

Elimination Virulent-pathogenic-biofilm Bacteria Using Highland-wild *Salvia officinalis* Preserve Bacterial-infection-control

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Abstract

This work for this title "Elimination Virulent-pathogenic-biofilm (VPB) Bacteria Using Highland-wild (HLW) *Salvia officinalis* (*S. officinalis*) Preserve Bacterial-infection-control (BIC)"; the aim was to prove the importance of HLW *S. officinalis* extracts have therapeutic herbal importance. Through its effected on the isolated VPB bacteria caused infection diseases that may preserve BIC for individuals, which proved the effectiveness of the HLW *S. officinalis* daily use or therapeutic use. *S. officinalis* specimens were collected during the flowering period from HLW, Taif, KSA. Essential oils (EOs) were equipped and biofilms preparation, then laboratory methods deputy for anti-biofilms formation activity and biofilms elimination activity, finally biofilms metabolic grades measurement. The bacterial metabolic grades of anti-biofilms formation activity showed the HLW *S. officinalis* EOs extracts eliminated VPB bacteria and effects were greater. Anywhere *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes* (*S. pyogenes*) were eliminated until 60 hours. While *Pseudomonas aeruginosa* (*PA*) was eliminated at 72 hours. The bacterial metabolic grades of biofilms elimination activity found the HLW *S. officinalis* EOs extracts eliminated within 8 hours (*S. aureus* and *S. pyogenes*), *PA* was to 10 hours. Concluded the HLW *S. officinalis* EOs extracts had proven its ability to eliminate VPB bacteria, and from that, it proven on the type used with healthy characteristics to maintain health and BIC. Recommendation: That topic recommend using the appropriate HLW *S. officinalis* EOs extracts for individuals daily to maintain the general health. In cases of illness, person must ask the "Specialized Physician" to determine the healthy and curative amount to use.

Key words: virulent-pathogenic-biofilm bacteria, highland-wild salvia officinalis, bacterial-infection-control, essential oils, therapeutic herbal importance, metabolic grades measurement

Abbreviation:

BIC: Bacterial Infection Control,

CE: Crude Extract,

EOC: Essential Oil Compounds,

EO: Essential Oils,

HLW: Highland Wild,

PA: Pseudomonas Aeruginosa,

PB: Pathogenic Bacteria,

SO: Salvia Officinalis,

S, Aureus: Staphylococcus Aureus,

S. Pyogenes: Streptococcus Pyogenes,

VPB: Virulent Pathogenic Biofilm.

Introduction

S. officinalis, called in ordinary community "Maramayah" also "Sage", it fits Lamiaceae (Labiatae) family. It recognized as harmless (GRAS) for its U.S. envisioned Food and Drug Administration (FDA) as per 21 CFR unit 182.20 then 21CFR977029-66-5, used in medicinal arrangements [1]. A traditional medicinal herb characterized as a recurrent low plant creating in the Mediterranean area [2]. Also as native Middle Eastern plant has subordinate metabolites. Alcoholic and aqueous extracts contain flavonoids and phenolic acids as bactericidal and bacteriostatic activities against all pathogenic bacteria. That polyphenols found in Middle Eastern plants with antibacterial action [3]. Pathogenic biofilm-associated bacteria are a big challenge to the healthcare, the potential biofilm-inhibiting, virulence factor-reducing and biofilm-eradicating activities of EOs and single EOCs using *S. aureus* and *PA*. They reported to be able to modulate the expression of genes that are involved in the formation of autoinducer molecules, biofilms, and virulence factors. Of great interest was the discovery that enantiomeric monoterpenes affected the quorum sensing regulation system in different ways. For the successful

