growth factors. One can realize that thymol and carvacrol are able to modulate all phases of wound healing, presenting an important role in this process.[42]

Conclusion

According to our results, thymol and carvacrol could be strong candidates for developing future drugs for the management of tissue repair. However, other studies are needed to understand the molecular mechanisms by which these monoterpenes can act in the wound healing process in detail.

Funding

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Competing interests

The authors declare that they have no competing interests.

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