eradication of biofilms and the bacteria living inside them, it is necessary that the lipophilic volatile substances can penetrate into the aqueous channels of biofilms. As shown in recent work, hydrophilic nanodelivery systems encapsulating EOs/EOCs with antibacterial effects may contribute to overcome this problem as BIC [4]. The antimicrobial characteristics of extract by using root dentin samples with a biofilm model, which to evaluate the antibacterial characteristics against PB [5]. More specifically, monoterpenes, diterpenes, triterpenes, and phenolic components have anti PB activities [6]. EOs contains major terpenes; manool, viridiflorol, eucalyptol, borneol, and thujone. In turn, carnosol, carnosic acid, rosmarinic acid, flavonoids, polysaccharides, tannic acid, oleic acid, ursonic acid, ursolic acid, fumaric acid, chlorogenic acid, caffeic acid, and estrogenic in leaves had eliminated *S. pyogenes* [7]. This were applied as many medical uses showed the anti PB effects against numerous strains and that cause pharyngitis [8]. Anti PB glycolic, extract concentrations against *S. aureus*, which eliminated 100% strains without toxicity [9]. Used against cariogenic bacteria, EOs associated with glassionomer cement (GIC) inhibits PB [10]. EOs dichloromethane CE were promising anti PB managers and may deliver beneficial explanations for periodontal infections [11]. EOs against *S. aureus* biofilms removal after 90 min of exposure, EOs potential use sanitizers as alternative or in support in the disinfection of contaminated *S. aureus* surfaces [12]. EOs evaluated for the phytochemical profile, antibacterial, and anti-biofilm against *S. pyogenes*. The microscopic visualization of biofilms treated with EOs have shown morphological and density changes compared to the untreated control. EOs inhibited the growth and biofilm formation [13]. EOs against *PA* biofilm showed good efficacy towards strong and weak biofilm producers, 81.8% showed lower biofilm production. The obtained results showed high potential EOs for the treatment of persistent infections caused by *PA* biofilms [14].

The aim of this experiment was to prove the importance of the extract for HLW of *S. officinalis* that have a therapeutic herbal importance. Through it was effect on the isolated pathogenic bacteria with virulence so that biofilms formation and caused infection diseases. That may preserve the BIC of the bacterial pathogens of infectious diseases for individuals, which proved the effectiveness of the *S. officinalis* daily use or therapeutic use.

Methodology

I. Plant specimens' deputy:

- Examples collection:** *S. officinalis* L. specimens were collected during the flowering period from HLW, Taif, KSA. Taxonomist according to classification and official plant inspectors authenticated them. They were deposited of Plant Dept.; fresh samples were dried

at 50 °C for 3 days, ground into fine particles, and stored in airtight containers [8].

- EOs extractions:** EOs were equipped from specimens in "Plant Chemistry Dept.", the processed were mixed with DW as (1:15 ratio) and were exposed to hydro distillation "Clevenger Apparatus" for about 3 h. Composed EOs were dried with anhydrous sodium sulfate and filled in an airtight amber vials at 4 °C [15].

II. Bacterial strains deputy:

- Biofilms preparation:** Bacterial strains were identified as pathogenic bacteria in HL, Taif; they were included (*S. aureus*, *S. pyogenes* and *PA*). They were stored at -80 °C in BHI (Oxoid Ltd., Nepean, ON, Canada), with 20% glycerol. Colonies were transferred to BHI and cultured at 37 °C for overnight to mid-log phase. The turbidity of cell suspensions was stately by absorbance at 600 nm, and were attuned consistent to ~1×10⁹ CFU/mL. The consistent cell suspensions were diluted in BHI to ~1×10⁶ CFU/mL [16].

III. Laboratory methods deputy:

- Anti-biofilms formation activity:** EOs G were prepared 100 µL was added then 100 µL diluted bacteria (~1×10⁶ CFU/mL) in BHI media into each well of the "Microtiter Plate"; (Falcon, Corning Inc., NC, USA). After incubation for (12, 24, 36, 48, 60 and 72) h at 35 ± 2 °C, the plates planktonic bacteria were removed by carefully washed 3 times with sterile PBS. Measure bacterial metabolic activity for each 12 h [17].
- Biofilms elimination activity:** Biofilms were pre-formed by 100 µL addition diluted bacteria in early log phase (~1×10⁶ CFU/mL) in BHI media into each well of "Microtiter Plate"; (Falcon, Corning Inc., NC, USA). After 24 h incubation at 35 ± 2 °C, the non-adherent planktonic cells were removed by gently pipetting the liquid from each well and were carefully washed 3 times with sterile PBS. EOs were added to pre-formed bacterial biofilms, 100 µL was added to wells containing pre-formed biofilm in 100 µL fresh BHI media. Following (2, 4, 6, 8, 10 and 12) h incubation at 35 ± 2 °C, the metabolic activity remaining in the biofilm in each were measured for each two h [18].
- Biofilm metabolic grades measurement:** "Arginine Degradation Test" were used for biofilm metabolic grades before and after exposed to EOs extract [19].

IV. Statistical examination understudy:

Data were planned and were inspected by "Microsoft Outclass" [8].

Results and discussion

Entry	Days in h						Mean
	12h	24h	36h	48h	60h	72h	
Bacteria	12h	24h	36h	48h	60h	72h	
<i>S. aureus</i>	4	3	2	1	0	0	1.7
<i>S. pyogenes</i>	4	3	2	1	0	0	1.7
<i>PA</i>	4	4	3	2	1	0	2.3
Mean	4.0	3.3	2.3	1.3	0.3	0.0	

*h: Hour, *S. aureus: *Staphylococcus aureus*, *S. pyogenes: *Streptococcus pyogenes*, *PA: *Pseudomonas aeruginosa*

Entry	Days in *h						Mean
	12 h	24 h	36 h	48 h	60 h	72 h	
<i>Bacteria</i>	12 h	24 h	36 h	48 h	60 h	72 h	
* <i>S. aureus</i>	4	3	2	1	0	0	1.7
* <i>S. pyogenes</i>	4	3	2	1	0	0	1.7
* <i>PA</i>	4	4	3	2	1	0	2.3
Mean	4.0	3.3	2.3	1.3	0.3	0.0	

*h: Hour, *S. aureus: *Staphylococcus aureus*, *S. pyogenes: *Streptococcus pyogenes*, *PA: *Pseudomonas aeruginosa*

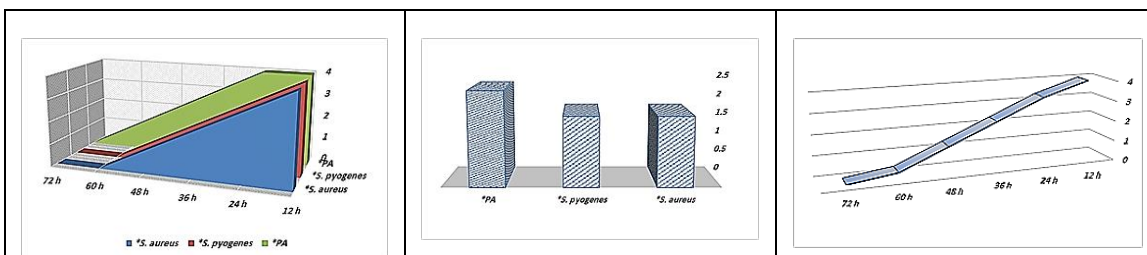


Table 1 and graph (1, 2 & 3): Bacterial metabolic grades of anti-biofilms formation activity

Table 1 and graph (1, 2 & 3) indicated bacterial metabolic grades of anti-biofilms formation activity, the results of using the HLW *S. officinalis* EOs extracts showed that the elimination of the VPB bacteria and the effect was greater. Where (*S. aureus* and *S. pyogenes*) bacteria were eliminated until 60 hours. While the other *PA* bacteria was eliminated on the third day at 72 hours. From the metabolic grades, it found that both *S. aureus* and *S. pyogenes* bacteria were affected by the HLW *S. officinalis* EOs extracts faster than *PA* bacteria. It reported that

the HLW *S. officinalis* EOs extracts maintained excellent qualities to eliminate VPB bacteria. This confirmed that the HLW *S. officinalis* EOs extracts had good properties to eliminate them outside the body, which indicated that it had medicinal properties in eliminating them and BIC. It is necessary to go to the "Pharmacy Dept.," to determine the quantities or levels that are beneficial for health to maintain the occurrence of bacterial pathogenic diseases by eliminate bacterial causes and protect BIC and the body health [4-14].

Entry	*h						Mean
	2 h	4 h	6 h	8 h	10 h	12 h	
*S. aureus	4	2	1	0	0	0	1.2
*S. pyogenes	4	2	1	0	0	0	1.2
*PA	4	3	2	1	0	0	1.7
Mean	4.0	2.3	1.3	0.3	0.0	0.0	

*h: Hour, *S. aureus: Staphylococcus aureus, *S. pyogenes: Streptococcus pyogenes, *PA: Pseudomonas aeruginosa

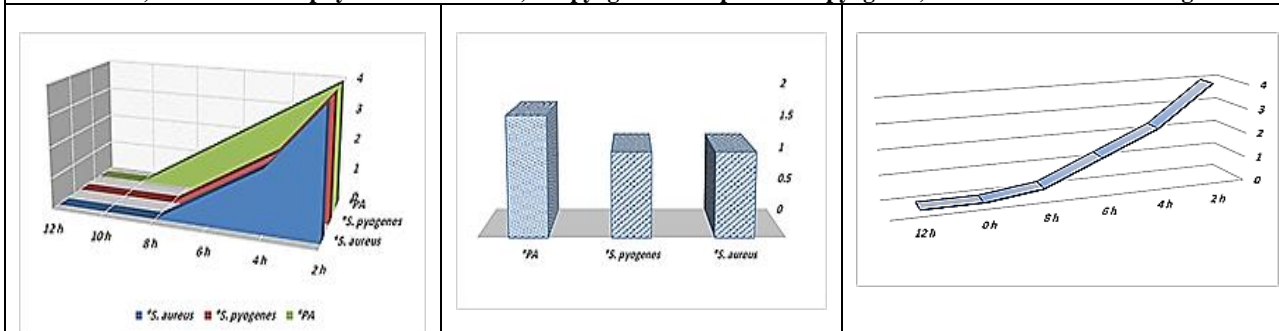


Table 2 and graph (4, 5 & 6): Bacterial metabolic grades of biofilms elimination activity

Table 2 and graph (4, 5 & 6) indicated bacterial metabolic grades of biofilms elimination activity, as a result, it was found that the HLW *S. officinalis* EOs extracts removal of the VPB bacteria was faster than the elimination of the bacteria themselves. As it was eliminated within only 8 hours for (*S. aureus* and *S. pyogenes*) bacteria, while the other *PA* bacteria to only 10 hours. It known that the VPB bacteria were formed inside the infected patient, so this indicated that the HLW *S. officinalis* EOs extracts might help in treatment in a short time. Through the effect of the HLW *S. officinalis* EOs extracts on the VPB bacteria, which proved the necessity of precipitating the appropriate proportions to eliminate the VPB bacteria inside the body during the case of disease through the of "Pharmacy Dept.,". By this, it will show the type of goal in HLW *S. officinalis* EOs extracts and the extent of benefit to the health of people and BIC [4-14].

Conclusion

The HLW *S. officinalis* EOs extracts had proven its ability to eliminate VPB bacteria, and from that, it proven on the type used with healthy characteristics to maintain health and BIC.

Recommendation: That topic recommend using the appropriate HLW *S. officinalis* EOs extracts for individuals daily to maintain the general

health. In cases of illness, person must ask the "Specialized Physician" to determine the healthy and curative amount to use.

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That send thanks to the all individuals who participated in this theme.

Future Works: I hope to work on medicinal plants and their effects on the VPB bacteria, and contact the "Pharmacy Dept.," to estimate the healthy and therapeutic quantities of EOs extracts.

Fundus: That was spent on enquiry from the author.

Conflict of interest: There did not.

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